FUNCTIONAL DIVERSITY OF INDIGENOUS DIETS IN COASTAL PAPUA NEW GUINEA:

ROLE IN THE NUTRITION TRANSITION AND NONCOMMUNICABLE DISEASE RISK

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DEDICATION

To my mother, Roselyne De Baets.

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ABSTRACT

Simplification of diets and reduced consumption of traditional foods and medicines play an important role in the increased prevalence of noncommunicable disease (NCD) in developing countries undergoing the nutrition transition. Empirical evidence on the consequences of reduced dietary diversity and exposure to functional dietary elements is sorely lacking in the literature. To address this, a food frequency and ethnomedical survey of 365 participants stratified according to age, gender and area of residence was conducted in coastal Papua New Guinea (PNG), and anthropometry, blood pressure and fasting blood glucose (FBG) recorded. Our results support the hypothesis that reduced food variety, especially in the fruits and vegetables group, is associated with increased obesity, hypertension and FBG, particularly in urban people. Application of the quantitative index for dietary diversity (QUANTIDD) showed that this was a superior indicator of nutrient adequacy and was more closely associated with reduced NCD risk. To test the effects of food functionality, a new compound indicator, the dietary functionality index (DFI) was developed. Application of the DFI to our population showed significant trends with improved health parameters, providing novel support for the functionality of indigenous foods towards health maintenance.

From comparative ethnobotanical surveys, five plant species that were used in one community significantly more often than another were selected for laboratory analysis to assess whether differential consumption patterns might explain disparate rates of NCD. Crude extracts of betel quid (BQ), comprised of areca nut (AN; *Areca catechu* L.) and *Piper betle* L. inflorescence (PBI), guava bud (GB; *Psidium guajava* L.), noni (*Morinda citrifolia* L.) and mangrove bean (MB; *Bruguiera gymnorrhiza* (L.) Lam.) were tested for their ability to mediate glucose transport in cultured 3T3-L1 adipocytes, their antioxidant activity, their capacity to prevent Cu^{2+} -catalyzed low density lipoprotein (LDL) oxidation or reduce the cytotoxicity of oxidized LDL (oxLDL) towards cultured bovine aorta endothelial cells (BAEC). Our results demonstrate that BQ and its constituents AN and PBI inhibit insulin action while GB and noni mimic or potentiate it, suggesting that consumption of the latter offsets the diabetogenicity of the former. Betel quid was also

highly toxic towards BAEC and exacerbated oxLDL cytotoxicity, despite being a potent free radical scavenger. In contrast, GB and the root of noni (NR) were able to inhibit LDL oxidation and effectively protected BAEC.

Our findings suggest that communities who consume more plants that possess antiatherogenic and/or antidiabetic functionality, which in itself is contingent on adequate dietary diversity, may be protected from NCD risk. This has important implications for public health policies aimed at reinforcing traditional food habits as a strategy to lessen the NCD burden that is usually associated with economic development.

RESUMÉ

La simplification des diètes et la consommation réduite d'aliments et de médecines traditionnelles jouent un rôle important dans la prédominance de maladies non-transmissibles (NTD) dans les pays en voie de développement exposés à une transition alimentaire. L'évidence empirique sur les conséquences d'une variation alimentaire réduite et d'une exposition aux éléments fonctionnels manque considérablement de littérature. À cette fin, un sondage de fréquence alimentaire et ethnomédical sur 365 participants regroupés selon l'âge, le genre et le secteur de résidence ont été menés sur la côte de Papouasie-Nouvelle Guinée (PNG), au cours desquels l'anthropométrie, la tension artérielle et la glycémie à jeun (FBG) furent enregistrés. Nos résultats appuient l'hypothèse qu'une variété réduite en aliments, particulièrement dans le groupe de fruits et légumes, est associée à l'obésité, à l'hypertension et à la glycémie accrues, en particulier dans les zones urbaines. L'usage d'un index quantitatif de variété alimentaire (QUANTIDD) a démontré qu'il s'agissait là d'un indicateur supérieur de suffisance alimentaire qui était plus étroitement liés au risque réduit de maladies non transmissibles. Afin de mieux observer les effets de la fonctionnalité alimentaire, un nouvel indicateur composé, l'index de fonctionnalité alimentaire (DFI) fut développé. L'application de cet index sur notre population a révélé des tendances marquées à l'amélioration de sérieux paramètres de santé, fournissant ainsi un nouveau support de fonctionnalité des aliments indigènes pour l'entretien de la santé.

Parmi les sondages ethnobotaniques comparatifs effectués, cinq plantes qui furent utilisées plus sensiblement souvent dans une communauté que dans les autres, furent sélectionnées pour analyse en laboratoire afin d'évaluer si les diverses habitudes de consommation pourraient expliquer des taux disparates de maladies non transmissibles. Des extraits bruts de chiques de bétel (BQ), composées de noix d'arec (AN : *Areca catechu* L.) et d'inflorescence de *Piper betle* L. (PBI), des bourgeons de goyave (GB : *Psidium guajava* L.), du noni (*Morinda* citrifolia L.) et des doliques de mangrove (MB : *Bruguiera gymnorrhiza* (L.) Lam.) furent étudiés pour leur capacité de diffusion de glucose dans les adipocytes 3T3-L1 cultivés, pour leur activité antioxydante, pour leur capacité à prévenir l'oxydation de lipoprotéine de faible densité (LDL) catalysée par Cu^{2+}

ou de réduire la cytotoxicité du LDL oxydé (oxLDL) vers les cellules endothéliales cultivées d'aorte de bovin (BAEC). Nos résultats démontrent que les chiques de bétel (BQ) et ses constituants AN et PBI entravent l'action de l'insuline tandis que les bourgeons de goyave (GB) et le noni l'imitent ou la renforcent, impliquant que la consommation de ces derniers compensent pour la diabétogénicité de l'ancien. La chique de bétel était également fortement toxique vers les cellules endothéliales et aggrava la cytotoxicité de l'oxLDL, bien qu'elle soit un désactiveur efficace de radical libre. En revanche, les bourgeons de goyave (GB) et la racine de noni (NR) purent empêcher l'oxydation de la lipoprotéine de faible densité (LDL) et protéger efficacement les cellules endothéliales cultivées d'aorte de bovin (BAEC).

Nos résultats démontrent que les communautés qui consomment plus de plantes à fonctionnalité antiathérogénique et/ou antidiabétique, qui en soi dépendent d'une variation alimentaire adéquate, peuvent être protégées contre les risques de maladies non transmissibles (NCD). Ceci comporte d'importantes implications pour les politiques de santé publique visant à renforcer les habitudes alimentaires traditionnelles en tant que stratégie pour diminuer la charge de maladies non transmissibles habituellement reliée au développement économique.

STATEMENT OF ORIGINALITY

To date, there is little information on functional non-nutrient intake from fruits, vegetables and other plants in human populations and how this relates to long term health. One of the principal strengths of this study is the transdisciplinary approach to a common phenomenon among developing nations. By combining approaches from the fields of anthropology, nutrition, pharmacology, epidemiology, and ethnobotany, we obtain a more holistic perspective by integrating molecular biochemistry with human health and nutritional ecosystems. From this, we present a new paradigm on how foods should be regarded health wise. Emphasis on food functionality as a nutritional tool is used here to support the intuitive concept that eating a variety of foods rich in bioactive phytochemicals promotes health and reduces the risk of chronic disease. The model is tested where it is most needed: in a developing country that is experiencing a rapid rise in noncommunicable disease as a result of changes in lifestyle, activity patterns and the nutrition transition. Our results provide scientific support for the functionality of indigenous foods and medicinal plants which is needed for public health policies centered on reducing NCD prevalence. Reinforcing the incorporation of traditional elements within Westernizing food systems is a socioeconomically feasible strategy that would enable development without incurring a chronic health burden.

Specifically, some of the original contributions from this study include:

- Application of an ecosystems research approach in identifying biophysical and socioecological determinants of dietary diversity and its relationship to obesity, adiposity, hypertension, and impaired glucose tolerance. Biochemical processes are linked to epidemiological dietary and disease patterns in order to demonstrate the complex interconnectedness between systems at the molecular level and at the human population level.

- First application of the recently developed quantitative index for dietary diversity (QUANTIDD) outside of Japan. We demonstrate that QUANTIDD provides dietary information that is not detected by the more conventional food variety score

(FVS) and dietary diversity score (DDS), and is a superior predictor of nutrient adequacy. The index is also tested, for the first time, for its association with health indicators of noncommunicable disease risk (obesity, abdominal adiposity, hypertension and hyperglycemia).

- Conception, construction and validation of the dietary functionality index (DFI), designed to address the need to quantify the pharmacological properties of food in relation to human health and chronic disease. Significant associations between the DFI and proxy-indicators of NCD risk indicate the importance of food functionality in maintaining health. The index also highlights the contribution of cultivated and non-cultivated traditional fruits, vegetables and medicinal plants as essential sources of bioactive phytochemicals.

- Inclusion of medicinal plants, masticants, spices, herbs and dietary adjuncts in all nutritional surveys, nutrient composition analyses, variety scores and functionality indices. Most nutritional and agricultural studies ignore these elements of traditional food systems because of their quantitative insignificance. This study however, emphasizes their role as sources of vitamins, minerals and bioactive compounds.

- Application of the phytochemical index (PI), which was conceived to quantify food functionality but never tested prior to this study. The PI is the ratio of calories derived from plant foods, fish, probiotics and some alcohols, to total caloric intake. The strengths and limitations of the PI are explored and its association with some NCD risk parameters is compared to that of the DFI.

- The nutritional composition of mangrove bean hypocotyl (*Bruguiera gymnorrhiza* (L.) Lam), hitherto unknown, was determined. This study also contributes new pharmacological information about the plant and its relation to local consumption patterns. To date, very little compositional and pharmacological data exists for this species.

- Support for the health benefits of guava bud (*Psidium guajava* L.), particularly as an antidiabetic, and noni (*Morinda citrifolia* L.). From our laboratory findings, we are the first to propose that consumption of these species offsets the diabetogenicity of

betel quid and its constituents, area nut (*Areca catechu* L.) and *Piper betle* L. inflorescence, and more broadly, to other environmental diabetogenic factors.

- The first to subject the aforementioned species to the bioassays included in this study. This not only helps build the pharmacological knowledge base of these plants, but contributes directly to PNG's efforts to catalogue and document the pharmacology of indigenous drugs.

AUTHORS' CONTRIBUTION

Patrick L. Owen designed the study and prepared and submitted all documents required for ethics approval, research visas and travel permits in order to conduct field research in Papua New Guinea. Dietary surveys, ethnobotanical collections, and the measurement of anthropometry, blood pressure and fasting blood glucose were carried out by the candidate. All laboratory protocols, except for 3T3-L1 and BAEC cell culture assays, were developed and conducted by him. The candidate was also responsible for all statistical analyses and the conception and application of the dietary functionality index (DFI).

Teatulohi Matainaho provided housing, resources and facilities in Papua New Guinea, as well as access to the laboratories, libraries and services at the University of PNG. Dr. Matainaho facilitated the acquisition of research permits from the PNG Medical Advisory Board and the Department of Conservation and the Environment, as well as approval for the exportation of biological material to Canada.

Dayna Caves carried out all cell culture experiments on 3T3-L1 adipocytes and aided in protocol modification and analysis. She also contributed to the literature review and tabulation of functional foods required for the construction of the DFI.

Louis C. Martineau was responsible for the 3T3-L1 protocol designed to analyze antidiabetic medicinal plants. His expertise and laboratory techniques facilitated cell culture experiments.

Pierre S. Haddad provided the laboratory space, equipment and facilities at the Université de Montréal to conduct the 3T3-L1 experiments.

Martin Sirois provided the laboratory space, equipment and facilities at the Institut de Cardiologie de Montréal to conduct the bovine aorta endothelial cell (BAEC) culture experiments. Dr. Sirois and his graduate students acquired the cell line, designed the BAEC protocol and provided technical advice on cell culture.

Timothy Johns proposed the original thesis hypothesis and suggested that PNG serve as a model. Dr. Johns provided the laboratory space, equipment and facilities to prepare plant extractions, perform *in vitro* antioxidant assays and LDL oxidation studies. He provided advice on the direction, content and presentation of the manuscript, as well as financial support.

ABBREVIATIONS

%BF:	Body fat percentage
AGE:	Advanced glycation end products
AN:	Areca nut
BAEC:	Bovine aortic endothelial cells
BHT:	Butylated hydroxytoluene
BMI:	Body Mass Index
BQ:	Betel quid
CVD:	Cardiovascular disease
DBP:	Diastolic blood pressure
DDS:	Dietary diversity score
DFI:	Dietary Functionality Index
DFI_V:	Dietary Functionality Index – Variety
DFI_W:	Dietary Functionality Index – Weight
DM1:	Type 1 diabetes mellitus
DM2:	Type 2 diabetes mellitus
DMEM:	Dulbelco's modified Eagle's medium
DMSO:	Dimethylsulfoxide
DPPH:	1, 1-diphenyl-2-picryl-hydrazyle
EDTA:	Ethylenediaminetetraacetic acid
EE:	Energy expenditure
FBG:	Fasting blood glucose
FBS:	Fetal bovine serum
FFI:	Food Functionality Index
FFQ:	Food frequency questionnaire
FVS:	Food variety score
GB:	Guava bud
IGT:	Impaired glucose tolerance
IR:	Insulin resistance
LDH:	Lactate dehydrogenase
LDL:	Low density lipoprotein
MAMC:	Mid-arm muscle circumference
MAR:	Mean Adequacy Ratio
MBC:	Mangrove bean, cooked
MBR:	Mangrove bean, raw
MDA:	Malondialdehyde
MUAC:	Mid-upper arm circumference
NAR:	Nutrient Adequacy Ratio
NCD:	Noncommunicable Disease
NF:	Noni fruit
NJ:	Noni juice
NL:	Noni leaf
NO:	Nitric oxide
NR:	Noni root

oxLDL:	Oxidatively-modified low density lipoprotein
PAF:	Physical Activity Factor
PBI:	*Piper betle* inflorescence
PI:	Phytochemical Index
PNG:	Papua New Guinea
QFFQ:	Quantitative food frequency questionnaire
QUANTIDD:	Quantitative Index for Dietary Diversity
REE:	Resting energy expenditure
ROS:	Reactive oxygen species
SDA:	Seventh-Day Adventist
SBP:	Systolic blood pressure
TBARS:	Thiobarbituric acid reactive substances
TSF:	Tricep skinfold thickness
WHR:	Waist: hip ratio

CHAPTER 1
INTRODUCTION

Let food be thy medicine and medicine be thy food

Hippocrates (460-377 B.C.)

The eradication of hunger in the developing world is the first and primary objective of the UN Millennium Development Goals. Although the number of people going hungry is increasing in parts of sub-Saharan Africa and Southern Asia, the total proportion of people suffering from chronic hunger has declined from 25% in 1970 to 16% in 2005 (United Nations, 2006). Ironically, the developing world is also where the greatest increase in noncommunicable disease (NCD) prevalence is occurring. This is largely a consequence of the nutrition transition and its attendant simplification of diets, especially seen among the urban poor who shift from traditional staples towards energy-rich foods bereft of micronutrients. High-input agriculture, reduced transportation costs and agricultural subsidies have resulted in unprecedented access to refined carbohydrates (e.g. wheat, rice and sugar), where fried food now form a significant portion of the diet. The availability of cheap cereal foods has contributed to the attrition of agricultural biodiversity and a concomitant reduction in dietary diversity which is further narrowed by public health strategies that emphasize technological options (supplementation, fortification) that focus on a few staple crops rather than those based on indigenous and traditional food systems (Frison *et al.*, 2004).

Epidemiological studies support the benefits of a varied diet in relation to nutrient adequacy, improved cognitive and physical function (Clausen *et al.*, 2005) as well as reduced risk of chronic disease (La Vecchia *et al.*, 1997) and all-cause mortality (Kant *et al.*, 1993). In developing countries, where evidence is more scarce, links between dietary diversity and human health have highlighted the contribution of indigenous foods, including wild vegetables, to the traditional food supply base (Hatløy *et al.*, 1998; Ogle *et al.*, 2001; Savy *et al.*, 2005). Integrated within these food systems are a variety of uncultivated fruits and vegetables, spices, herbs, masticants and dietary adjuncts that are

seldom considered in modern agricultural and nutritional surveys and intervention programmes. Although quantitatively insignificant relative to dietary staples, these impart a measurable health benefit as purveyors of vital nutrients and bioactive phytochemicals.

Mounting evidence points towards various phytochemicals in fruits and vegetables as the responsible agents for prolonging human lifespan (Agudo *et al.*, 2007) and reducing the risk of NCDs (Lock *et al.*, 2005) including type 2 diabetes mellitus (DM2) (Sargeant *et al.*, 2001), cardiovascular disease (CVD) (Bazzano *et al.*, 2002), and cancer (Talalay & Fahey, 2001). That a greater variety of plant foods in the diet would provide the necessary phytochemicals needed to stave off NCDs is an intuitive assumption that is sorely lacking empirical evidence. The phytochemical composition of most dietary plants, not to mention those of indigenous origin, is to a large extent unknown and fraught with experimental and analytical inconsistencies. To date, no method exists to quantify the functionality of foods or whether a relationship exists between the amounts or variety of functional foods in the diet and how this affects NCD risk.

Papua New Guinea (PNG), a Pacific nation belonging to the Melanesian group of islands, offers an ideal setting in which to investigate associations between dietary diversity, the nutrition transition and NCD prevalence. Since independence in 1975, socioeconomic shifts towards wage labour have influenced diet and other lifestyle factors such that obesity and DM2 are now commonplace (Ulijaszek, 1993). One cultural group in particular, the Wanigela, have received much attention after having been identified as being especially susceptible to DM2 compared to culturally related groups surrounding them. Of note is that both rural and urban Wanigelans have similar rates of DM2, despite higher rates of obesity in the latter (Martin *et al.*, 1980; Martin *et al.*, 1981; Dowse *et al.*, 1994), which suggests a genetic disposition towards insulin resistance.

The present dissertation offers empirical support that dietary diversity and functionality, interfaced with agro-ecological and sociocultural factors, are more plausible explanations that account for the disparate rates of DM2 observed in coastal Papua New Guinea. A multidisciplinary ecosystems approach incorporating an epidemiological and

laboratory element was employed to illustrate the potential benefits of indigenous food functionality to NCD risk. Comparative dietary and ethnomedical analyses between urban and rural Wanigela, and a culturally related semi-rural village, Kalo, located equidistant between the two, revealed divergent plant use. Mangrove bean (*Bruguiera gymnorrhiza* (L.) Lam.) is unique among the rural Wanigelans in that the starchy hypocotyls form their primary staple. Noni (*Morinda citrifolia* L.) is a popular remedy for a variety of ailments in urban Wanigela. Betel quid, a psychoactive masticant comprised of areca nut (*Areca catechu* L.), pepper vine inflorescence (*Piper betle* L.) and slaked lime (calcium hydroxide) is valued throughout the country, but due to religious principle, is not chewed by Wanigelans. In Kalo, one of the most often cited traditional remedies prescribed for an array of disorders is an infusion of the buds and young leaves of guava (*Psidium guajava* L.). These plants serve as a model to illustrate the hypothesis that reduced intake or outright elimination of certain indigenous traditional foods from the diet of communities undergoing nutritional transition has consequences on NCD risk.

Study Objectives

The objective of this project is to demonstrate an association between reduced exposure to functional dietary elements and increased risk of NCD. Specifically our objectives are:

1. To determine sociodemographic predictors of dietary diversity and whether dietary diversity is an indicator of nutrient adequacy and is associated with fasting blood glucose, hypertension, obesity and visceral adiposity.
2. To develop a quantitative index able to predict dietary functionality and assess its association with fasting blood glucose, hypertension, obesity and visceral adiposity.
3. To investigate the insulin-mimetic and potentiating effects of selected plants and relate findings to the health status of the community who uses them.
4. To assess the antioxidant potential of selected plants and whether this is related to their ability to preserve endothelial integrity when confronted with atherogenic/cytotoxic concentrations of oxidatively-modified low density lipoprotein.

CHAPTER 2
LITERATURE REVIEW

DIETARY DIVERSITY AND HEALTH

With the exception of human breast milk in the first months of life, no one food can provide all essential nutrients. This concept underlies the pervasive nutritional recommendation of consuming a wide variety of foods, one of the principle elements of food-based dietary guidelines established by several countries (Savide et al., 1997). Japan, for example, has the most quantitative variety guidelines, which recommends consuming more than 30 different kinds of foods each day. Greater food variety not only ensures nutritional adequacy (Hsu-Hage & Wahlqvist, 1996; Hatløy *et al.*, 1998), but has been associated with decreased risk of mortality and reduced risk of chronic noncommunicable disease (Kant *et al.*, 1995). On the other hand, diversity in certain selected food groups – energy-dense foods, for example – has been associated with body mass index (BMI) and obesity (Kennedy, 2004; Ponce *et al.*, 2006). This underscores the importance of moderation and balance, while requiring a refined definition of dietary diversity and its measurement.

Although epidemiology indicates a strong inverse relationship between dietary diversity and noncommunicable disease, no particular nutrient, food, or dietary pattern, aside from hypercaloric consumption, has been consistently identified as a causative or protective factor. Focused attention on fruits and vegetables suggest that this food group may be the key to health assurance. A diet diverse in fruits and vegetables provides the vitamins, minerals and bioactive compounds that offer both generic (e.g. antioxidant) and disease-specific (e.g. insulin mimetic) effects that can plausibly reduce NCD risk (Bloch *et al.*, 1995). Low consumption of fruits and vegetables was estimated to be responsible for 2.4%, 2.8% and 3.5% of the burden of disease in New Zealand (Tobias, 2001), Australia (Mathers *et al.*, 1999) and the European Union (National Institutes of Public Health, 1997; Pormerleau *et al.*, 2006), respectively. Although no attempt has been made to estimate this contribution in developing countries, total worldwide mortality

attributable to low fruit and vegetable consumption is estimated to be up to 2.635 million deaths per year, or 1.8% of the global burden of disease. Ischemic heart disease and ischemic stroke would especially be affected by increasing fruit and vegetable intake to optimal levels, reducing the disease burden by 31% and 19%, respectively. The burden of stomach, esophageal and lung cancer would also be reduced by 19%, 20% and 12%, respectively (Lock *et al.*, 2005). Epidemiological studies have observed a decreased incidence of DM2 in individuals who consume more fruits and vegetables (Colditz *et al.*, 1992; Feskens *et al.*, 1995; Williams *et al.*, 1999; Ford & Mokdad, 2001; Sargeant *et al.*, 2001). In the Nurses Health Study, risk of DM2 was inversely associated with vegetable, but not fruit intake (Colditz *et al.*, 1992), while the National Health and Nutrition Examination Survey (NHANES I) found that a higher mean daily intake of both fruits and vegetables provided protective benefits, even after correcting for body mass index, level of physical activity, age, race, cigarette smoking and alcohol consumption (Ford & Mokdad, 2001). Increased consumption of vegetables and legumes was inversely associated with 2 h glucose level in a prospective study of middle-aged men (Feskens *et al.*, 1995) as well as with glycosylated hemoglobin (HbA_{1C}) levels in a cross-sectional study of middle-aged men and women (Sargeant *et al.*, 2001). This latter effect was not explained by dietary fiber, and dietary or serum vitamin C.

As a diet-related disorder of glucose metabolism, the global burden of DM2 would doubtless be reduced with adequate fruit and vegetable intake, if only to reduce the risk of late-stage cardiovascular complications responsible for the majority of DM2 deaths. Type 2 diabetes, with or without co-occurring obesity, is one of the first metabolic diseases to surface from a dietary imbalance in indigenous populations of developing countries (O'Dea *et al.*, 1980), and for this reason, serves as an ideal focal point for a review on the effects of dietary diversity and functionality on NCD risk.

DIETARY FUNCTIONALITY OF INDIGENOUS FOOD SYSTEMS

The concept of "physiologically functional food" originated in Japan from a 1984 systemic, large-scale national project aimed at exploring the physiological efficacies and potential health benefits of antioxidant phytochemicals, digestion-resistant oligosaccharides and bioactive oligopeptides, generally recognized as belonging to non-nutritive compounds (Swinbanks & O'Brien, 1993). Recognition of food's pharmacological properties quickly lead to political action affecting functional food development, commercialization, and marketing health claims, pioneered with Japan's "foods for specific health use" (FOSHU), and now part of the regulatory framework of the EU, USA and Canada, among others (Health Canada, 1998). Agriculture and Agri-Food Canada (Health Canada, 1998, Section 2.2) defines functional foods as:

> "...similar in appearance to, or may be, a conventional food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions".

The term "nutraceutical", often used interchangeably with functional foods, is used to describe a product isolated or purified from food rather than the whole food. In this dissertation, functional foods refers to unaltered, unprocessed foods, including probiotic bacteria from fermented dairy products, prebiotics (substances that promote growth of certain bacteria), fish, and certain alcohols (wine, beer and cider) even though the term in the broader sense encompasses fortified, processed or engineered foods in which functional ingredients have been added.

Relevant to the field of functional food science but beyond the scope of this review is the application of genetic information to assess dietary functionality at the mRNA and/or protein level by transriptomics and/or proteomics. The emerging field of nutrigenomics will help define individual genetic responses to functional elements. For example, some food consumers with a single nucleotide polymorphism in the gene for β-oxidation have an expression product, W64R, that is no longer able to interact with the ligand adrenalin (Kagawa *et al.*, 2002). In such cases, the adrenalin inducing phytochemical capsaicin from chili peppers would not be able to elicit its ability to reduce

obesity through body fat oxidation. Nutrigenomic research will not only enable personalized nutrition but will clarify the link between diet and differential subgroup susceptibilities to degenerative diseases (Arai, 2005).

Traditional diets, usually characterized by foods considered less palatable and potentially more toxic than modern foods, are important sources of essential nutrients and functional phytochemicals. Moreover, wild and cultivated species of herbal medicines, beverages, masticants and supplements, often neglected by modern nutritionists, are unquestionably related to nutritional adequacy. In this context, the functionality of such underutilized and/or little-known dietary elements may have more relevance to human health than their nutritional qualities, and may have serious implications for diseases of basic metabolism. As with carbohydrates, protein, fats, vitamins and minerals, non-nutrient phytochemicals have been a part of human dietary history, and are increasingly recognized as significant mediators of health and physiology (Johns, 1996). These may elicit a physiological effect in their own right, or affect the digestion, absorption and metabolism of other nutrients as to influence their bioavailability and bioactivity. Humans have had to adapt to various environmental conditions via genetic, physiological, behavioural and cultural mechanisms, in order to meet dietary needs and maintain physiologic homeostasis (Ulijaszek, 2002). Humans display a degree of phenotypic and genotypic plasticity, such that dietary patterns can vary significantly, for example from the high protein, high fat diet of the Inuit, to the low protein, low fat diets of PNG Highlanders. Rapid divergence from such nutritional adaptations may have detrimental consequences, as can be observed in rapidly modernizing nations who are experiencing rapid increases in chronic disease prevalence. On the other hand, nutritional adaptation is a continuous process, and Diamond (1992) claims that genetic adaptation to Western high-sugar diets has already developed in various Pacific Island populations.

Noncommunicable diseases have a multi-factoral etiology and do not arise from changes in single factors. The importance of phytochemicals in such processes is difficult to conceptualize due to complex interactions with nutrients, non-nutrients and other environmental factors (Johns, 1996). Long-term exposure to specific non-nutrient phytochemicals elicits different forms of adaptive mechanisms in order to detoxify,

metabolize, excrete or incorporate particular compounds. It is therefore likely that changes in non-nutrient phytochemical intake affect dietary homeostasis and disease progression through disruption of the balance between humans and the chemical environment to which they are genetically adapted. The role of functional phytochemicals in NCD is shadowed by the quantitatively more important nutrients, but as mediators of human genetics and metabolism, their absence or presence must be considered when contemplating human dietary ecology.

Traditional diets are considered protective against NCD development, due in part to their higher diversity in fruits, vegetables, course cereals and root crops. In Australian Aborigines, a temporary 2-week reversion to a traditional diet resulted in small but significant improvements in glucose tolerance and insulin response (O'Dea & Spargo, 1982). Reversion to a traditional lifestyle for 7 weeks resulted in marked improvements or normalization in carbohydrate and lipid metabolism in diabetic Australian Aborigines (O'Dea, 1984). In contrast, conversion from a traditional to an affluent diet for only 5 weeks had detrimental effects on weight, plasma lipids and lipoproteins levels in Mexico's Tarahumara Indians (McMurry *et al.*, 1991). The protective effects of traditional diets against noncommunicable diseases has been attributed in part to its similarities with the dietary behaviour of Paleolithic humans – the nutrition on which *Homo sapiens* survived and evolved since their appearance on Earth about 100,000 years ago (Eaton & Konner, 1985). Paleolithic nutrition differs substantially from the typical modern American diet, and it also differs, albeit to a much lesser extent, from that advocated by nutritionists and health agencies to avert chronic diseases (WHO Study Group on Diet, 1991). Low-fat diets high in complex carbohydrates that have a low glycemic index are recommended for DM2 management by nutritionists (Wolever *et al.*, 2000).

Fiber and the Glycemic Index

The glycemic index of a food refers to the glycemic response of a fixed amount of available carbohydrate from a test food to the same amount of carbohydrate from a standard food (glucose or white bread) consumed by the same individual (Jenkins *et al.*, 2002). Foods that are more slowly absorbed may have biological benefits in relation to

DM2, as supported by laboratory, animal, clinical, and epidemiological findings (reviewed in Willett *et al.*, 2002). Fiber influences the glycemic response to ingested carbohydrates through its physical action in the gut, where it tends to decrease transit time and slow the absorption of nutrients (Jenkins & Jenkins, 1985). Fiber also produces short-chain fatty acids from colonic bacterial fermentation, which enter the portal circulation and increase glucose oxidation, decrease fatty acid release and increase insulin clearance, thereby creating an environment conductive to insulin sensitivity (Thorburn *et al.*, 1987). This is complicated by the fact that different foods produce a wide range of glycemic responses that are not predicted from their chemical composition (Jenkins *et al.*, 1981). Sucrose and some sugary foods, for instance, give a lower glycemic response than many starchy foods, while high- and low-fiber foods often have similar glycemic indices.

What is certain is that traditional use of low-glycemic-index carbohydrate foods in the diet was prevalent among cultures who now experience high rates of DM2 after having recently changed to high-glycemic-index foods (O'Dea *et al.*, 1980; Boyce & Swimburn, 1993). The majority of traditional Australian and Pacific bushfoods have a lower glycemic index than common "western" foods, which is consistent with the hypothesis that carbohydrates in traditional diets are more slowly digested and absorbed, thus providing a protective advantage against diabetes (Thorburn *et al.*, 1987).

Antidiabetic phytochemicals in food plants

The notion that food can convey a health promoting benefit beyond its nutritional value has gained increasing scientific acceptance (Bloch *et al.*, 1995). This has been reinforced by evidence showing the protective effects of antioxidant flavonoids and polyphenols against CVD (Hertog *et al.*, 1993) and some forms of cancer (Hertog *et al.*, 1994). Although antioxidants may be beneficial for diabetes pathologies derived from glucose toxicity, their involvement in DM2 development is less clear (Rösen *et al.*, 2001). Pancreatic β-cells are highly susceptible to free radical damage and are characterized by low levels of endogenous antioxidants. Vitamin C and E may provide benefits against DM2 development, as suggested by some epidemiological studies (Feskens *et al.*, 1995; Salonen *et al.*, 1995), but these failed to improve insulin sensitivity in men and women of various ethnicities (Sanchez-Lugo *et al.*, 1997). In addition to antioxidant vitamins, fruits

and vegetables are good sources of minerals. Magnesium may lower the risk of DM2 in light of its role in insulin action, such that hypomagnesemia impairs insulin activity and promotes insulin resistance (Paolisso *et al.*, 1990). Chromium, required for the glucose tolerance factor, improves insulin sensitivity and glycemic control in diabetic patients (Morris *et al.*, 2000), but not all studies report an association (Althuis *et al.*, 2002).

Food is the major source of exposure to health-mediating components in our environment. Although the cumulative outcome of this exposure on health remains difficult to quantify, there is a growing body of evidence that phytochemicals can modify the risk of developing DM2. These act by stimulating β-cell insulin secretion, increasing peripheral tissue insulin sensitivity, modifying glucose metabolism, or by reducing carbohydrate absorption via inhibition of the intestinal glycolytic enzyme α-glucosidase. Several classes of phytochemicals, including alkaloids, polysaccharides, terpenoids, steroids, and flavonoids have been found to possess antidiabetic activity (Bailey & Day, 1989). Several common food plants consumed in PNG contain such phytochemicals. Sweet potato, (*Ipomoea batatas* (L.) Lam.), an important staple, contains an acidic glycoprotein isolated from the cortex which displayed antidiabetic activity in streptozotocin (STZ)-induced and genetically diabetic rats (Kusano & Abe, 2000; Kusano *et al.*, 2001). Dioscoretine, isolated from the aqueous fraction of African yam (*Dioscorea dumetorum* (Kunth) Pax.) produced significant hypoglycemic activity when injected intra-peritoneally in normal and alloxan-induced diabetic rabbits (Iwu *et al.*, 1990), as did the glycan dioscoran C isolated from glutinous yam (*Dioscorea japonica* Thunb.) when injected intra-peritoneally in alloxan-induced hyperglycemic mice (Hikino *et al.*, 1986). The outer seed coats of rice (*Oryza sativa* L.) contain the polysaccharide oryzabran A, which has a hypoglycemic effect in normal and alloxan-induced hyperglycemic mice (Hikino *et al.*, 1988).

One food plant that has received considerable attention as an antidiabetic aid is bitter melon (*Momordica charantia* L.) (reviewed in Patel & Srinivasan, 1997). A 50 ml serving of bitter gourd juice consumed with a 50 g oral glucose challenge reduced glucose concentrations of DM2 patients by approximately 20% within 1 h and also improved glycemic control after 2-3 months of routine use (Leatherdale *et al.*, 1981). The fruit

contains a variety of hypoglycemic principles, including "charatin", a homogenous mixture comprised mainly of β-sitosterol-D-glucoside and stigmadine glucoside. Moreover, an insulin-like peptide, polypeptide-d, isolated from the fruit, seeds and tissue of *M. charantia* displayed hypoglycemic effects when administered subcutaneously in type-1 diabetic patients (Khanna *et al.*, 1981). The plant does not act by enhancing insulin release (Leatherdale *et al.*, 1981), although an aqueous extract stimulated insulin release from normal isolated islets *in vitro* (Welihinda *et al.*, 1982). Rather, it seems to inhibit hepatic gluconeogenesis and decrease intestinal glucose uptake (Meir & Yaniv, 1985).

Onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) contain the volatile oils allyl-propyldisulphide and diallyldisulfide oxide, which contain disulfide bonds similar to insulin and may bind to insulin-degrading enzymes, thereby prolonging insulin life (Jain & Vyas, 1974). At an oral dose of 10 mg/kg, onion produced a more potent hypoglycemic effect than tolbutamide in glucose-induced hyperglycemic rabbits (Karawya *et al.*, 1984).

A variety of other food plants have been reported to contain hypoglycemic agents. However, practically all have been examined in the context of diet-based medicines. Onion and garlic have long been used as dietary supplements for the traditional treatment of diabetes in Asia, Europe and the Middle East. Bitter melon is widely used as a traditional therapy for diabetes in India, China, Central America and Australia. Both the African yam and glutinous yam are traditional diabetes remedies in Nigeria and the Orient, respectively (reviewed in Bailey & Day, 1989), and the sweet potato is used traditionally in Shikoku, Japan (Kusano *et al.*, 2001). This conceptual duality of foods as medicines has cultural importance and gives insight into the functionality of foods. Many cultures do not clearly categorize certain plants as food and medicines since the same species may be perceived as one, the other, or both simultaneously depending on the stage of plant development, method of preparation, and the state of health or pathology of the individual (Etkin & Ross, 1982).

Antidiabetic phytochemicals in medicinal plants

The origins of medicine can be viewed as a specialized form of dietary behaviour, such that materials are deliberately ingested to engender a state of health separate from that provided by basic nutrition needed for growth and maintenance. Alternatively, phytochemicals can be passively ingested as part of a normal diet and if consumed routinely, may elicit a long-term biological effect. The distinction between passive or deliberate ingestion can be illustrated by routine behaviours that contribute to the prevention or maintenance of health, versus intentional efforts to alleviate a disease state (Johns, 1999). For example, the Maasai of East Africa supplement their high fat and cholesterol diet with herbs and barks with hypocholesterolemic (Chapman *et al.*, 1997) and antioxidant (Lindhorst, 1998) properties that help avert thrombogenesis. The Maasai perceive these as dietary components of their traditional food system, likely as flavouring agents, and do not necessarily recognize their preventative pharmacological properties. This suggests an adaptation to local dietary conditions in which phytochemicals in food or other ingested substances contribute an essential component of dietary ecology. Conversely, the Luo of Kenya specifically and deliberately ingest *Aphania senegalensis* (Juss. ex Poir.) Leenh. as a remedy for stomach ailments (Johns, 1999), which is ingested only when symptoms appear. Although these plants lack the quantitative importance of food plants, they unquestionably affect well-being.

Over 1,000 plants have been used to treat diabetes in different cultures from around the world (Marles & Farnsworth, 1995). Of the 295 traditional medicinal plants screened using cell cultures, 81% were potentially antidiabetic, and over 200 pure phytochemicals displayed hypoglycemic activity. Unfortunately, most do not lend themselves readily to pharmaceutical development (Day, 1998) since perhaps 30-60% of investigated plants exert a hypoglycemic response through toxic mechanisms (Marles & Farnsworth, 1995). Certain phytochemicals may also exacerbate diabetic complications. For instance, taro (*Colocasia esculenta* (L.) Schott) an important staple of PNG, has been found to aggravate nephropathy in diabetic rats (Grindley *et al.*, 2001).

Broadhurst et al. (2000) evaluated the insulin-like activity of several culinary herbs and spices commonly incorporated in foods using cultured rat epididymal

adipocytes. Of the 49 species examined in their study, cinnamon (*Cinnamomum verum* J. Presl.) displayed strongest activity. Chalcone polymers in cinnamon have previously been shown to improve insulin receptor function by activating insulin receptor kinase and inhibiting insulin receptor phosphatase, resulting in increased insulin sensitivity (Imparl-Radosevich *et al.*, 1998). Green and black tea (*Camellia sinensis* (L.) Kuntze) also had activity due to the tea's polyphenolic and polysaccharide content, and displayed hypoglycemic activity in STZ-induced diabetic rats (Gomes *et al.*, 1995). However, since STZ induces diabetes by free radical damage to β-cells, these tea polyphenolics may have protected the rat from STZ damage, and may not necessarily represent true antidiabetic action. A variety of other common herbs, spices, foods and medicines were screened by Swanston-Flatt et al. (1989; 1990; 1991) using STZ diabetic rats. Those that elicited a hypoglycemic response were further evaluated for their ability to mediate glucose transport, metabolism and glycogenesis using cell culture, and included agrimony (*Agrimony eupatoria* L.) (Gray & Flatt, 1998a), elder (*Sambucus nigra* L.) (Gray *et al.*, 2000), eucalyptus (*Eucalyptus globules* Labill.) (Gray & Flatt, 1998b), coriander (*Coriandrum sativum* L.) (Gray & Flatt, 1999) and alfalfa (*Medicago sativa* L.) (Gray & Flatt, 1997). All plants demonstrated the presence of antihyperglycemic, insulin-releasing, and insulin-like activity. Metformin, a commonly prescribed insulin sensitizer and hypoglycemic agent, with its origins in traditional European folk medicine, is derived from goat's rue (*Galega officinalis* L.).

In areas of developing nations in which conventional medicines are not readily available, traditional treatments for DM2 remain the major form of therapy. Few traditional plants have been thoroughly scrutinized scientifically or medically, stimulating the WHO to implore greater research efforts (WHO Expert Committee on Diabetes Mellitus, 1980). Such plants may represent the most practical and effective forms of primary and secondary modes of treatment, and has the added advantage of being culturally sensitive.

THE NUTRITION TRANSITION IN PAPUA NEW GUINEA

Food systems of Papua New Guineans have continuously adjusted over the last 10,000 years to changing environmental, demographic, cultural and social pressures. Only two staple food crops are indigenous to PNG: palm sago (*Metroxylon* spp) and taro (*Colocasia* spp). Agriculture, which appeared roughly 9,000 years ago and is believed to have evolved independently in PNG, may have involved additional crops introduced from Polynesia and South East Asia (Allen, 1983) such as yams (*Dioscorea* spp) and plantains (*Plantago* spp). Subsistence practices and food systems changed dramatically with the introduction of sweet potato (*Ipomoea* spp) by Portuguese traders which were subsequently brought to West Papua by Malay traders. Sweet potato has a higher yield and faster maturation time relative to other PNG crops and could be cultivated at higher altitudes, thereby allowing further population growth. Up until then, changes in diet had been due to availability of rainforest resources, introduction of new edible species and advances in agriculture.

With European contact, introduction of steel and new crops allowed greater population expansion due to facilitated land clearing and garden tending. More importantly, the introduction of a cash economy created a new subsistence option: wage-earning and access to a variety of imported foods (Spencer & Heywood, 1983). By independence in 1975, imported food had already become an important item on the national budget. Transition to wage-based economies meant that selection and acquisition of food depended on income. To meet the demand of urban growth, rural communities were encouraged to "market garden" and sell surpluses at urban markets (Ulijaszek, 1993). However, prices of traditional foods were high and urban wages low, allowing more reasonably-priced imported items such as rice and tinned fish to displace other staple foods. Other new items in the diet included edible fats and oils, refined foods and alcohol. In Simbu Province, where unprecedented cash prosperity had resulted from the cash-cropping of coffee, the consumption of rice had increased dramatically, while sweet potato consumption decreased (Harvey & Heywood, 1983). Nationally, annual per capita availability of starchy foods and fruits and vegetables has steadily decreased since

1964, while the supplies of cereals have increased (Food and Agriculture Organization of the United Nations, 1999) (**Figure 2.1.**).

Urbanization and population migrations to urban centers impose considerable non-sustainable demands on ecosystems by increased market demands, encroachment on arable land, generation of wastes and pollutants, and destruction of forest and ocean resources. Such socioeconomic and environmental challenges result in reduced access to a healthy varied diet needed to resist infectious disease, toxicity and avert malnutrition and chronic disease (Johns, 2002). Reduced access to fresh fruits and vegetables, and declines in traditional garden food production are thought to be the primary causes for the high prevalence of DM2 among Torres Straight Islanders, who reside between Australia and PNG (Leonard *et al.*, 1995).

NONCOMMUNICABLE DISEASES IN PACIFIC ISLAND POPULATIONS

According to the latest WHO estimates (2005), more than 180 million people worldwide have DM2, and this is assumed to double by 2030. Roughly 1.1 million deaths in 1995 are believed to be from DM2, 80% of which have occurred in low and middle-income countries. However, this is likely a gross underestimation. The risk of macrovascular disease is two- to four-fold higher in people with DM2 compared to non-diabetics, and this is usually recorded as the underlying cause of death (Gerstein & Yusuf, 1996). The association between DM2 and CVD in the Asia-Pacific Region is so strong that one study demonstrated that each 1 mmol/L increment drop in fasting blood glucose resulted in a 21% (95% CI 18-24%) and 23 % (19-27%) reduced risk of total stroke and ischemic heart disease, respectively (Asia Pacific Cohort Studies Collaboration, 2004). If DM2 is taken into account as a contributory condition, standardized WHO estimates rise to approximately 2.9 million deaths per year. Statistics for DM2 in PNG should also be considered an underestimation. The WHO PNG country profile reported a DM2 prevalence of 152,000 in 2000, although only 1.3 – 2.8% of these were correctly recognized according to hospital admittance records (Lelsey *et al.*, 2001).

Noncommunicable diseases have only begun to emerge in the last half-century in Pacific Island nations and have now reached epidemic proportions, especially in urbanized populations (Taylor *et al.*, 1989). A thrifty-gene hypothesis to explain heightened susceptibility to NCD is particularly applicable to Pacific populations when considering regional social, cultural and environmental history. Only after the art of open-sea navigation had been mastered could the Pacific arena be accessible for human settlement. Consequently, Oceania was the last continent in the world to have been populated by humans (as recently as 12-15 millennia ago) and for centuries, Islanders have remained more or less isolated. Social, cultural and economic patterns had remained more or less unaltered up until WWII, whereupon the region had become stepping-stones and battlefields for the conflict between industrialized nations. Within the space of a few years, new technology, new communications, and increased social contact had catapulted Pacific Islanders into an age that, in comparison, had come as a gradual process in Western societies (Zimmet, 1979). Inevitable conflict between traditional cultural systems and Western ideas and technologies brought about extraordinary change in disease patterns. Communicable diseases such as measles, whooping cough, tuberculosis, influenza, and venereal disease, introduced by early European mariners, drastically decimated the native Pacific population. In one extreme case, infectious disease killed up to 64% of New Zealand Maoris who, until the early 20th century, were considered a dying race. At present, the annual growth rate of Maoris is double that of New Zealand Europeans although it is projected to slow in the future, from 4% in 1971 (Prior, 1971), to 1.4 % in 2002, to 1.2% in 2021, (Statistics New Zealand, 2006).

Communicable diseases, especially HIV-AIDS, malaria, tuberculosis and viral/bacterial gastrointestinal infection, along with persistent micronutrient (iron, iodine, folic acid, vitamin A) deficiencies remain a health priority in the Pacific, but these are quickly giving way to NCDs, currently the primary cause of death in the Western Pacific region (**Figure 2.2.**). Atherosclerotic vascular disease is thought to become the greatest regional health problem in the coming decade, along with a growing global concern for mental health and depression (Wahlqvist *et al.*, 2001).

In a comparative longitudinal analysis of DM2 and impaired glucose tolerance (IGT) incidence in Pacific and Indian Ocean populations, Dowse (1996) reported considerable variation between nations, ethnicities and urban-rural areas. The Wanigela people of Koki, Port Moresby, PNG, are suggested to have one of the world's highest recorded prevalence of impaired glucose tolerance (IGT) and DM2 (Dowse *et al.*, 1994). When the prevalence data of IGT was age-standardized, this population had a higher prevalence than Nauruans, even though the latter were more obese, leading the investigators to suggest that Wanigelans might be the most diabetes-susceptible population yet identified (**Figure 2.3.**).

In the present dissertation, Koki was selected to represent the urban sample. Progressively more traditional villages include Kalo and Wanigela village, located approximately 135 km and 200 km SE of Port Moresby respectively. Because of geographical proximity and cultural similarities, these communities are ideally placed to emphasize environmental and socioeconomic influences and account for the effects of genetics and certain sociocultural elements (**Table 2.1.**).

Koki was first established in Port Moresby in the 1950s as a canoe settlement by the Wanigela people. Consequently, a clear majority of residents of Koki today originate from Wanigela village located in the Marshall Lagoon area (Hodge *et al.*, 1996c). Koki has ready access to trade stores and supermarkets and many residents work for wages. Houses are built over the water on stilts using modern materials, and generally have electricity and running water. Kalo and Wanigela lifestyles are comparatively more traditional, where fishing and gardening remain important food sources. Imported products such as rice, tinned meat and fish, and biscuits are purchased by men who work and commute to Port Moresby. Houses are built from traditional materials and most lack electricity and running water. Greater detail on the study area and population characteristics is presented in Manuscript 1 (page 83).

INSULIN RESISTANCE IN THE ETIOLOGY OF TYPE-2 DIABETES MELLITUS

According to the American Diabetes Association (ADA), DM2 is almost always associated with insulin resistance (IR) and either relative or absolute insulin deficiency, although not requiring insulin treatment for survival (American Diabetes Association, 1997). There is general agreement that the primary lesion in diabetes is IR of peripheral tissues. The primary effects of insulin on glucose metabolism are to suppress hepatic glucose production via inhibition of gluconeogenesis and glycogen breakdown, and to promote the transport of glucose into peripheral tissues, most notably, skeletal muscle (80-90%). Insulin resistant individuals who have normal glucose tolerance are hyperinsulinemic relative to more insulin-sensitive control subjects; a mechanism which allows them to overcome the defect in insulin action. Individuals who can maintain insulin hypersecretion and sufficiently high levels of circulating insulin to overcome the defect in insulin action remain normoglycemic (Hollenbeck & Reaven, 1987), whereas those with a co-existing β-cell abnormalities are unable to do so and develop hyperglycemia and clinical DM2.

Genetic determinants of type 2 diabetes mellitus

O'Dea et al. (1980) observed that obesity and DM2 were among the first diseases to appear with economic development. However, there are dramatic differences in the ways different populations respond to acculturation, which suggest an important genetic component to DM2. Neel (1962) postulated that polygenic forms of DM2 are the consequences of having evolved a thrifty genotype in order to cope with food scarcity and physical exertion during ancient human migrations and settlements. The thrifty genotype model was not designed to construe causality in all populations, but rather, incorporates the evolutionary history of certain populations, such as Pacific Islanders and Amerindians, in situations creating a strong selective advantage for individuals with a hyperinsulinemic response. In the 40 years since Neel's thrifty gene hyptothesis, researchers have found polymorphisms in the β_3-adrenergic receptor gene that significantly affected susceptibility to obesity and diabetes (Silver *et al.*, 1997). Many more diabetogenic genes await further elucidation.

Environmental mediators of type 2 diabetes mellitus

Significant differences in DM2 prevalence and incidence have been noted between rural and urban settings in several Pacific nations (King *et al.*, 1984; Eason *et al.*, 1987; Russel-Jones *et al.*, 1990; Taylor *et al.*, 1991; Collins *et al.*, 1994). In virtually all of these studies, urban living was associated with obesity (BMI>27), hypertension, elevated total triglycerides and cholesterol, and greater prevalence of IGT and DM2. In PNG, rural - urban differences have been profound in the Wanigela people residing along the coast surrounding the capital, Port Moresby. As previously mentioned, those living in the city (Koki) had an extraordinarily high prevalence of DM2 while rates among those residing a few hundred kilometers away, in the more traditional village Kalo, were comparatively low (**Table 2.2.**).

Although obesity rates are comparatively lower than other Melanesian societies, urbanized Wanigelans had a higher BMI relative to their rural counterparts. Likewise, this population exhibited a thrombogenic blood profile characterized by elevated serum total triglycerides, total cholesterol (Erasmus *et al.*, 1993) and LDL cholesterol (Hodge *et al.*, 1996a) concentrations. Such urban / rural discrepancies indicate the enormous importance of the environmental components of DM2. Changes in physical activity and diet seem to have the most impact, as these are important determinants for the likelihood of obesity, the most significant risk factor for DM2.

Obesity and visceral adiposity

Among environmental factors, obesity is most strongly associated with insulin resistance of skeletal muscle and adipose tissue. However, it is also apparent that only a proportion of obese subjects develop diabetes. In the context of the thrifty genotype, it has been suggested that the relationship between obesity and DM2 is not that one factor is the cause of the other, but rather that both are separate results of a common defect (Neel *et al.*, 1998). This may explain why obesity is not as strongly associated with DM2 in developing countries as it is in industrialized nations. The relationship between obesity and DM2 is difficult to infer from cross-sectional data, since diabetes itself may cause weight loss, and patients are specifically encouraged to lose weight. In this respect,

incidence data is more relevant in the context of a causal relationship. Moreover, the manner in which adiposity is distributed has more relevance to DM2 prevalence.

Fat distribution, clinically assessed using the waist-hip ratio (WHR), is more important than BMI in conferring risk for death from all causes, and specifically from ischemic heart disease (Fujioka *et al.*, 1987). Greater risks are attached to the "pot-bellied" abdominal deposition of fat in subcutaneous and especially intra-abdominal visceral sites, compared with fat deposition around the hips, thighs and buttocks. Truncal obesity is associated with insulin resistance, hypertension, dyslipidemia and accelerated atherogenesis in the metabolic "syndrome X" described by Reaven (1988). Genetics partially determines truncal fat deposition, which is also favoured by overeating, reduced physical activity, and likely smoking, excessive alcohol consumption and the neuroendocrine responses (particularly cortisone secretion) which result from psychological stress (Björntorp, 1988). That is, all factors associated with acculturation and urban living.

Intrauterine and infant malnutrition

Hales and Barker (1992) have proposed a "thrifty phenotype" which maintains that intrauterine and/or early postnatal malnutrition predisposes an individual to insulin resistance in adulthood. Low birth weight and obesity in adulthood are both independently associated with insulin resistance, with those who were both thin as babies and obese as adults the most susceptible. Continued malnutrition onto adulthood has no adverse effects. Such patterns are seen in societies that have become affluent after periods of nutritional deprivation, such as in the Pima Indians (McCance *et al.*, 1993) and Nauruans (Zimmet, 1991). In PNG, it is estimated that 25% of newborns weigh less than 2.50 kg. Indices of maternal nutrition were predictors of birth weight, and disparities were noted among highland and coastal mothers, such that babies born in the Highlands had a higher mean birth weight than those in coastal areas (Primhak & MacGregor, 1991).

OXIDATIVE STRESS IN THE ETIOLOGY OF DM2

In the last decade, research has indicated that oxidative stress is a direct consequence of hyperglycemia and plays an important role in the pathophysiology of diabetes (Tesfamariam, 1994; West, 2000). The diabetic state is associated with depleted cellular antioxidant defense systems and enhanced production of reactive oxygen species (ROS). Clinical studies show subnormal levels of plasma α-tocopherol, ascorbate (Sinclair, 1993), total glutathione, and serum malonaldehyde (Sharma *et al.*, 2000), along with increased levels of oxidation biomarkers such as TBARS (Griesbacher *et al.*, 1995), 8-epi-$PGF_{2\alpha}$ (Gopaul *et al.*, 1995; Davi *et al.*, 1999), 8-OHdG (Dandona *et al.*, 1996) and oxidized low-density lipoproteins (oxLDL) (Altomare *et al.*, 1992; Sundaram *et al.*, 1996) in diabetics compared to control subjects. Some of the major mechanisms by which hyperglycemia induces oxidative stress include nonenzymatic glycosylation of proteins and formation of advanced glycated end products (AGEs), autoxidative glycosylation, alterations in sorbitol pathway activity, and enhanced protein kinase C activity. Metabolic stress resulting from changes in energy metabolism and inflammatory mediators also play a major role in free radical production. There is also increasing evidence that oxidative stress may play a role in the development and progression of type 1 diabetes mellitus (DM1) (Eizirik *et al.*, 1996) and IR in type 2 diabetes mellitus (DM2) (Rudich *et al.*, 1998b).

Insulin resistance may be associated with increased intracellular concentrations of free radicals and depleted antioxidant defenses. Compensatory hyperinsulinemia may induce a rise in plasma free radical production, while hypertriglyceridemia and hypercholesterolemia, observed in obese individuals, may exacerbate free radical generation. It has also been proposed that increased cytosolic triglyceride stores in non-adipose tissues associated with central obesity increases oxygen free radical production (Bakker *et al.*, 2000). Low levels of α-tocopherol (Salonen *et al.*, 1995) and ascorbate (Vijayalingham *et al.*, 1996) were strong predictors of DM2 in human prospective studies. Oxidative stress may act by impairing insulin-mediated GLUT-4 translocation from internal vesicles to the plasma membrane (Rudich *et al.*, 1998a). The mechanism may involve redistribution of insulin receptor substrate-1 (IRS-1) and

phosphatidylinositol 3-kinase (PI-3 kinase) as demonstrated using 3T3-L1 adipocytes (Rudich *et al.*, 1998b; Tirosh *et al.*, 1999) and L6 muscle cells (Rudich *et al.*, 1998b). Administration of α-lipoic acid improved or reversed insulin resistance in these cell cultures, as well as in murine diabetic models (Jacob *et al.*, 1996; Henrikson *et al.*, 1997; Khaimisi *et al.*, 1997) and DM2 patients (Jacob *et al.*, 1999). Other antioxidants such as α-tocopherol (Paolisso *et al.*, 1993), ascorbate (Paolisso *et al.*, 1995) and glutathione (Laurenti *et al.*, 1997) also improved glucose homeostasis, establishing the beneficial role of antioxidants in stimulating glucose uptake via activation of the insulin signaling pathway.

Oxidative stress and diabetic complications

Oxidative stress associated with hyperglycemia impairs cellular function and contributes to the pathophysiology of diabetes by altering vascular and neural function. As a result, peripheral vascular disease accounts for the majority of deaths related to diabetes, and up to 50% of diabetic patients develop diabetic neuropathy. High glucose concentrations promote free radical production via three biochemical pathways: (1) increased formation of glucose-derived advanced glycation end products (AGEs) (Singh et al., 2001); (2) glucose induced activation of protein kinase C isoforms (Koya & King, 1998), and (3) increased glucose flux through the aldose reductase pathway (Lee & Wolever, 1998).

Advanced glycation end products

In their open chain form, reducing sugars such as glucose react nonenzymatically with the free amino groups and lysine residues of proteins. Glycated proteins, in the presence of transitional metals, are able to donate an electron to molecular oxygen, leading to the formation of reactive oxygen species. When the protein half-life is longer than ten weeks, glycated proteins undergo irreversible modifications, forming advanced glycation end products (AGEs). Diabetic vascular tissues have a four-fold increase in AGE concentrations compared to non-diabetics, and like glycated proteins, they are also able to produce oxygenated free radicals. If oxidation accompanies glycation, glycoxidation products such as N^{ε}-[carboxymethyl]-lysine (CML) and pentosidine are formed (Bierhaus *et al.*, 1998b). These products in turn facilitate free radical production

by binding redox reactive transition metal ions (Saxena *et al.*, 1999). Elevated levels of CML have been observed in DM1 patients preceding micro- and macro-vascular complications (Berg *et al.*, 1997), and detected in the perineurium, endothelial cells, pericytes of endoneurial microvessels as well as in myelinated and unmyelinated fibers compared with control subjects (Giardino *et al.*, 1994).

Structural components of the connective tissue matrix or basement membrane, such as collagen, are prime targets of AGEs but can also include myelin, complement C3, tubulin, plasminogen activator and fibrinogen. Binding of AGEs to the extracellular matrix leads to cross-link formations, resulting in increased stiffness of the protein matrix, hence impeding function as well as increasing resistance to proteolytic removal (Paul & Bailey, 1999). Physiological consequences of cross-link formation include sclerosis of renal glomeruli, thickening of the capillary basement membrane and atherosclerosis development (Monnier *et al.*, 1996). Cross-linking of sub-endothelial structural proteins may also trap lipoproteins, which impairs cholesterol efflux leading to lipoprotein accumulation and atherogenesis (Chappy *et al.*, 1997).

Receptors for AGEs (RAGE), have been identified on several tissues, including smooth muscle cells, monocytes, macrophages, and endothelial cells (Thornally, 1998) and are expressed at higher concentrations in diabetics compared to controls (Bierhaus *et al.*, 1998a). The binding of AGE-RAGE leads to oxidative stress and activation of the transcription factor NF-κB in endothelial cells, mesangial cells, neurons and smooth muscle cells. NF-κB is a free radical-sensitive transcription factor and modulates gene expression of endothelin-1 (pro-coagulant tissue factor), VCAM-1 (adhesion molecule), and thrombomodulin (Bierhaus *et al.*, 1998b), and is thereby able to transform the endothelial environment to a pro-coagulant, vasoconstrictive state.

High glucose concentrations are also able to induce NF-κB expression, suggesting an association between postprandial hyperglycemia and the development of vascular complications (Du *et al.*, 1999). In macrophages, activation of NFκB also induces release of the cytokines interleukin-1α (IL-1α), interleukin-6 (IL-6) and TNF-α, which induce an inflammatory response and exacerbate endothelial dysfunction (Neumann *et al.*, 1999). Antioxidants, such as α-lipoic acid (Bierhaus *et al.*, 1997; Hofmann *et al.*, 1998), α-

tocopherol (Rösen *et al.*, 1995) and acetylcysteine, have been observed in experimental and clinical studies to inhibit NF-κB activation or decrease its binding activity, and ameliorate complications in diabetic patients. Such findings provide further evidence of the benefits of antioxidants in the modulation of redox-sensitive transcription factors, which play an important role in vascular and neural diabetic complications.

Protein Kinase C Activation

High serum glucose concentrations, as well as AGEs, are able to enhance activation of protein kinase C (PKC). Several isoforms have been observed in diabetic patients (Koya & King, 1998), of which PKCβ and α are predominant in vascular tissues (King *et al.*, 1997). Elevated PKC activity enhances the synthesis of prostaglandins by releasing arachidonic acid (AA) from phospholipids and by *de novo* synthesis of prostaglandin H synthase in endothelial cells. Such vasoconstrictor prostanoids, like thromboxane A_2 (TXA_2), prostaglandin H_2 (PGH_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) promote vasoconstriction and play a role in endothelial cell dysfunction. Free radicals themselves are also able to activate arachidonic acid metabolism (Tesfamariam & Cohen, 1994). Cyclooxygenase, the enzyme that converts PGG_2 to PGH_2, is capable of generating $O_2^{\bullet -}$ by a mechanism that involves the conversion of NADPH to an intermediate radical form, which then interacts with oxygen to produce the ROS (Tesfamariam, 1994). Therefore, hyperglycemia is related to an augmented flux of AA and vasoconstrictor prostanoid synthesis that is coupled to increased generation of ROS.

Aldose Reductase Pathway

Glucose at abnormally high intracellular concentrations is preferentially metabolized through the polyol pathway since hexokinase is saturated. Aldose reductase catalyzes the reduction of glucose by NADPH to sorbitol, which can in turn, be oxidized to fructose by sorbitol dehydrogenase (SDH). Increased flux through this pathway depletes intracellular NADPH, rendering it unavailable for regeneration of reduced glutathione, thereby reducing the antioxidative capacity of cells and contributing to the oxidative stress in diabetes (Kashiwagi *et al.*, 1994). Moreover, the ratio of cytosolic NADH / NAD^+ is disturbed due to enhanced SDH activation and augments production of

$O_2^{\bullet-}$ via reduction of PGG_2 to PGH_2 by prostaglandin hydroperoxides that use NADH as a reducing cosubstrate (Williamson *et al.*, 1993).

As a cofactor of nitric oxide synthase (NOS III), NADPH is also required for the generation of nitric oxide (NO) from L-arginine. Depletion of NADPH caused by increased flux through the polyol pathway therefore decreases NO synthesis. Nitric oxide is not only a potent endothelium-derived vasodilator, but also inhibits expression of adhesion molecules (VCAM-1, ICAM-1) and the proliferation of smooth muscle cells (Lee et al., 1989). It is therefore an essential component of normal endothelial function and vasotonus.

Nitric oxide is able to react with $O_2^{\bullet-}$, generated via hyperglycemia-induced AGE formation and PKC activation, to form peroxynitrite ($OONO^{\bullet}$), which is a potential oxidizing agent due to its decomposition to NO_2, and $OH^{\bullet}$, a highly reactive ROS (Tesfamariam & Cohen, 1994). It has also been observed that AGE-containing collagen could scavenge NO and thus limit its diffusion into smooth muscle cells (Bucala *et al.*, 1991). An elevated intracellular glucose concentration therefore results in several abnormalities in NO metabolism and promotes endothelial dysfunction and thrombogenesis

Diabetic endotheliopathy

Microvascular complications, such as retinopathy and nephropathy, are noticable symptoms that directly affect a diabetes patient's quality of life. In tissues where glucose uptake is independent of insulin, including retina, lens, kidney, and peripheral nerves, all tissue sites of diabetic complications, exposure to elevated glucose levels causes an increase in intracellular sorbitol and fructose levels due to increased activity of AR and SDH (Giugliano & Ceriello, 1996).

Although diabetic patients frequently suffer from microvascular complications, the majority succumb to macrovascular disease expressed as accelerated atherosclerosis often leading to myocardial infarction and stroke. Diabetic vascular injury is not uniformly caused by a single factor such as hyperglycemia, but rather from complex interactions involving hyperglycemia, hyperlipidemia, oxidative stress, accelerated aging,

hyperinsulinemia and/or hyperproinsulinemia, and alterations in coagulation and fibrinolysis (Calles-Escandon *et al.*, 1999).

Strong evidence suggests that the initial step leading to atherosclerosis involves changes in endothelial cell function. Such changes, including the up-regulation of surface and soluble cell adhesion molecules and the release of cytokines, are triggered by a variety of inflammatory stimuli encountered in the blood including oxidized low-density lipoproteins (oxLDL), free radical species, lipopolysaccharide (LPS) and cytokines such as TNF-α. This process, termed endothelial activation, plays an integral role in the development of atherosclerosis. During endothelial activation, adhesion molecules are expressed and circulating monocytes are attracted to the endothelium by cytokines, bind to the adhesion molecules, adhere and transmigrate to the subendothelial space, where they become macrophages. Within the subendothelium, macrophages scavenge oxLDL, transform into lipid-laden foam cells, and contribute to the fatty streak in the early stages of atherosclerosis (Calles-Escandon & Cipolla, 2001).

Endothelial cells line the internal lumen of all the vasculature and serves as an interface between circulating blood and vascular smooth muscle cells (VSMC). The endothelium participates in the local regulation of vascular smooth muscle tone via vasoconstriction and vasodilation. Several molecules crucial for its vasomotor function are synthesized by the endothelium in response to local mechanical stimuli (e.g., flow and shear stress), metabolic conditions (e.g., hypoxia) and receptor-mediated agonists (e.g., acetylcholine) (Vogel, 1997). Major vasoconstrictors synthesized by the endothelium include thromboxane A_2, prostaglandin H2 and endothelin 1 (Shimokawa, 1999). Major endothelium products which act as vasodilators include nitric oxide (NO), endothelium-derived hyperpolarizing factor and prostacyclin. Under normal physiological conditions, smooth muscle vascular tone is determined by the balance of local vasoconstricting and vasodilating agents synthesized by the endothelium. The primary compound responsible for vasodilation in arteries is NO, which also plays an important role in vascular homeostasis and neuronal and immunological functions. Nitric oxide also inhibits platelet aggregation, modulates leukocyte-endothelium interactions by altering adhesion molecule expression and reducing monocyte adherence and inhibits the proliferation of

smooth muscle cells. Several physiological changes associated with diabetes such as hypercholesterolemia, hyperglycemia and oxidative stress potentiates endothelial dysfunction through decreased NO production or NO inactivation (reviewed in Garcia & Stein, 2006; Rask-Madsen & King, 2007).

Oxidation of LDL and its role in atherogenesis

Several studies have shown that a diet high in saturated fats leads to elevated low density lipoprotein (LDL) levels in serum (Nicolosi *et al.*, 2001). LDL carries on average three fourths of the serum total cholesterol. Each LDL particle has a hydrophobic core composed of cholesterol esters and triglycerides, and a hydrophilic coat composed of phospholipids, free cholesterol and a molecule of apolipoprotein B-100. Upon oxidation, the LDL particle will have newly formed lipids and lipid oxidation products, some of which sufficiently polar to leave LDL. Other lipid oxidation products however, can react with various amino acid residues of the apolipoprotein and alter it structurally and in composition. These oxidatively modified LDL have several new biological actions. It is a potent chemoattractant to circulating monocytes due to the lysophosphatidylcholine generated upon oxidation; it significantly inhibits the motility of tissue-localized macrophages; it is cytotoxic to cultured fibroblasts and endothelial cells (Steinbrecher *et al.*, 1990), and may also promote platelet aggregation, modulate growth factor production by cultured cells, and modify prostaglandin synthesis (Bankson *et al.*, 1993).

The current hypothesis on the role of oxidized LDL in atherogenesis involves its role in the development of fatty streaks. With high LDL concentration in the serum, the intimal LDL concentration is also increased. According to the "response-to-retention" hypothesis, the first modification of LDL once in the vessel wall is aggregation and fusion (Williams & Tabas, 1998). Once intimal LDL is oxidized, circulating monocytes are recruited, enter the arterial wall, and undergo a phenotypic modification and are converted to macrophages. The oxidized LDL inhibits the motility of the macrophages, preventing them from returning to circulation. Since macrophages are able to oxidize LDL themselves, the magnitude of LDL oxidation is increased dramatically. Macrophage-colony stimulating factor, which is released by endothelial and smooth muscle cells, induces the expression of the scavenger receptor, which binds oxidized LDL

particles (Hajjar & Nicholson, 1995). It is important to note that these scavenger receptors do not bind normal LDL. Oxidized LDL is then engulfed by the macrophage, where the LDL contributes to the cell's transformation into a foam cell. These lipid-laden foam cells contribute to the formation of a fatty streak, which increases in size with the accumulation of cells and lipids. The fatty streak may ultimately become mineralized to from an atherosclerotic plaque (Selwyn *et al.*, 1997).

Free radicals are, by definition, any chemical species with one or more unpaired electron in its outer shell (Bankson *et al.*, 1993). The oxidation of LDL *in vitro* is initiated with the superoxide anion ($O_2^{\bullet-}$) and propagation is dependent on lipid oxyradicals ($ROO^{\bullet}$). The first biochemical change within LDL involves the peroxidation of polyunsaturated fatty acids, which results in the rapid generation of free radicals. *In vivo*, oxidized LDL is rapidly removed from circulation by sinusoidal endothelial cells in the liver, spleen and bone marrow. The degree of LDL oxidation in the serum is usually thought to be insignificant due to the presence of relatively high amounts of endogenous antioxidants. Therefore, LDL oxidation occurs most frequently in areas where antioxidant concentration is low, such as the subendothelial space of the arterial wall (Bankson *et al.*, 1993; Hajjar & Nicholson, 1995).

The role of antioxidants in CVD prevention

Because oxidized LDL is implicated in the pathogenesis of atherosclerosis, treatment with antioxidants could conceivably retard the progress or actually regress the atherosclerotic lesion. Supplementation with ascorbic acid, α-tocopherol or β-carotene has been shown *in vitro* and *in vivo* to increase LDL resistance to oxidation (Jialal & Fuller, 1995). Epidemiological studies have also shown a negative correlation between plasma concentrations of these antioxidants and the risk of CVD (Riemersma *et al.*, 1991). The Nurses' Health Study (Stampfer *et al.*, 1993) and the Health Professional Follow-Up Study (Rimm *et al.*, 1993) support the use of vitamin E supplementation in reducing the risk of coronary heart disease. In a randomized, placebo-controlled trial, the Cambridge Heart Antioxidant Study (CHAOS) (Stephens *et al.*, 1996) demonstrated a significant reduction of cardiovascular events by vitamin E. However, other intervention trials have yielded rather mixed results (Steinberg & Witztum, 1998). Observations that

vitamin E supplementation showed only a modest effect on the progression of atherosclerosis in humans (Hodis *et al.*, 1995) suggests that the benefits of antioxidant supplementation are not due to a reduced propensity of macrophages to transform into foam cells or reverse the increased susceptibility of foam cells to oxLDL-induced cell lysis (Asmis & Jelk, 2000). Rather, vitamin E exerts its protective effects in the endothelium or the inhibition of smooth muscle cell proliferation (Keaney *et al.*, 1999). This is the basis for the relatively recent suggestion that antioxidants should be part of the human diet for the prevention of CVD (Hajjar & Nicholson, 1995).

In addition to these conventional antioxidants, there is currently a considerable amount of interest in the flavonoids, phenylpropanoids and phenolic acids of plant foods which may act as antioxidants or as agents of other cardioprotective mechanisms (Rice-Evans *et al.*, 1996). Flavonoids are ubiquitous in plants and constitute a large class of phytochemicals with free radical-scavenging properties attributed to their phenolic hydroxy groups. They are able to inhibit non-enzymatic lipid peroxidation and NADPH-induced lipid peroxidation *in vitro* and inhibit LDL oxidation by macrophages (Brandi, 1992). Since these bioactive phytochemicals are found in fruits and vegetables, a diet high in these foods would be considered beneficial for the prevention and management of CVD. Several epidemiological studies found a lower risk of cancer and CVD among individuals whose diets were relatively high in fruits and vegetables (Greenberg & Sporn, 1996). However, flavonoid intake was not strongly associated with a reduced risk of CVD in a prospective study and mean follow-up of 6.9 y (Sesso *et al.*, 2003).

The *in vivo* protective effects of flavonoids has been suspect since quercetin was found to be poorly absorbed through the gut and thus unable to reach plasma levels that are able to inhibit LDL oxidation and platelet aggregation. Although Hertog (1993) calculated an average daily intake of 30 mg of quercetin per day, more recent reports suggest that the systemic availability of quercetin depends on the form by which it is ingested. For instance, quercetin-4'-O-β-D-glucoside (Q-4-G) is preferentially absorbed from the gut compared to other forms such as quercetin 3-O-β-rutinoside (Aziz *et al.*, 1998; Hollman & Katan, 1999; Cermak *et al.*, 2003). Supplementation of Q-4-G to

human subjects inhibited platelet aggregation via interference with platelet cell signaling pathways and thrombus formation (Hubbard *et al.*, 2003).

Small dietary intervention trials have shown that consumption of flavonoid-rich foods such as tea and onions were associated with a significant increase in plasma levels of flavonoids in diabetic patients (Hollman & Katan, 1999; Lean *et al.*, 1999). Since ROS and abnormal antioxidant status facilitates the progressive impairment of β-cell function in the pathogenesis of DM2, flavonoids may plausibly incur a benefit. A prospective study in Finland reported that intake of specific flavonoids such as quercetin and myricetin were inversely correlated with risk of incident DM2 (Knekt *et al.*, 2002). Also, oxidative stress may be involved in the pathogenesis of chronic inflammation underlying IR, DM2 and CVD (Esposito *et al.*, 2002). However, a prospective and cross-analysis of the Women's Health Study found no association between intake of flavonols and flavones and plasma concentrations of fasting insulin, Hb_{A1C}, CRP or IL-6 (Song *et al.*, 2005).

PLANTS SELECTED FOR LABORATORY ANALYSIS

The following plant species were selected for analysis on the basis of their quantitative and qualitative importance, determined by surveys and personal interviews. These are: betel quid (BQ) comprised of areca nut (AN: *Areca catechu*) and the inflorescence of *Piper betle* (PBI), guava (*Psidium guajava*), noni (*Morinda citrifolia*) and mangrove bean (*Bruguiera gymnorrhiza*) (**Figure 2.4.**). A review of their ethnobotany, phytochemistry and ethnopharmacology pertaining to NCD follows.

Betel Quid

Betel quid is a psychoactive masticant chewed by 200-600 million people, roughly a tenth of the human population, and is the fourth most widely used 'addictive' substance after caffeine, tobacco and ethanol (Marshall, 1987). This ancient practice is thought to originate from Malaysia (Norton, 1998) and eventually spread to PNG. One of the

earliest reports of betel quid chewing in PNG was made by Atkinson et al. (1964) who noted its popularity among coastal populations where children first began chewing as young as 3 years of age. Typically, betel quid in PNG is comprised of three ingredients; the nut of *Areca catechu* (areca nut), the inflorescence of *Piper betle* (betel vine) and slaked lime (calcium hydroxide). The tip of the betel inflorescence is moistened and dipped into lime, then applied to the buccal mucosa of the inner cheek along with the areca nut. The mixture produces a blood-red juice which stains the teeth and gums. Long-time users eventually develop permanent black stains.

While betel quid chewers report feelings of increased alertness, stamina, well-being and some euphoria while chewing, there are some inherent health risks with habitual use. Betel quid chewing is intimately associated with the occurrence of oral leukoplasia, oral submucous fibrosis, hepatocarcinoma, oropharyngeal and esophageal cancer in China, India and other-betel-chewing countries (Norton, 1998). Indeed, oral cancer is the most common form of cancer in PNG (IARC, 2004). The carcinogenicity of areca nut is attributed to the nitrosamines derived from its four major alkaloids arecoline (0.30-0.63% dry weight), arecadaine (0.31-0.66% dry weight), guvacoline (0.03-0.06% dry weight) and guvacine (0.19-0.72% dry weight). Several experiments have demonstrated that areca nut extract or its alkaloids possess cytotoxic or genotoxic properties in numerous cell lines including mammalian cells, Chinese hamster V79 cells and ovary cells, oral fibroblasts and keratinocytes (Stich *et al.*, 1981; Shirname *et al.*, 1984; Jeng *et al.*, 1994; Jeng *et al.*, 1999). When exposed to cultured buccal mucosal epithelial cells, AN extract induced DNA strand breaks, DNA protein cross-links and cell differentiation (Sundqvist & Grafström, 1992). In addition, BQ components have been shown to induce inflammation by stimulating prostanoid, interleukin-6 and TNF-α production of gingival keratinocytes and KB cancer cells (Jeng *et al.*, 2000; Jeng *et al.*, 2003; Chang *et al.*, 2004). Based on existing evidence the WHO International Agency for Research on Cancer recently classified betel quid and areca nut as human carcinogens (IARC, 2004). The carcinogenicity of arecal alkaloids is attributed to the generation of ROS (Stich & Anders, 1989), and although these have a direct relevance to exposed buccal squamous cells and keratinocytes, there is reason to suppose that systemic arterial endothelial cells may also be affected. Arecoline could be detected by GC-MS in blood

plasma of eight fasting men fed freshly dried areca nuts, confirming that areca nut components are absorbed through the gastrointestinal tract or directly from the oral mucosa into the blood stream (Strickland *et al.*, 2003). Indeed, there is mounting evidence that habitual BQ chewing is related to increased risk of metabolic disorders such as obesity, CVD and DM2.

Betel quid chewing and cardiovascular disease

The most common acute ill effects observed in betel quid users are tachycardia/palpitation, followed by tachypnea/dyspnea, sweating, dizziness and nausea (Deng *et al.*, 2001). Although heart rate increases in all users, only neophyte users ever show increased systemic blood pressure (Chu, 1993). The cardioacceleratory response is due to stimulation of the parasympathetic nervous system by arecoline, resulting in a reduced RR interval variation (Chu, 1995).

The mechanisms by which areca nut and betel quid increase cardiovascular risks are not clear (Trivedy *et al.*, 1999). One possibility is via elevated homocysteine concentrations, which are associated with increased risk of ischemic heart disease. A study of a Bangladeshi population living in east London found raised homocysteine and reduced folate concentrations that strongly correlated with regular betel quid chewing (Mannan *et al.*, 2000). Another possibility is via upregulation of the copper-dependent enzyme lysyl oxidase caused by the relatively high copper concentrations found in areca nuts. Lysyl oxidase is involved in the cross-linking of collagen and atherogenesis in the major vessels (Trivedy *et al.*, 1997).

In seeming contradiction, areca nut extract, areca tannins and arecaidine induced vasodilation when exposed to isolated rat aorta (Goto *et al.*, 1997). In addition, propargyle esters of arecaidine induced 6-keto-$PGF_{1\alpha}$ and cGMP production in bovine aortic endothelial cells, however not in rabbit vascular aortic smooth muscle cells (Jaiswal *et al.*, 1991). Cyclooxygenase-2 (COX2), an inducible enzyme responsible for PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesis, plays an important role in inflammation and vascular integrity. Areca-nut extract induced COX2 mRNA and protein expression in primary human gingival keratinocytes (Jeng *et al.*, 2000), as did its major alkaloid arecoline in

human buccal mucosal fibroblasts (Tsai *et al.*, 2003a). The differences in vascular response and prostaglandin synthesis reflects the influence other betel quid components have on the actions of areca-nut.

Deng et al. (2001) reported at least one case of myocardial infarction resulting from betel quid chewing. One of the major risks for arterial occlusion resulting in myocardial infarction is arterial plaque instability. Platelets are well known to play crucial roles in the homeostasis of plaque formation and blood coagulation processes. In an *in vitro* study, an aqueous extract of areca nut stimulated platelet aggregation with induction of thromboxane B_2 (TXB_2) (Jeng *et al.*, 2000). Although TXB_2 strongly promotes platelet aggregation, areca nut was able to induce its effect independently via a pathway requiring activation of tyrosine kinase, phospholipase C and calcium mobilization. Production of TXB_2 itself was caused indirectly by ROS produced from areca nut.

Formation of reactive oxygen species from betel quid constituents

The astringent taste of areca nut is due to the large proportion of polyphenols (tannins, hyroxychavicol and safrole) that make up its dry weight (17-29.8%) (Wang *et al.*, 1997). In alkaline conditions (pH $\geq$9.5) such as that produced in the oral cavity because of slaked lime, these polyphenols undergo autoxidation to form superoxide anion ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) (Nair *et al.*, 1990; Nair *et al.*, 1992). Areca polyphenols, including the tannin fraction, were genotoxic at alkaline pH in *Saccharomyces cerevisiae* due to ROS production (Rosin, 1984).

The relatively high concentration of the transitional metals copper (3-188 μg/g) (Trivedy *et al.*, 1997) and iron (~75 μg/g) in areca nut and slaked lime (190 μg/g Fe) (Zaidi *et al.*, 2002) exacerbate ROS formation. This is achieved by redox cycling via quinine semiquinone radicals and iron-catalyzed Haber-Weiss and Fenton reactions. Nair et al. (1992) demonstrated that the catechin fraction of an areca nut extract was the most active producer of ROS, enhanced by Fe^{2+}, Fe^{3+}, and Cu^{2+} but inhibited by Mg^{2+}. In the presence of DNA, areca nut extract at alkaline pH formed 8-OH-deoxyguanosine (8-OHdG), as quantified by HPLC-electrochemical detection (Nair *et al.*, 1987).

Hydroxychavicol, a phenol found in areca nut and in greater amounts in *P. betle* inflorescence (9.74 mg/g fresh weight) (Hwang *et al.*, 1992) displayed pro-oxidant activity on oral KB carcinoma cells *in vitro* at concentrations higher than 0.1 mM, resulting in induced intracellular production of ROS and depletion of GSH. Lower concentrations however, were able to scavenge H_2O_2 and $O_2^{-\bullet}$ and elicit an antioxidant effect (Chang *et al.*, 2002). During its metabolism, an *ortho*-quinone is produced, which subsequently produces ROS via redox cycling (Iverson *et al.*, 1995).

Antioxidant effects of betel quid constituents

The inflorescence of *P. betle* contains a high concentration of phenolic compounds that have displayed antioxidant activity. The major phenolic compounds, expressed as mg/g fresh weight, are safrole (15.35), hydroxychavicol (9.74), eugenol (2.51), eugenol methyl ester (1.81), isoeugenol (1.81) and quercetin (1.11) (Hwang *et al.*, 1992).

Aqueous extracts of betel inflorescence proved to be effective scavengers of ROS, with an IC_{50} of 80, 28 and 73 μg/ml towards H_2O_2, $O_2^{-\bullet}$ and $OH^{\bullet}$ radicals, respectively (Lei *et al.*, 2003). Eugenol, a well-establish hydrophobic antioxidant found also in clove oil, is able to inhibit lipid peroxidation at the initiation and propagation phase and inhibit TBARS formation in Cu^{2+}-induced LDL oxidation. Lower concentrations, however, were found to possess pro-oxidant activity towards LDL (Naidu & Thippeswamy, 2002). Eugenol's strong antioxidant capacity is due to its methoxyphenolic structure that is able to trap chain-carrying peroxy radicals much faster than peroxy radicals can react with lipid substrates. Furthermore, eugenol compounds are able to form complexes with reduced metals, further preventing metal-catalyzed oxidation (Ito *et al.*, 2005). Eugenol also appears to act as an *in vivo* antioxidant as demonstrated in rats intoxicated with CCl_4 (Kumaravelu *et al.*, 1995).

Interestingly, epidemiological findings suggest that chewers who include piper inflorescence rather than the leaf in their masticant have increased risk of developing oral cancer (Ko *et al.*, 1995). In an animal study, the presence of PBI in BQ exacerbated the occurrence of hyperkeratosis to AN alone despite an increase in survival rate (Chiang *et*

al., 2004). This may be due to the high concentrations of carotenes (80.5 mg/g fresh weight) and to a lesser degree, ascorbic acid (1.9 mg/g fresh weight) in the leaf that may confer extra antioxidant protection (Wang & Wu, 1996). Administration of β-carotene (180 mg/wk) to habitual betel quid chewers significantly reduced the frequency of micronuclei in the oral mucosa and also inhibited the development of new oral leukoplasias (Stich & Anders, 1989). Betel-leaf extract was able to inhibit radiation-induced lipid peroxidation effectively and when fed to male Swiss albino mice at 1, 5 and 10 mg/kg b.w., enhanced hepatic concentrations of superoxide dismutase (SOD) and glutathione peroxidase (GSH) in a dose-dependent manner. Catalase activity, however, was inhibited at higher concentrations (Choudhary & Kale, 2002). An earlier study demonstrated that high concentrations of betel-leaf extract (400-2000 mg/kg b.w.) fed to the same animal model resulted in increased lipid peroxidation and decreased activity of catalase and SOD in the thyroid. This was inhibited when betel leaf extract was fed at lower concentrations (Panda & Kar, 1998).

The enhanced carcinogenicity of *Piper* inflorescence may be attributed to its content of the human hepatocarcinogen safrole (IARC, 1976). At high concentrations (1mg/mL), an aqueous extract of PBI induced DNA breaks of oral mucosal fibroblasts (Jeng *et al.*, 1994) and was cytotoxic towards oral keratinocytes (Jeng *et al.*, 1999). These concentrations are markedly higher than that required for PBI's antioxidant activity, suggesting that the benefits or risks of including PBI in the quid are a matter of dosage. This physiological significance is further complicated by findings that demonstrated the ability of PBI phenolics to inhibit the formation of carcinogenic areca nut nitrosamines and thus mediate the genotoxic and cytotoxic effects of betel quid. At concentrations up to 1.8 mg/mL, crude PBI inhibited the nitrosation of arecoline and formation of *N*-nitrosoguvacoline (Wang & Peng, 1996). Hydroxychavicol from betel-leaf reduced the formation of *N*-methyl-*N*-nitrosurea, a mutagenic product of nitrite and methylurea by scavenging nitrite (Nagabhushan *et al.*, 1989). Additional benefits may arise from the fact that PBI extract can inhibit platelet aggregation by inhibiting ROS-mediated TXB_2 and PGD_2 production at concentrations above 10 μg/mL (Lei *et al.*, 2003). However, when in the presence of areca nut, *P. betle* leaf was not able to prevent platelet aggregation (Jeng *et al.*, 2002).

Whether components of *Piper* can induce an antioxidant and antithrombotic effect *in vivo* is uncertain. Complex reactions occur during the mastication of betel quid, where the antioxidant potency of PBI is compartmentalized and may act primarily to quench the ROS produced from AN. Interestingly, saliva itself was found to inhibit both H_2O_2 and $O_2^{-\bullet}$ formation due to salivary peroxidases (Nair *et al.*, 1987). Ultimately, the level of oxidative stress and carcinogeneity produced from chewing betel quid depends on the balance between the rate of ROS generation from areca nut and the rate at which they can be quenched by *P. betle* polyphenols and salivary enzyme systems.

Betel quid chewing and type 2 diabetes mellitus

Areca nut nitrosamines target tissues that originate from the embryonic foregut, including the naso- and oropharynx, the stomach, liver, lungs, and relevant to DM2, the pancreas. Several other nitrosamines induce diabetes, most notably streptozotocin (STZ), which induces DM1 in animals when administered in high doses, and DM2 at lower doses. Their diabetogenicity is attributed to a structural moiety that has a 'ring' configuration similar to that of the chair-shaped 'ring' of hexomeric glucose. This moiety is able to bind with islet β-cell glucose receptors and damage, or at high doses destroy, β-cells as a result of free radicals generated from the instability of such nitroso-compounds (Okamoto *et al.*, 1988).

There is evidence that dietary sources of nitrosamines cause diabetes in humans. High dietary nitrosamine intakes in mothers is associated with increased incidence of childhood DM1 (Dahlquist *et al.*, 1990). In Iceland, imposed regulations on the amount of nitrosamines permitted in smoked cured mutton in 1980 may have explained the reduced incidence of childhood diabetes in relation to other European countries (Helgason *et al.*, 1992). Although smoked cured mutton was never confirmed to be diabetogenic in humans, it demonstrated strong diabetogenicity in adult CD1 mice (Helgason *et al.*, 1984).

Compelling epidemiological and animal studies suggest an association between betel quid chewing and DM2. In Papua New Guinea, chewing betel quid was the predominant independent risk factor for DM2 (odds ratio 3.4; 95% CI. 2.0-5.9), along

with age. Body mass index and region of residence (coastal inhabitants had a higher incidence than highlanders) were also relevant factors (Benjamin, 2001). Among a Bangladeshi population residing in east London, waist size and weight, longstanding predictors of hyperglycemia, increased with increasing use of *paan* betel quid, independent of other established risk factors such as central obesity, age, smoking and parity (Mannan *et al.*, 2000). The authors caution that the widespread vitamin D deficiency found in 90% of those at risk of diabetes and in 45% of those not at risk in their sample population may have obscured their analyses in relation to betel quid use. Vitamin D deficiency has been shown to be associated with hyperglycemia and hypoinsulinemia in an earlier study by the same authors (Boucher *et al.*, 1995).

Young adult CD1 mice were fed areca nut in standard feed for 2-6 days and glucose tolerance and pancreatic islet were investigated. Permanent diabetes developed in 8.3% of the animals, in association with obvious central obesity and pancreatic islet histology characteristic of human DM2 (enlargement and vacuolation of islet cells). The offspring of the areca-fed mice, especially males, developed diabetes in 10.6-30% of the various test litters and all were obese and had similar islet changes. The same phenomena was seen in subsequent F_2-F_4 generations, leading the researchers to suggest that consumption of areca nut induces heritable abnormalities resulting in increased DM2 risk (Boucher *et al.*, 1994).

Epidemiological data suggest that there is no obvious relationship between betel quid chewing and DM2 susceptibility. Betel quid has been used since before 500 BC, and DM1 or DM2 have been historically uncommon in the populations who chew. Modernization of lifestyle, mainly obesity and physical inactivity were the major determinant factors of DM2 and IGT in Pacific populations where betel quid chewing is not practiced (Dowse, 1996). In PNG, an extraordinarily high incidence of DM2 was recorded in the non-chewing Seventh Day Adventist Wanigela communities, whereas a low incidence was recorded in Kalo where betel quid chewing is indulged (Dowse *et al.*, 1994). However, the hypothesis that betel quid chewing causes DM2 may be justified if the myriad of confounders are considered. Betel quid may increase susceptibility to DM2 indirectly by promoting weight gain and increased waist size. The association may also

be compounded by genetically determined susceptibility to areca nut alkaloids in humans, much like that shown in mice where susceptibility to low-dose STZ-induced DM2 was determined by HLA type (Tanaka *et al.*, 1990). In addition, the carotenoids in betel leaf may provide some protection. When CD1 mice were fed the most carcinogenic of the arecal nitrosamines, 3-methylnitrosaminopropionitrile (MNPN), added β-carotene diminished the hyperglycemic effect that developed in males but not in females (Motahar & Boucher, 1997).

Sociocultural and economic aspects of betel quid chewing in PNG

The areca palm is a coastal tree grown in lowland villages and is now consumed virtually all over PNG. It is the most important cash crop not destined for export and is grown and marketed without any involvement from the state (Benediktsson, 2002). Areca nut is so embedded into the national culture that it shares the same sociocultural importance as food. It plays a symbolic role in reaffirming social ties, sanctions religious beliefs and practices, and is an expression of cultural identity. It is often exchanged as gifts to visitors, in appreciation of kind deeds or as a sign of agreement between individuals or clans, and is shared in social gatherings. Weddings, funerals, birthdays and other social occasions would not be complete without a gift of areca nut (Williams *et al.*, 2002). Not surprisingly, it forms an important component of traditional medicine; the nut's astringency is purported to cure dysentery, eliminate worms and treat malaria. A disturbing trend among some healers is the prescription of BQ chewing as a treatment for DM2, a practice that is increasingly demonstrated to be pro-diabetogenic (Benjamin, 2001).

According to the 1996 PNG Household Survey (Gibson, 2000), Papua New Guineans consumed 11 kg areca nut/person/year, costing households close to 300 million Kina (in 1996, 1PGK= approx US$0.76 or CDN$1.03). Approximately 10% of the population participated in the market, with cash incomes in the rural sector forming 9.5% of their total income. In respect to caloric contributions, a single average area nut weighs approximately 300 g, of which 0.4% is edible and provides 33 kilocalories. According to the 1996 survey, AN consumption contributed 0.6% of a person's daily caloric intake. Whether the consumption of AN displaces more subsisting nutritional items from the diet

is a concern, since many workers claim that chewing suppresses appetite and stimulates productivity. Considering its popularity, the red spit stains that blemish public buildings and structures have little hope of disappearing anytime soon. This is despite repeated efforts on behalf of the government and townships intent to curb the sale and consumption of BQ with the goal of reducing urban litter. Betel quid chewing thus remains an ingrained and integral aspect of Papua New Guinean culture that must be considered in any economic and social development stratagem that aims to improve long-term health.

Guava

Guava (*Psidium guajava*) is a small tree native to Central and South America that now grows throughout all tropical regions. In addition to its edible fruit, the plant has a long history of medicinal use for a variety of ailments. In Asia and Africa, the plant is used for the prevention and treatment of scurvy (Watt & Branehwizk, 1969). The leaves are used to treat cough and pulmonary disease in Bolivia, India and Egypt (Khan & Ahmad, 1985). In China and Japan, the leaves are used externally as an anti-inflammatory and hemostatic agent (Hon-Ning, 1988). By far its greatest and most common pharmacological use is to treat gastroenteritis, dysentery and diarrhea. Many cultures, including some coastal Papua New Guinean groups, habitually consume an infusion of young guava leaves and buds as a gastrointestinal tonic. The antispasmodic and antimotility effect of guava leaves are due primarily to its flavonoid content, most notably quercetin, which can affect smooth muscle fibers as calcium antagonists (Meli *et al.*, 1990; Morales *et al.*, 1994; Gálvez *et al.*, 1996; Lozoya *et al.*, 2002). Antibacterial activity against organisms that cause diarrhea further substantiates its traditional use. Various extracts of guava leaves inhibited the growth of *Vibrio cholera, V. parahaemolyticus,* (Gritsanapan & Chulasiri, 1983), *Aeromonas hydrophilia, Shigella* spp. (Chulasiri *et al.*, 1986), *Staphylococcus aureus, Sarcina lutea, Mycobacterium phlei* (Malcolm & Sofowora, 1969) *Salmonella enteritidis, Bacillus cereus* (Arima & Danno, 2002) and *Escherichia coli* (Vieira *et al.*, 2001).

Guava leaves contain several compounds including various terpenoids (Meckes *et al.*, 1996; Begum *et al.*, 2002a; Begum *et al.*, 2002b), the flavonoids quercetin,

guaijavarin, leucocyadin, and amritoside (Seshadri & Vasisha, 1965; Lozoya *et al.*, 1994), coumarin, gallic acid, alkaloids and tannins (Okuda *et al.*, 1984; Okuda *et al.*, 1987). Although several of these compounds exert some biological effects, it is the phenolics quercetin and gallic acid that are thought to be the responsible agents for the therapeutic qualities of guava leaf. This is especially relevant to cardiovascular disease, since intake of quercetin and gallic acid has been reported to be inversely correlated with mortality from coronary heart disease, presumably due to their antioxidant properties (De Whaley *et al.*, 1990; O'Reilly *et al.*, 1997; Wiseman, 1999; Kim *et al.*, 2003a).

Antioxidant and cardioprotective properties of guava leaf

The guava fruit is high in soluble fiber and a rich source of potassium. In a randomized, single-blind, controlled trial, hypertensive patients fed 100g/day guava fruit had lower blood pressure, significant decreases in serum total cholesterol, triglycerides and an increase in HDL after 4 weeks compared to a control group (Singh *et al.*, 1993). Similarly, rabbits fed guava fruit had minimal coronary artery plaque sizes associated with increased serum levels of vitamin E, C, A and β-carotene and low lipid peroxides (Singh *et al.*, 1992). Not surprisingly, the pulp and peel fraction of the fruit were reportedly rich in antioxidant phenolics able to inhibit lipid peroxidation and free radical damage (Jiménez-Escrig *et al.*, 2001; Hassimotto *et al.*, 2005). From what little research that has been carried out on the leaf, we can ascertain that the leaf may also possess cardioprotective benefits. Using isolated perfused rat hearts, Yamashiro et al. (2002) demonstrated that various extracts of guava leaf reduced ischemia-reperfusion injury by significantly attenuating ischemic contracture during ischemia and improving myocardial dysfunction after reperfusion. The extracts also possessed strong free radical scavenging activity (Qian & Nihorimbere, 2004), able to significantly decrease TBARS in the reperfused hearts. Isolated quercetin and gallic acid also demonstrated these effects in an equipotent manner, suggesting that these were the bioactive phytochemicals (Yamashiro *et al.*, 2002). The crude extract and particularly the acetic acid fraction of guava leaf showed cardiac activity in the guinea pig atrium by depressing myocardial inotropism in a dose-dependent manner (Conde Garcia *et al.*, 2003).

Although these studies demonstrate the effects of guava leaf extract on mammalian myocardial tissues, more information is required as to whether intake affects *in vivo* vascular tissues. It can be assumed that the quercetin content would have a beneficial cardiovascular effect. Quercetin has been shown to modify eicosanoid synthesis with ramifications for both inflammation and vascular disease (Reiterer *et al.*, 2004). Its effects on platelet aggregation (Hubbard *et al.*, 2003), LDL oxidation and vasodilation suggest an ability to interrupt the pathophysiology of atherosclerotic plaque formation (reviewed in Formica & Regelson, 1995).

Effects of guava leaf on DM2

Considering the soluble fiber and polyphenolic content of guava fruit, a hypoglycemic effect could be assumed. To date, there are conflicting reports of whether guava fruit can prevent DM2. A hypoglycemic effect was observed in healthy and alloxan diabetic mice, as well as in healthy and diabetic volunteers (Cheng & Yang, 1983). However, Alarcon-Aguilara et al. (2003) reported a lack of effect in normal rabbits, and in alloxan-induced IGT rabbits the fruit juice actually increased glycemia. Similarly, guava fruit juice had no effect in temporarily hyperglycemic rabbits (Roman-Ramos *et al.*, 1995). The authors suggested that the mature fruits used in the study may have had a high quantity of absorbable carbohydrates that contributed to the observed glycemia. Research on guava leaves on the other hand, seems to suggest an antidiabetogenic effect. At the intestinal level, guava leaf tea had a modest effect on prolonging sucrose absorption (Matsura *et al.*, 2004). The butanol-soluble fraction of the leaves were found to be potent inhibitor of protein tyrosine phosphatase 1B (PTP1B), a major negative regulator of insulin signaling (Hong *et al.*, 2004). This property was considered responsible in part for the significant hypoglycemic effect observed in genetically diabetic $Lepr^{db}/Lepr^{db}$ mice when the fraction was administered via intraperitoneal injection at 10 mg/kg (Oh *et al.*, 2005). Similarly, (Maruyama *et al.*, 1985) reported that this fraction from a 50 % (v/v) ethanol extract inhibited the increase in plasma glucose level in alloxan diabetic rats and decreased levels in the oral glucose tolerance test. Guava leaf's main flavonoid, quercetin, stimulated insulin release and enhanced calcium uptake from isolated islet cells (Hif and Howell, 1985). However,

quercetin was also found to inhibit glucose transport following TPA or phospholipase C stimulation of rat adipocytes (Chriensen et al., 1987), suggesting a pro-obesity effect. In any case, the free radical scavenging activity of the flavonoid has shown to be beneficial in DM2 complications such as cataract formation and renal diseases (Havsteen, 1983; Varma, 1986)

It is difficult to say whether such hypoglycemic effects would occur in humans eating a varied diet, but preliminary findings suggest a promising health benefit.

Noni

Noni (*Morinda citrifolia*) has an extensive history of traditional medicinal and food use in the Pacific. The current distribution of noni throughout most of the Pacific islands is thought to have resulted from intentional transport of the medicinal plant during early human colonization (Whistler, 1992). Today noni, arguably the most important medicinal in the region, is prescribed for a wide array of indications (McClatchy, 2002). Traditionally the juice squeezed from the fruit was used for the treatment of mouth ulcers, hemorrhoids, hernia, headaches, body pain, diarrhea and dysentery, fever, intestinal worms, filariasis and leprosy. The leaves were commonly used topically as a poultice or bandage for broken bones and sprains. An infusion of the root was used externally to treat a variety of skin diseases, including bites, sores, abscesses, infections and inflammations. The root tea was ingested as a treatment for urinary disorders. Young fruits were consumed as a treatment for halitosis, menstrual cramps gastric and oral ulcers, toothache and indigestion (WHO, 1998; McClatchy, 2002). As a food, noni was commonly eaten in parts of Polynesia and Australia, although it never formed a significant portion of the diet. The fruit is an excellent source of vitamin C, providing up to 258 mg/100 g dried fruit. The leaves are equally rich in nutrients, providing a Daily Value (DV) of 70% vitamin C, 60% vitamin A (entirely as β-carotene), 45% calcium, 8% iron and 35% fiber based on a 2,000 calorie diet (Dignan *et al.*, 2004). Some of the phytochemicals identified to date in the plant include anthraquinones (nordamnacanthal, damnacanthal, morindone, rubiadin andrubiadin-1-methyl ether), β-sitosterol, scopoletin, caproic acid, caprylic acid, ursolic acid, rutin, flavonol and flavone glycosides, iridoid

glycosides, lipid glycosides, triterpenoids, and alkaloids (Liu *et al.*, 2001b; Sang *et al.*, 2003; Kamiya *et al.*, 2004).

Noni's popularity in Western markets has surged since the mid-nineties, propelled by the aggressive efforts of Tahitian Noni International, a Provo, Utah-based business also known as Morinda Inc. Since 1997, annual sales of the commercial fruit juice preparation have soared at an average rate of 116%, totaling US$ 471 million in 2003 (Smillie, 2004). Concomitant with its increasing popularity is the number of new indications for which it can be used for, most of which are not substantiated scientifically. Advocates claim that the juice can be used to increase energy, dampen allergy symptoms, improve asthma, lose weight, eliminate headaches and body pains, reduce blood pressure, and to treat or cure arthritis, cancer, AIDS, fibromyalgia, multiple sclerosis and diabetes. Several web sites selling the product and making such unproven claims have been targeted by the US Food and Drug Administration (FDA), warning that the claims cause the juice to be considered a drug under the Federal Food, Drug and Cosmetic Act. In fact, the FDA has no information that the juice is generally recognized as safe (GRAS), and thus must be considered a "new drug" under the act. As such, new drugs cannot be legally marketed in the US without prior approval from the FDA (FDA web site).

The hype behind noni originated with the work of University of Minnesota's Dr. Ralph Heinicke (Heinicke, 1985) who attributed bioactivity to an alkaloid he termed xeronine, derived from a precursor prexeronine. According to Heinicke's theory, all body tissue cells have proteins that contain receptors for the absorption of xeronine, whose primary function is to regulate the rigidity and shape of specific proteins resulting in broad physiological reactions such as cell repair, up-regulation of the immune system and protection from cancer. Since then, the number of new indications for which noni can be used to treat or cure has steadily risen to the point that may have us believe that the plant is a miraculous cure-all panacea. To date, the molecular structure of xeronine has not been elucidated and a search of the term on Medline (as of May 2006) returned zero results. The failure to substantiate or reproduce any of Heinicke's findings and claims brings his work into question, although the lack of scientific validity has not hampered the sale and popularity of the product.

Noni and Cancer

Despite the many fraudulent health claims attributed to noni, emerging scientific research supports its efficacy against certain cancer lines. Two glycosides that occur in noni juice were found to be the bioactive agents able to suppress 12-*O*-tedtradecanoylphorbol-13-acetate (TPA)- and epidermal growth factor EGF-induced Activation Protein-1 (AP-1) transactivation and cell transformation in mouse epidermal JB6 cells (Liu *et al.*, 2001b). A polysaccharide-rich substance in the juice was found to possess antitumor activity in the Lewis lung peritoneal carcinoma model (Hirazumi *et al.*, 1994; Hirazumi *et al.*, 1996) and the Sarcoma 180 (S180) ascited tumor in mice (Furusawa *et al.*, 2003). This substance was found to stimulate an immune response from murine effector cells via the release of several mediators including TNF-α, interleukin-1β, interleukin-10, interleukin-12, interferon-γ, and nitric oxide (Hirazumi *et al.*, 1996). The water and butanol extract of the fruit was found to display anti-proliferative activity against the breast carcinoma cell line (MCF-7) and a colon carcinoma line (HCT-116). Activity was attributed to six active compounds and a novel glycoside that was able to inhibit cancer growth and promote apoptosis by affecting several genes of the TNF apoptotic pathway (Fong *et al.*, 2001). Damnacanthal, an anthraquinone isolated from the root, was found to interfere with *ras* function in K-Ras-NRK cells (Hiramatsu *et al.*, 1993). The *ras* oncogene is involved in the signal transduction of several human cancers. This anthraquinone was also found to inhibit several tyrosine kinases involved in oncogenesis such as Lck, Src, Lyn and EGF receptors (Hiwasa *et al.*, 1999). Incubation of the commercial Tahitian Noni Juice® with cultured leukemia cell line induced cancer cell necrosis at high concentrations and apoptosis at lower doses, while co-incubation with other anticancer drugs Taxol and prednisolone resulted in more potent synergistic effects (Wang *et al.*, 2002). The fruit juice was also reported to inhibit tumor angiogenesis and suppress the vasculature of tumors, in part by promoting apoptosis of newly formed vessel networks (Hornick *et al.*, 2003). Such findings have prompted the National Institutes of Health to provide financial support for a phase I clinical trial of noni fruit extracts. The mechanisms by which noni prevents cancer are still being elucidated. One obvious avenue to explore is the plant's antioxidant potential, as free radicals are involved in carcinogenesis (Bartsch & Nair, 2000).

Free-Radical Scavenging Activity of Noni

Biochemical analysis of noni fruit composition showed a high content of antioxidant molecules, although the investigators doubted that these explained the plant's medicinal qualities (Chunhieng *et al.*, 2005). A 50 ppm MeOH and EtOAc fraction of noni juice was able to inhibit 88 and 96 % *in vitro* TBARS production from Cu-catalyzed LDL oxidation. The isolated active compounds were found to be a series of lignans whose potency depended on the number of phenolic hydroxyl groups (Salleh *et al.*, 2002; Kamiya *et al.*, 2004). Similarly, a lignan isolated from the n-BuOH soluble portion of noni fruits was found to be a potent DPPH and $ONOO^-$ scavenger (Su *et al.*, 2005). Using the lipid hydroperoxide (LPO) and tetrazolium nitroblue (TNB) assays to evaluate antioxidant activity, Wang and Su (2001) demonstrated that Tahitian Noni Juice® effectively inhibited lipid peroxidation and effectively scavenged superoxide radicals in a dose-dependent manner. Conversely, a 12.5 ppm MeOH extract of noni leaf failed to inhibit Cu-induced LDL oxidation and only reduced TBARS by 5 % (Salleh *et al.*, 2002). A lack of antioxidant activity in the MeOH extract of the leaf and fruit was also observed using the ferric thiocyanate method (FYC) and TBARS assay. However, these plant parts showed positive antioxidant activity comparable to ascorbic acid and BHT when extracted with ethyl acetate, suggesting that the antioxidant compounds were non-polar in nature. The most potent plant part according to these bioassays was the root which displayed powerful antioxidant activity for both the MeOH and ethyl acetate fraction (Zin *et al.*, 2002). The investigators retested the same leaf, fruit and root extracts after extensive fractionation and found that all fractions displayed various degrees of antioxidant activity, some comparable to BHT and α-tocopherol. Interestingly, the antioxidant activity was independent of the fraction's phenol content, suggesting different antioxidative mechanisms from non-phenolic compounds (Zin *et al.*, 2006).

The anthraquinone content of the roots has received some attention, as these were thought to account for its hypotensive, antibacterial, antiviral, anticancer and analgesic effects (Younos *et al.*, 1990; Hiramatsu *et al.*, 1993). Anthraquinones are a group of phenolic compounds that possess antioxidant or pro-oxidant activity depending on the number and position of hydroxyl groups and glycosides on the phenolic structure (Cai *et*

al., 2004). Nordamnacanthal, damnacanthal, 2-formyl-1-hydroxyanthaquinone, morindone and alizarin, anthraquinones that occur in the roots of noni, showed strong antioxidant activity in the FTC and TBARS assays, surpassing that of α-tocopherol (Nor Hadiani *et al.*, 2002). The hypotensive and antioxidant activity of anthraquinones was demonstrated on isolated perfused rat hearts, where preincubation with an anthraquinone-rich plant extract produced a dose-dependent protection against myocardial ischemia-reperfusion injury as evidenced by a significant decrease in LDH leakage. Protection in this case was found to be associated with enhanced myocardial glutathione antioxidant status (Yim *et al.*, 1998). The anthraquinone content of noni may also promote pro-oxidant activity, as suggested by case reports of acute hepatotoxicity in patients who consumed noni products (Millonig *et al.*, 2005; Stadbauer *et al.*, 2005). Cytotoxicity and apoptosis is induced by anthraquinones, producing ROS via redox cycling resulting in depletion of cellular glutathione, decreased mitochondrial membrane potential, initiation of lipid peroxidation and eventually, cell death (Bironaite & Ollinger, 1997; Kagedal *et al.*, 1999).

Noni research is still in its infancy and no clinical trials have yet been completed to validate any of noni's therapeutic claims. In PNG, the popularity of noni stems in part from its traditional use as a topical agent, although global influences have encouraged some street sellers to expand their products to treat a wider variety of diseases **(Figure 2.4.)**. With the successful and widening market of commercial noni products being developed in neighbouring islands such as Vanuatu and Palau, PNG is currently considering government-subsidized noni cultivation. Noni products intended for topical use such as soaps, creams, shampoos and other cosmetic products are molded on traditional practices and may be more culturally acceptable. Whether noni cultivation constitutes a future profitable venture may very well depend on the science behind the products, as growing international pressure to curb unsubstantiated claims or ban imports may cause a decrease in sales. Despite the controversy surrounding noni in the Western herbal supplement industry, laboratory experiments show that the plant has medicinal value. Strong antioxidant activity in some but not all noni parts or extracts suggest that the plant may have some effect on atherogenesis, although the benefit-cost ratio must be weighed against such risks as hepatotoxicity.

Mangrove bean

Mangroves are halophytic intertidal woody trees that are common along tropical and subtropical coasts. Their recognized ecological importance in maintaining and building soil, as a reservoir in the tertiary assimilation of waste, and in the global cycle of carbon dioxide, nitrogen and sulfur has led to increased conservation efforts by international, national and non-government agencies. Unfortunately these plant communities, including some in PNG, are threatened due to population pressures, mining, urbanization and conversion to agricultural land, salt pans or plantations (Chan & Salleh, 1987; Amarasinghe, 1988). In PNG, mangroves provide numerous products for human populations who have settled in or adjacent to mangrove habitats. Such habitats are favorable for settlement since the diversity of flora and epifauna provide an abundant source of food, fuel, traditional products and medicine (Bandaranayake, 1998). Furthermore, mangrove typically border streams and river mouths, thereby making fresh water accessible and available. Villages and dwellings erected near or adjacent to mangroves are typically built from materials almost entirely derived from mangroves and are often propped on stilts.

One common mangrove species, *Bruguiera gymnorrhiza* (Rhizophoraceae), is notable for being the staple food of some coastline Papua New Guinean populations. The species of Rhizophoraceae possess distinctive vivipary, meaning that the seed germinates within the fruit while still attached to the mother tree, forming a bean-like seedling (hypocotyl) that eventually drops off and floats in the saline water until a suitable site is found in which to take root.

The starchy hypocotyls are rendered palatable and free of tannins after considerable processing. Wanigelans boil the hypocotyls for 10 minutes, peel the outer dermis, slice the core into shavings, soak the shavings in salt water for 2-3 hours and boil them again for another 10 minutes. The resulting product is a sustaining, bland pasta-like food that is flavoured with coconut cream. The tannin-rich water left over from the boiled mangrove beans is a common traditional remedy for diarrhea and other gastrointestinal disorders. Macronutrient analysis **(Table 2.3.)** of the cooked hypocotyl shows that it is 90 % carbohydrate, 7 % protein and 1 % fat, and is an excellent source of

dietary fiber (23.10 g / 100 g dried weight). An average daily intake of 380 g provides the entire carbohydrate needs for an adult person requiring 2,000 kcal / day, and at least three times the recommended fiber intake (25 g / d) according to FAO/WHO guidelines. However, the food is a poor source of vitamins.

Analyses of select minerals show that mangrove beans are a good source of calcium, providing 20% of a person's average daily requirements per serving (Certispec Food Laboratory nutrient analysis, unpublished). The sodium content is of interest since high concentrations in the water imposes an osmotic stress and exerts toxic effects due to ion excess. In order to thrive, mangroves have evolved mechanisms to contend with saline environments. Mangroves can be divided into two groups based on their adaptation strategy; those that absorb salt through their roots and excrete it via salt glands on the leaves, and those that lack salt glands and largely exclude salt uptake in the roots by a special barrier (Tomlinson, 1986). *Bruguiera* belongs to the latter group, where hypocotyls are protected by having the surrounding tissues, the fruit and persistent pericarp, accumulate salt (Joshi *et al.*, 1972; Lin, 1988). Sodium, along with other minerals such as chloride, potassium, calcium and magnesium, is gradually reduced during the maturation of *Bruguiera* hypocotyls and accumulates in maturing leaves (Zheng *et al.*, 1999). According to our nutrient analysis, processing of the mangrove bean removed some of the mineral content, reducing Ca and Na by 37% and 82% respectively according to the values reported by Zheng and colleagues.

The pharmacological properties of *B. gymnorrhiza* are largely unknown (Bandaranayake, 2002). Traditionally, other cultures have used the fruits to treat a variety of eye diseases, extracted perfumes and condiments from the pneumatophores of the species, and produced adhesives from the bark (Rollet, 1981; Field, 1995). Tannins, produced by the plant to deter herbivores and microorganisms from predation, have demonstrated potential as cytotoxic, antineoplastic and antimicrobial agents. Crude *n*-butanol extract of *B. gymnorrhiza* exhibited weak antitumour activity against A-549 and HL-60 (Sun & Guo, 2004). Conversely, bark extracts of the related species *B. sexangula* and *B. exaristata* demonstrated active antitumor activity against the Sarcoma 180 and Lewis Lung carcinoma cell lines (Loder & Russell, 1969). The bark and leaves of *B.*

rumphii were recommended as a traditional remedy for diabetes (Rollet, 1981). To date, phytochemical analyses have detected anthocyanins, carotenoids, diterpenes, triterpenes, flavans, phenolic compounds, procyanidins condensed and hydrolysable tannins and catechins in various parts of *B. gymnorrhiza* (Ghosh *et al.*, 1985; Shinoda *et al.*, 1985; Han *et al.*, 2004; Han *et al.*, 2005a; Han *et al.*, 2005b). Recently, an unusual novel macrocyclic polydisulfide, gymnorrhizol, has been isolated from the stem and leaves of *B. gymnorrhiza* (Sun & Guo, 2004). Such a molecule belongs to an uncommon class of compounds that have been described to possess a variety of biological activities, such as chelators for metal ions, binucleating ligands and phase-transfer catalysts (Wolf *et al.*, 1987). The discovery of novel compounds emphasizes the need to promote further research into mangrove chemistry. A number of known and novel biologically active compounds or extracts have been isolated from various mangroves and mangal associates but have yet to be tested for potential medicinal and agricultural applications (Bandaranayake, 2002). The need to enhance mangrove phytochemical and ethnopharmacological exploration may also be a matter of urgency in light of the threat to biodiversity through the destruction of wetland ecosystems.

ETHICAL CONSIDERATIONS AND INTELLECTUAL PROPERTY RIGHTS

The present research adheres to all guidelines of professional ethics as outlined by societies with which the author is affiliated. The *International Society of Ethnobiology* (www.ise.org) and the *Society of Economic Botany* (www.econbot.org) have stringent codes of ethics that endevour to prevent the wrongful expropriation of cultural knowledge and intellectual heritage. These guidelines are accordant with the Convention on Biodiversity (www.biodiv.org) and the Papua New Guinea Institute of Biodiversity (PNG BioNET) (Matainaho, 2001). Of immediate relevance to this project, no research, collection, database or publication was initiated without complete informed consent, after full disclosure has been obtained using Motuan, Tok Pisin, Wanigela or English language from the persons solicited by the researchers (**Appendix I**). Parental consent was obtained for participants who were 18 years old and younger. Ethics approval was

obtained from both McGill University and the University of Papua New Guinea prior to the commencement of fieldwork. Research permits to conduct scientific investigations in Central Province, PNG were obtained at the national, provincial and municipal level.

The present study is aimed at examining community health and ecological sustainability and does not have any commercial objectives. In the unlikely event that our results reveal a compound for potential commercial or pharmaceutical development, no action shall be taken without consultation and written approval from the host institution. In all instances, all research, collection, databases and publications respects what is regarded as sacred, secret or confidential by tradition, and any prior informed consent specifically acknowledges what understanding has been reached between the researching parties and the indigenous practitioner.

STUDY RATIONALE AND STUDY DESIGN

The link between NCD and the simplification of diets and the loss of indigenous medicines in areas undergoing nutritional transition requires empirical data to support public health, agricultural and environmental policies aimed at reducing the global burden of chronic disease. The fields of nutrition and ethnobotany play crucial roles in this respect due in part to their multidisciplinary nature that combines both the social and natural sciences. Integrated projects provide a broad perspective on the interconnections between people, their ecosystem and diet, enabling the identification of areas that require concentrated research. With this in mind, the present dissertation attempts to connect laboratory findings to field conditions in order to explore whether the presence or absence of functional foods from the diet affects NCD risk (**Figure 2.5.**).

The first question was whether a relationship existed between the variety of foods consumed and the risk of NCD. Manuscript 1 provides a comparative overview of food variety and medicinal plant use in the Papua New Guinean communities investigated and highlights differential consumption patterns between genders and among age groups. Manuscript 2 provides support for the association between food variety, health and NCD risk. Importantly, it shows that certain indicators of food variety can reliably predict

nutrient adequacy. Sociodemographic determinants of food variety were also investigated and the application of the Quantitative Index for Dietary Diversity (QUANTIDD) was tested and compared between the three communities Koki, Kalo and Wanigela.

To investigate the role of foods with functional properties towards reducing NCD risk necessitated the development of a new quantitative index. Manuscript 3 introduces the Dietary Functionality Index (DFI), modeled after the compound indicator Mean Adequacy Ratio (MAR) which is commonly used to assess nutrient adequacy in populations. Construction of the DFI required an exhaustive review of the literature in order to substantiate the pharmacological properties of foods and medicinal plants consumed by Papua New Guineans. Despite the limited literature on indigenous and traditional Melanesian plants, we nevertheless show that the DFI is associated with favourable health parameters that may reduce NCD risk.

The next phase of the project was to question whether the functionality of particular plants found in the food system of one community, but not the other, would be a contributing factor to explain the disparate rates of NCD. Manuscript 4 explores the relationship between the insulin-mimetic and potentiating effects of selected plants in cell culture and their consumption patterns in relation to diabetes. This paper thus bridges the epidemiological and laboratory components of the project and provides a discussion on the merits and limitations of multidisciplinary research designs.

Recognizing the important contribution of dietary antioxidants towards NCD prevention, we redirected our research focus towards vascular disease. Manuscript 5 concludes this dissertation by relating a plant extract's capacity to prevent LDL oxidation to its ability to mediate oxLDL cytotoxicity towards cultured endothelial cells. Cytotoxic analyses of the extracts themselves show a pattern that does not necessarily reflect its purported health effects in humans, but rather accentuates the merits and limitations of using multicomponent preparations in cultured cell systems.

DESCRIPTION OF *IN VITRO* BIOASSAYS

Free Radical Scavenging Activity

The stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) gives off a strong absorption band at 517 nm because of its unpaired electron, which vanishes if the electron is paired up. The degree of decolourization is stoichiometric with respect to the number of electrons taken up and therefore, reflective of the antioxidant ability of a sample. The DPPH assay is a relatively simple, useful and commonly used assay; it can accommodate a large number of samples in a short period, and is sensitive enough to detect active principles at low concentrations. However, it cannot detect whether oxidized free radical intermediates are created from redox reactions with antioxidants (Ursini *et al.*, 1994).

Protection from Cu^{+2}-catalyzed oxidation of LDL

The time course of LDL oxidation can be followed by measuring the increase in TBARS, conjugated dienes, lipid hydroperoxides, fluorescence of proteins or lipids, and aldehydes. Additionally, one could measure the disappearance of endogenous antioxidants, PUFAs, the fragmentation of apo B or the increase in electrophoretic mobility (Esterbauer *et al.*, 1992). None of theses parameters gives a full and satisfactory picture of lipid peroxidation on its own, and only a combination of two or more time-related analyses allows the prediction of oxidation stage and the interrelationships between different events. In this dissertation, conjugated diene formation and malonaldehyde production were chosen as appropriate parameters based on their time and energy efficiency, ability to accommodate an average number of samples and degree of agreement between the two methods (Slater, 1984).

Oxidation of LDL by Cu^{2+} can be divided into three consecutive phases: 1) lag; 2) propagation; and 3) decomposition phase. During the lag phase, LDL endogenous antioxidants progressively become depleted, beginning with α-tocopherol and ending with β-carotene. Once depleted of its antioxidants, LDL is subjected to autocatalytic chain reactions of lipid peroxidation that rapidly accelerate in an exponential manner. Once 70-80% of the LDL PUFAs are oxidized, decomposition reactions become prevalent and the

concentration of lipid hydroperoxides and conjugated dienes decrease (Esterbauer *et al.*, 1992).

Conjugated dienes result from the oxidation of PUFAs with isolated double bonds to PUFA-hydroperoxides with conjugated double bonds, with a UV-absorption at 234 nm. Kinetic diene measurement shows the three phases clearly, and any antioxidant added to the reaction will prolong lag time to a degree dependent on the molecular structure, functional groups, and phase solubility of the antioxidant. The assay, however, is prone to photointerference from aromatic amino acids and phytochemicals that absorb at 234 nm. Measurement therefore requires that the solvent be sufficiently transparent with an adequate concentration of dissolved test sample that will possibly prolong lag time without impeding measurement.

Aldehydes are a byproduct of lipid peroxide decomposition in all biological systems and can in themselves act as cytotoxic agents. In effect, it is the covalent binding between aldehydes and amino acid residues in the apo B moiety that are responsible for the formation of the characteristic epitopes that are recognize by the macrophage scavenger receptor (Parthasarathy, 1987). Almost all aldehydes, except malonaldehyde (MDA) are lipophilic and remain associated with the LDL particle. The MDA released into the aqueous phase is able to react with TBARS and forms a pink chromogen when heated which can be measured spectrophotometrically. The TBARS assay has often been criticized as being nonspecific for MDA and give falsely high readings. Experiments have shown, however, that at least 90% of TBARS measured in oxLDL using the conventional assay is in fact free MDA (Esterbauer *et al.*, 1987). Interfering compounds are more of a concern when measuring TBARS in serum, plasma or culture medium.

Cell culture

Cell culture allows the study of animal cell behaviour free of the systemic variations that arise *in vivo* under normal homeostasis or experimental stress. The ability to control the physiochemical environment (pH, temperature, osmotic pressure, and O_2 and CO_2 tension), and physiological conditions are two of the major advantages of cell culture that allows more detailed characterization of such cell processes as intracellular activity, intracellular flux, environmental interaction, cell-cell interaction, genetics, and

cell products and secretion. The validity of the cultured cell as an *in vitro* model of physiological function *in vivo* has often been criticized because of the effect of the micorenvironment on cell phenotype. Cultured cells lack the heterogeneity and three-dimensional architecture found *in vivo,* and the absence of the correct hormonal and nutritional stimuli can promote spreading, migration and proliferation of unspecialized progenitor cells rather than expression of differentiated functions. Recognition of this has lead to the inclusion of a number of different hormones in culture media. Also, glucose must be added to most culture media as a carbon source for glycolysis. Under normal culture conditions, oxygen is maintained at atmospheric pressure and consequently is in relatively short supply. Raising the oxygen pressure in the absence of a carrier such as hemoglobin would otherwise generate cytotoxic ROS. As a result, the cells are maintained in anaerobic conditions and rely on glycolysis for energy, generating lactic acid as an end product. The citric acid cycle remains active, albeit to a lesser degree, and some amino acids, particularly glutamine, can be utilized as a carbon source (Butler & Christie, 1994).

Regardless of these limitations, cell culture can be a valuable tool to measure specialized function and is most often used to test the effect and cytotoxicity of pharmaceuticals, cosmetics, anticancer agents and in this case, botanical extracts. It is very difficult to recreate the complex pharmacokinetics of drug exposure *in vitro*. In order for the model to be accepted as an alternative to *in vivo* testing, it must be demonstrated that the potential functional compunds reach the cells *in vitro* in the same form as they would in biological systems. This rarely happens due to significant differences in exposure time and concentration of the drug, its metabolism, transport, tissue penetration, clearance and excretion. Several nontoxic compounds become toxic after being metabolized by the liver and toxic compounds *in vitro* can be detoxified by hepatic enzymes. This is rendered increasingly more complex for multicomponent drugs such as plant extracts. Cytotoxicity assays tend to oversimplify the events they measure without delving into specific molecular targets or regulation of metabolism and are thus inadequate for modern drug development. Nevertheless, gross tests of cytotoxicity are still required to provide insight into an important aspect of pharmacokinetics and pathology.

Adipocytes and endothelial cells are involved in the pathology of diabetes and vascular disease, respectively, and are appropriate for preliminary pharmacological investigations of botanical extracts. Both cell types are mesenchymal cells derived from the embryonic mesoderm, which also includes connective tissue, bone, and muscle. Mature adipocytes are difficult to culture and must be prepared from fibroblasts induced to differentiate by adipogenic factors (Gregoire, 2001). Adipocytes are terminally differentiated cells whose primary function are as storage depots for triglycerides. They are also endocrine tissues that release adipokines that play an important role in appetite control and the regulation of carbohydrate and lipid metabolism (Badman & Flier, 2007). 3T3-L1 adipocytes have been used extensively to study the regulation of glucose transporters and insulin sensitivity. Derived from mouse embryonic tissue, 3T3-L1 cells differentiate from fibroblast-like cells and once mature expresses insulin-sensitive GLUT4. With prolonged exposure to insulin, GLUT4 expression and translocation is reduced, mirroring the hyperinsulinemic *in vivo* conditions of insulin resistant tissues (Thomson *et al.*, 1997). Pharmacological testing of natural products for their insulin-like or insulin potentiating activity (Liu *et al.*, 2001a; Kang & Kim, 2004), their ability to inhibit cell differentiation (Liu *et al.*, 2005; Huang *et al.*, 2006), lipolysis (Moreno *et al.*, 2003; Yang *et al.*, 2004a) or adipokine expression (Liu *et al.*, 2006) is commonly performed using 3T3-L1 adipocytes.

A single layer of endothelial cells line the interior of all vascular vessels, providing a smooth anticoagulant surface, contributing to blood vessel contractility, acting as a permeable barrier to allow the transport of nutrient and gases as well as influencing emigration of immune cells from the blood. Its involvement in vascular disease, repair of blood vessels and cancer angiogenesis has garnered much research interest in endothelial cell culture. Cells are often harvested from large blood vessels, predominantly from human umbilical veins (HUVEC) or bovine aorta (BAEC), which form a confluent "cobblestone" pattern monolayer that is anchored to the bottom of cell culture wells.

Some pharmacological studies using cultured endothelial cells have focused on the ability of compounds, usually antioxidants, to mediate expression of vasoconstrictive

factors and inflammatory cytokines as a model of endothelial dysfunction and atherosclerosis (Lorenz *et al.*, 2004; Martin-Nizard *et al.*, 2004). The ability of compounds to reduce the toxocity of particles such as oxLDL towards cultured endothelial cells is also found (Kapiotis *et al.*, 1997; Martin & Frei, 1997; Walters-Laporte *et al.*, 1998). Lacking in the literature, however, is the testing of botanical extracts or multicomponent drugs. This is possibly due to the complex interactions that take place in the extracellular matrix that make it difficult to ascribe activity to one of many constituents. Multicomponent reactions are disfavoured in pharmacologicalinvestigations for this reason. The merits of compound indigenous drugs and foods should be further inverstigated in light of the potential additive, synergystic and/or negating effects that can occur between constituents. In order to emulate conditions closer to their original state, crude plant extracts rather than isolated phytochemicals were tested for their ability to protect BAEC from oxLDL-induced cytotoxicity. To our knowledge, this is the first time this model has been used to test multicomponent preperations

Table 2.1. Age and sex specific prevalence (%) of impaired glucose tolerance and type 2 diabetes in three Wanigela communities at different stages of modernization (Dowse *et al.*, 1994).

		Impaired Glucose Tolerance			**Type 2 Diabetes**		
Age group		35-44	45-54	55-64	35-44	45-54	55-64
Koki (urban)	Male	24.6	16.7	31.4	28.3	47.9	42.9
	Female	28.9	23.4	29.4	28.9	53.2	52.9
Wanigela (rural)	Male	14.8	9.7	19.4	22.2	19.4	19.4
	Female	14.3	18.0	20.4	13.0	16.4	22.4
Kalo (semi-rural)	Male	0.0	7.7	10.5	0.0	3.8	0.0
	Female	6.9	4.3	0.0	0.0	4.3	0.0

Table 2.2. Characteristics of the study population, Papua New Guinea

	KOKI (urban)		WANIGELA (rural)		KALO (semi-rural)	
	Men	Women	Men	Women	Men	Women
Population (1996) [a]	401	349	541	378	198	106
Body mass index (BMI) (kg/m^2) [a]	27.7	29.3	25.8	24.0	24.6	24.8
Waist : hip ratio (WHR) [a]	0.889	0.819	0.895	0.836	0.889	0.832
Overweight (%) (BMI>25 kg/m^2) [b]	74.8	79.5	48.3	36.1	41.3	43.4
Diabetes (%)[b]	31.1	33.5	17.7	10.1	1.1	2.8
Systolic blood pressure (mmHg) [c]	134.6	129.1	130.4	126.5	115.2	116.1
Diastolic blood pressure (mmHg) [d]	81.1	80.0	-	-	74.8	75.8
Total cholesterol (mmol/L) [d]	5.5	5.1	4.9	4.8	4.9	5.0
HDL (mmol/L) [b]	0.97	1.15	0.93	1.19	1.02	1.27
LDL (mmol/L) [b]	3.92	3.45	3.46	3.28	2.98	2.95
Total triglycerides (mmol/L) [d]	1.20	0.91	0.98	0.87	1.07	1.00

[a] (Hodge *et al.*, 1995); [b](Hodge *et al.*, 1996b); [c](Dowse *et al.*, 1994); [d](Martin *et al.*, 1981)

Table 2.3. Nutrient composition of *Bruguiera gymnorrhiza* hypocotyl and % daily value (DV). Nutrient composition analysis was performed by Certispec Food Laboratory, Dorval, Québec, from samples brought back from Papua New Guinea.

Nutrient	Nutrient /100 g cooked dry wt	Average daily intake*	% Daily Value based on 2000 kcal/d diet
Energy (kcal)	352	1337.60	
Protein (g)	6.30	23.95	44 (♂) 53 (♀)
Carbohydrate (g)	80.20	304.75	102
Dietary fiber (g)	23.10	87.79	351
Total fat (g)	0.64	2.44	3.8
Saturated (g)	0.23	0.87	3.5
Monounsaturated (g)	0.11	0.42	
Polyunsaturated (g)	0.30	1.15	
Cholesterol (mg)	0.90	3.41	1.0
Vitamin A (IU)	1.70	6.46	0.1
Vitamin C (mg)	0.10	0.38	0.8
Ca (mg)	144.00	547.20	55
Fe (mg)	1.12	4.26	24
Na (mg)	97.92	370.95	16

*Average intake in Wanigela = roughly 3 cups/d or 380 g (1 serving = 1 cup = 126.0 g)

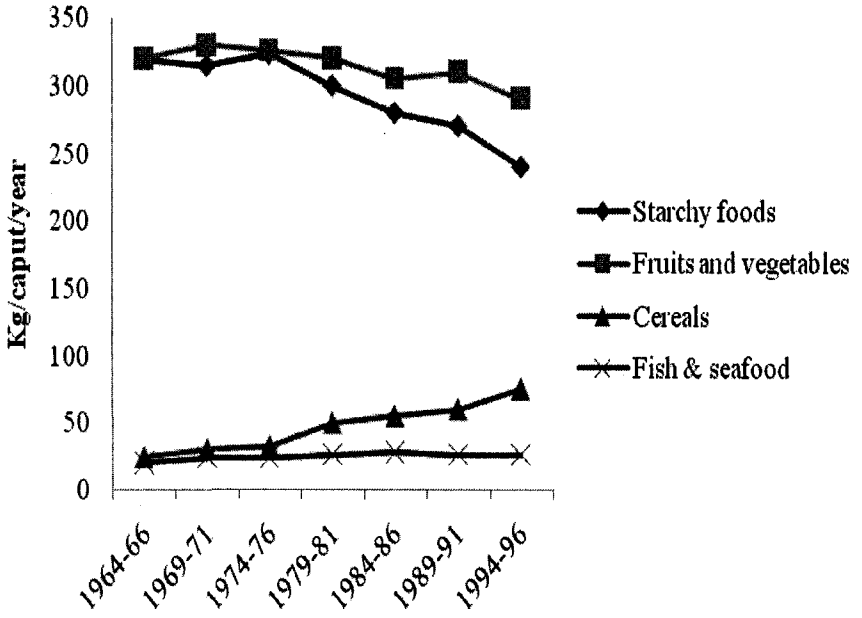

Figure 2.1. Supplies of some food groups (in kg/caput/year) in Papua New Guinea from 1964-66 to 1994-96 (Food and Agriculture Organization of the United Nations, 1999).

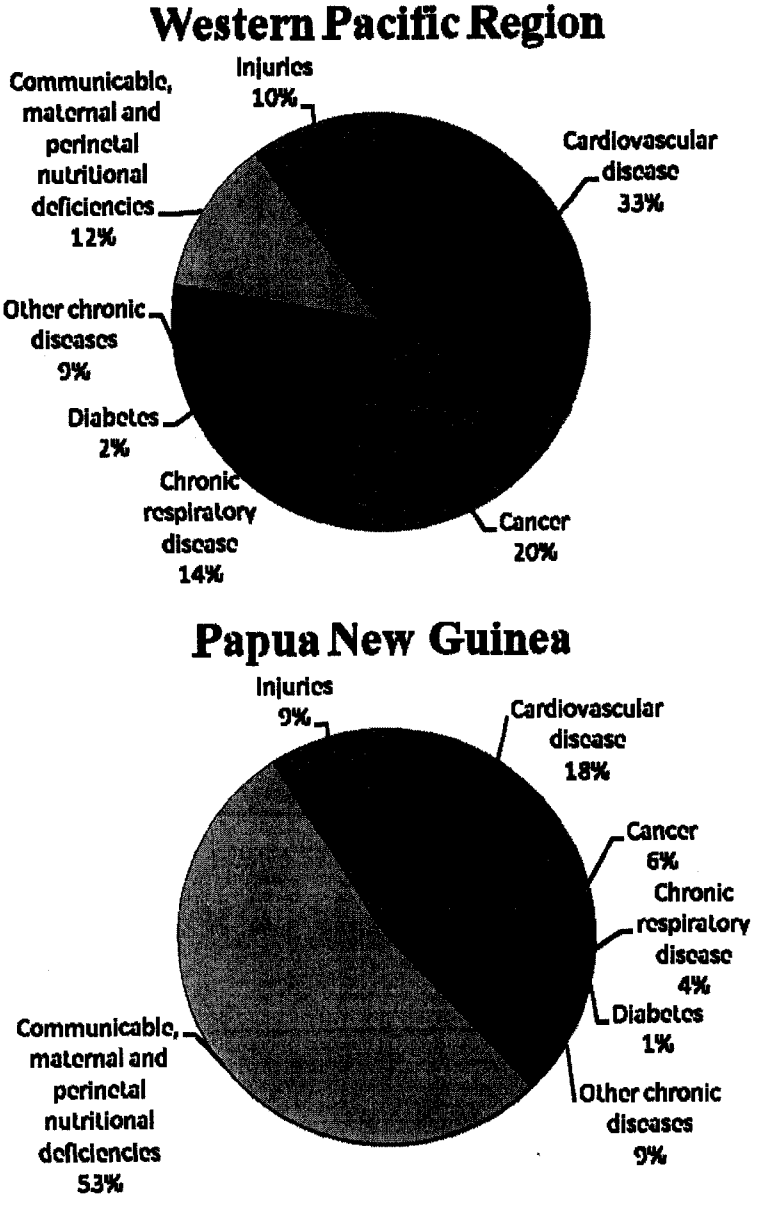

Figure 2.2. Death by cause, all ages, for the WHO Western Pacific region, 2005 and Papua New Guinea, 2002 (WHO, 2007).

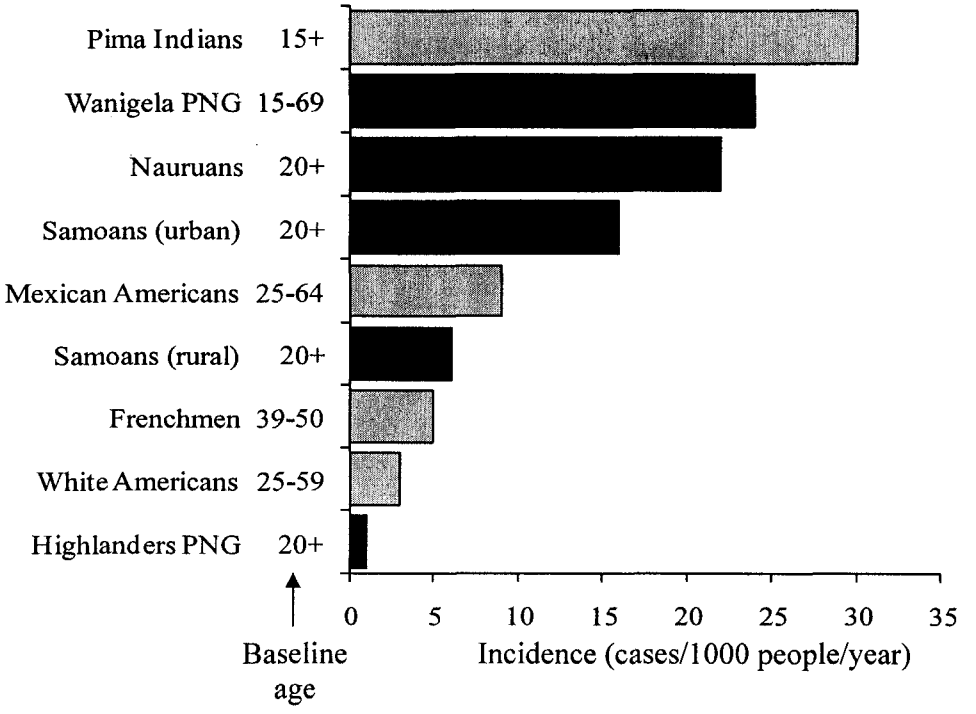

Figure 2.3. Incidence of type 2 diabetes in selected populations stratified according to BMI. Dark bars represent Pacific nations (Dowse, 1996).

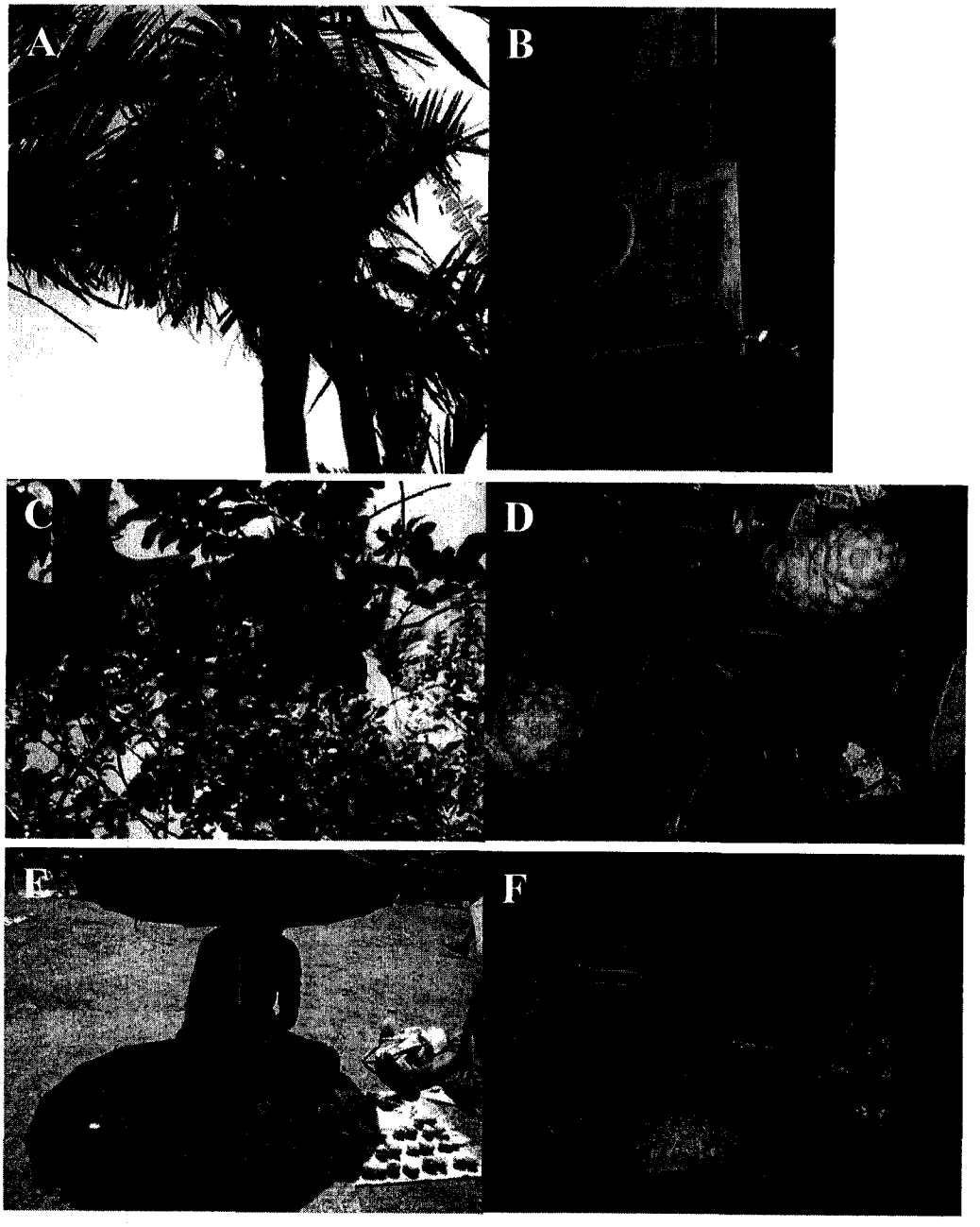

Figure 2.4. Plants selected for analysis. A) Areca nut (*Areca catechu*); B) Areca nut and inflorescence of *Piper betle* being sold along with a tin of homemade scones; C) Guava (*Psidium guajava*); D) Noni fruit and leaves (*Morinda citrifolia*); E) Woman selling homemade noni products beside fresh noni fruit; F) Family peeling and scraping mangrove beans (*Bruguiera gymnorrhiza*).

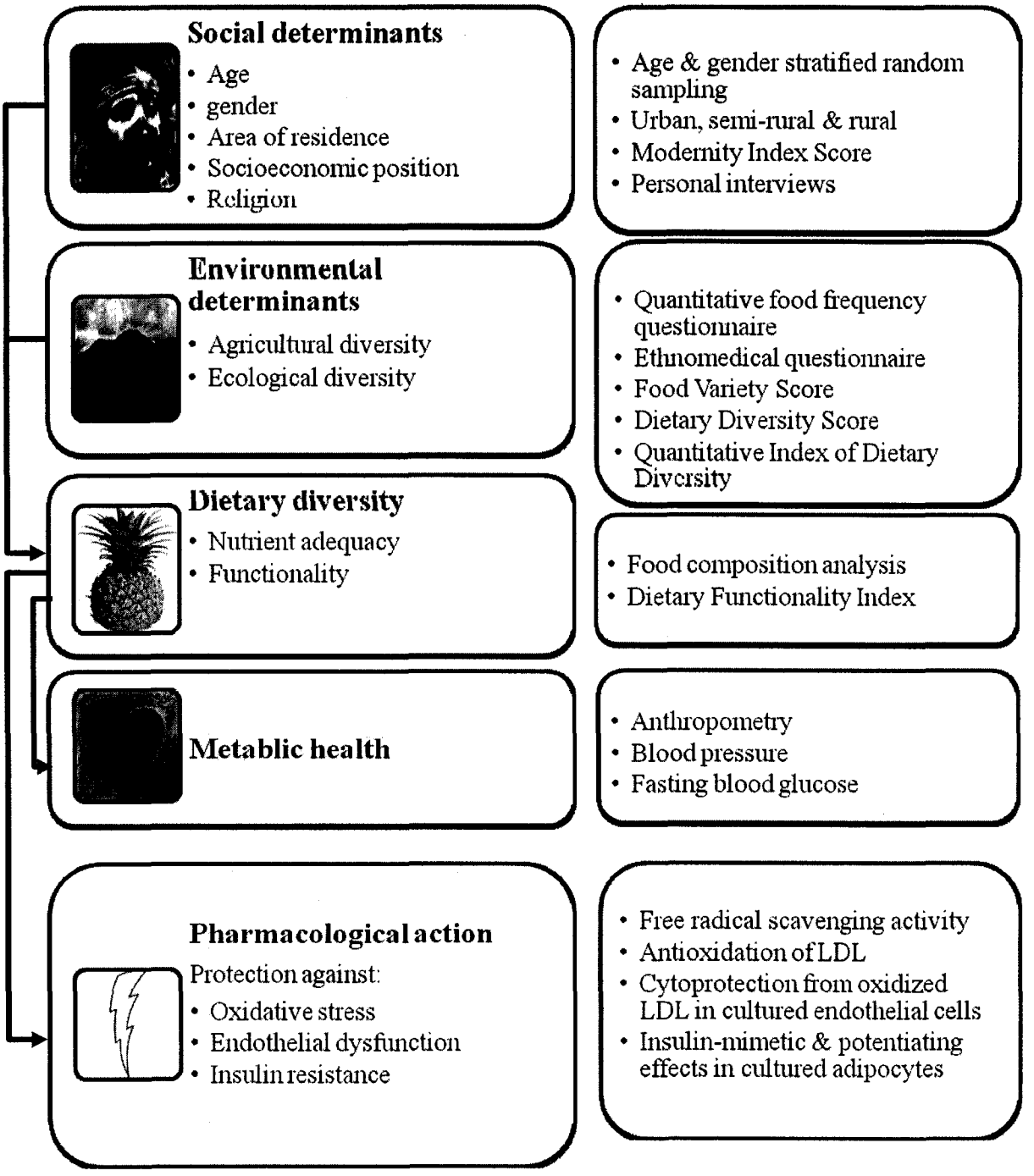

Figure 2.5. Schematic model of study design and methods. Dietary diversity is determined by social and environmental variables. Diet quality affects long-term metabolic health via adequate nutrition and exposure to bioactive phytochemicals. These may impart a pharmacological action on insulin-sensitive tissues and vasculature.

CHAPTER 3

MANUSCRIPT 1

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PLANT DIVERSITY AND HEALTH:

FOOD AND MEDICINAL PLANT USE IN TRANSITIONAL COASTAL COMMUNITIES OF PAPUA NEW GUINEA

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INTRODUCTION

Dietary guidelines of several nations recommend eating a variety of foods under supposition that nutritional adequacy and dietary quality is improved (Drewnowski *et al.*, 1997). Dietary diversity has demonstrated positive benefits against certain noncommunicable diseases such as cancer (Vecchia *et al.*, 1997), prolonged longevity (Kant *et al.*, 1995) and improved health status (Hodgson *et al.*, 1994). These findings relate to studies conducted in industrialized countries, where dietary assessment tools, albeit costly and time consuming, are fairly easily achievable. Such analyses are much more difficult in cultures such as those in Africa where families eat from a common food bowl (Hatløy *et al.*, 1998). In such cases, dietary diversity is a simple, cost effective alternative to nutrient intake analysis that can be used to assess dietary quality, food security and nutrient adequacy.

Diversity in food systems of indigenous and local populations depends on an intact ecosystem, as agricultural produce is regularly supplemented with foods and medicines obtained from forests, fields and swamps. The importance of environmental sustainability and biodiversity on the nutrition of developing countries has been emphasized in the policy framework of the Food and Agriculture Organization of the United Nations (FAO) (FAO, 2000) and the International Plant Genetic Resources Institute (IPGRI) (Convention on Biological Diversity - Subsidiary body on scientific, 2005); decision VII/32, paragraph 7. To date, scarce data exists to demonstrate the effects of urbanization on dietary diversity and noncommunicable diseases. Even fewer studies have considered the importance of non-nutrient ingestibles such as masticants, medicinal plants, supplements or spices on health status. Although not as quantitatively important as staples, such little-used foods and medicines may have long-term subtle effects on physiology and energy metabolism. In relation to transitional diets, these are often the first to be abandoned, and virtually no information exists to determine if their absence has an impact on noncommunicable disease etiology. In the current study, dietary diversity was assessed in three communities of a rapidly developing country, Papua New Guinea, that are in various stages of transition (urban, semi-rural, and rural). Clinical characteristics such as blood pressure, obesity, and glucose tolerance were

considered in relation to dietary diversity using an ecosystems approach. Data is thus analyzed in the context of social, environmental and economic paradigms. Included herein are preliminary results that do not include dietary nutrient/non-nutrient composition analysis or a thorough integration of medicinal plants within the model, but rather a cursory analysis of anthropometry, clinical characteristics, dietary diversity, and medicinal plant use.

MATERIALS AND METHODS

Study Site and Subjects

Field work was conducted in Koki, Kalo and Wanigela between January and August, 2004 (**Figure 3.1.**). Koki is an urban coastal settlement in Port Moresby, National Capitol District, where residents predominantly originate from Wanigela, Marshall Lagoon. Situated close to the city centre, Koki residents are within walking distance to several grocery stores, pharmacies and the Port Moresby General Hospital. Similar to Wanigela, most of the houses are stilted, constructed with modern materials, usually corrugated iron, and located along ocean-flanked wharfs. Most houses have plumbing or access to piped water. Though some have modern facilities, most have outhouses that release directly into the water. Additional refuse and garbage deposited offshore produces unsanitary and foul-smelling conditions. Most are wage workers or are self-employed as construction workers and subsist primarily on store-bought foods. The predominant religion is Seventh-Day Adventism (**Figure 3.2.A**).

Kalo is situated at the mouth of the Kemp Welsh River, Rigo District, Central Province (**Figure 3.3**) and residents have access to fresh water and marine resources. Located approximately 200 km from Port Moresby, several Kalo residents choose to travel regularly to Port Moresby to earn wages. Kalo communally owns several hectares of fertile riverine land and several crops are grown, the surplus either sold in Port Moresby or at Hula, a nearby coastal trading village. Houses are constructed of modern materials, mostly corrugated iron, and occur crowded together. Several of the houses on the periphery or close to the forest have pit-latrines that are shared by several other

families and are refilled every 1-4 years. The nucleus of Kalo is situated on sandy soil surrounded by coconut-betelnut plantations, nypa palm swamps, melaleuca savannahs and beaches. Rain water and well water are preferred to riverwater as a water source. Seven trade stores sell rice, tin meat, oil, sugar, tea and a variety of other incidentals. The major religion is United Church, though some follow the Salvation Army (**Figure 3.2.B**).

Wanigela is located about 400 km east of Port Moresby on the eastern bank of Marshall Lagoon, Abau District, Central Province (**Figure 3.3**) and is unique in that it is a stilt village positioned approximately 500 m in the water. Low tide occurs during most of the day and Wanigelans must walk through knee-deep mudbanks to access the mainland. Since all outhouses overhang the lagoon, the mud exudes a disagreeable odour. Local health workers have observed a higher than usual incidence of respiratory illnesses relative to neighbouring land-based communities. Their diet is also unique in that mangrove bean (*Bruguiera gymnorrhiza*) forms their primary staple food, a practice that may not be practiced anywhere else in the world. Mangrove beans are washed and boiled in the same lagoon water that serves as a reservoir, for laundry and for bathing. Although Wanigela has been granted several hectares of land in 1960s (Walsh, 1964), only a small proportion cultivate their land. From these, mostly low-maintenance crops such as sugarcane, banana and coconut are grown. Most subsist by fishing and bartering with neighbouring villages for fresh produce or trade-store goods while others rely on city relatives who regularly deliver urban food. Consequently, several Wanigelans eat an urban-type diet and have low energy expenditures despite their rural conditions, which may explain why several clinical characteristics resemble those of urban Koki. Only one trade store exists and is usually poorly stocked. Although some Wanigelans collect rainwater, most canoe to nearby Imila River to obtain fresh water. The Seventh-Day Adventist Church is the only denomination present in Wanigela (**Figure 3.2.C**).

Participants were selected by stratified random sampling according to gender and age. Each village was divided according to clan boundaries and every third household was approached and invited to participate until each clan was equally represented. All individuals above 16 years were eligible to participant except pregnant and lactating

women, and circumstances where communication or cognitive difficulties made it impossible for the participant to provide the necessary information.

A sample size of 120 individuals per community was calculated for multiple regression analysis using 5 independent variables (Dietary Diversity Score (DDS), Dietary Functionality Index (DFI), Mean Adequacy Ratio (MAR), Modernity Index Score (MIS) and age), a significance level of $\alpha = 0.05$ and a power of 0.80 in order to detect a minimum R^2 value of 12% and greater. In the present report, DFI and MAR were not calculated and thus not considered.

Anthropometric Measurements

Height was measured with a measuring tape to the nearest cm with participants standing straight against a wall without shoes. Weight was measured without shoes and in light clothing using a Seca Dial Scale (Vogel & Halke, Germany). Body Mass Index (BMI) was calculated as kg/m^2. Waist and hip circumference was obtained using a fiberglass measuring tape. Skin-fold measurements were obtained using Lange calipers and % body fat calculated according to formulae supplied by the manufacturer for four skin-fold sites: tricep, bicep, subscapular and suprailiac. All measures were repeated twice and averaged. Blood pressure was taken in duplicate after a participant was asked to sit quietly for five minutes. In cases where hypertension was detected, another reading was taken after ten minutes. If still hypertensive, the participant was referred to the local Community Health Worker or to the Port Moresby General Hospital. Participants were encouraged to retest their blood pressure at any time during the study. Resting energy expenditure (REE) was estimated using equations for tropical people (Henry & Rees, 1991). A rough estimate of energy expenditure was calculated by multiplying REE with WHO physical activity levels (PAL) as follows: 1.2 = sedentary; 1.375 = lightly active; 1.55 = moderatly active; 1.725 = very active; 1.9 = extremely active.

Blood Glucose Determination

Participants were tested after an overnight 12-hour fast. A single drop of blood from the side of the forefinger was extracted using a sterile lancet and deposited on a test strip. Blood glucose concentration was determined using a portable glucometer (CardioChek™ Analyzer, Polymer Technology Systems Inc., Indiana) which uses

reflectance photometry to measure changes in blood colour. Readings were obtained within 2 minutes. Participants with readings of glucose concentrations above 7 mmol/L were retested to confirm hyperglycemia and referred to the Port Moresby General Hospital Diabetes Clinic for further testing and treatment.

Modernity Index

To determine to degree of acculturation, a modernity index score (MIS), developed and validated in PNG (Hodge *et al.*, 1995) was administered. The score is based on seven questions pertaining to area of origin, father's employment, type and duration of individual's employment, education, etc., giving a maximum of 50 points. A high score indicated a more modernized participant.

Food Frequency Questionnaire

A modified semi-quantitative seven-day FFQ based on that developed and validated by the International Diabetes Institute for Papua New Guinea included additional food items such as masticants, nuts, herbs, spices and beverages. Participants were asked to estimate their intake during the week, and the process repeated to obtain greater detail. Measuring cups and spoons were used as visual aids for portion sizes.

Food Variety Score (FVS) and Dietary Diversity Score (DDS)

Food variety scdore is defined as the number of different food items eaten during the time frame of the food frequency questionnaire (7 days) (Hodgson *et al.*, 1994). The DDS defines the number of food groups consumed by each individual (Hatløy *et al.*, 1998) and includes 12 categories included in the FFQ (**Table 3.1.**) comprised of 135 food items.

Ethnomedical Questionnaire

Every participant was asked to list all medicinal plants that they used regularly, infrequently or at least were familiar with. Method of preparation, indicated use and other ethnologic information was gathered for each plant. Individuals who had more extensive knowledge of medicinal plants were asked to list plants that were used for diabetes or related symptoms according to the *Expanded Diabetes Diagnostic Criteria* (EDDC) (Carlson, 1995). Voucher specimens have been deposited at the herbariums of

the University of Papua New Guinea, the Forest Research Institute, PNG and McGill University.

Ethical Considerations and Intellectual Property Rights

Ethics approval was obtained from the McGill University Ethics Committee, the Papua New Guinea Medical Advisory Board, the Forest Research Institute and the Department of Environment and Conservation. All research was conducted according to the guidelines outlined in the Convention on Biodiversity (www.biodiv.org) and the PNG Institute of Biodiversity (PNGBIOnet, DEC, 2004). A letter of introduction describing in detail the objectives of the study and potential benefits was handed to the counselor, chairman, pastor and Community Health Worker of the villages, followed by an information session. Research commenced once approval was granted from the village local level government. Written prior informed consent was obtained for every participant. Completed questionnaires remained confidential and were kept in a locked bag, accessible only by the author.

Statistical Analysis

All values are represented as mean ± standard error and significance set at $p<0.05$. SPSS v.14.0 was used for all analyses. ANOVA and post-hoc Tukey's multiple comparisons was used to assess differences between villages and student's t-test between genders. Linear regression analysis was employed to examine the effects of modernity and dietary patterns on body composition indices.

RESULTS AND DISCUSSION

Participant characteristics

Population figures from the 2000 National Census (Office of Statistics, Government of Papua New Guinea) show that the urban community Koki had the highest population followed by Wanigela and Kalo (**Table 3.2.**). Koki and Wanigela officials agree with this figure, although Kalo councilors believe their population number grossly underestimated, the reason being that most villagers were absent during the census. A

figure closer to 4000 was suggested by the Kalo councilor. Modernity Index Scores correspond to the socioeconomic circumstances of the communities, where urban Koki had the highest score and rural Wanigela the lowest. In all communities, men had a higher MIS than women (**Table 3.2.**).

The World Health Organization (WHO) considers a BMI below 25 to be normal; from 25 to 30 overweight, and above 30 obese. Recognizing the dissimilar anthropometry of humans in Asia-Pacific areas, the FAO and WHO have adopted country-specific cut-off points to define overweight and obesity (International Diabetes Institute Steering Committee, 2000). For example, Pacific Islanders tend to be larger and more muscular than Caucasians, with less body fat at higher BMI levels (Weisell, 2002). However, this characteristic was not personally observed in Papua New Guinea, and thus original WHO classifications for Europids were retained. As with similar urban-rural comparative studies (Steyn *et al.*, 2000), obesity was most prevalent among urban dwellers and less common further from the city. The average BMI for Koki was borderline overweight for men and women, a population where virtually half of all men and women sampled were classified as overweight. This is in agreement with previous findings that recorded a 10 kg difference between Koki and Wanigela, despite Koki being, on average, shorter framed (Martin *et al.*, 1981). Average BMI was significantly different between village women, but not men, whereas the prevalence of obesity was significantly higher for both genders in Koki compared to Kalo and Wanigela, and Kalo compared to Wanigela.

Since being overweight is not necessarily due to excess body fat, additional measures such as waist circumference, waist: hip ratio (WHR), tricep skinfold thickness, and % body fat were calculated. A high WHR indicates abdominal obesity, a form of body fat distribution that is considered a greater risk factor for diabetes and cardiovascular disease compared to other fat deposition sites (WHO, 2000). According to leading health organizations, a WHR above 90 and 80 is considered a health risk for men and women, respectively. There is no standard agreement as to what constitutes a healthy amount of fat, but in general a % body fat below 20 is considered healthy for men, while 30% is considered normal for women. In all three villages, abdominal obesity and excess

body fat were very common, especially among women. Similar to the findings for BMI, abdominal obesity and excess body fat was significantly more common in the urban sample for men and women, with the exception of Kalo women who had the highest mean WHR and prevalence of abdominal obesity.

Cultural perception of what constitutes a healthy body size has been reviewed in the Pacific region, where a large size reflected status, power, wealth and attractiveness (Craig *et al.*, 1998). In New Caledonia, Snowdon and Schultz (2001) observed gender-specific differences in how islanders perceive their own body image. Males considered middle-ranged BMI shapes to be both the healthiest and most attractive for men and women, whereas females found middle-ranged figures the most attractive, but larger sizes healthier. This view may also pertain to coastal Papua New Guinean women. In informal interviews in Kalo, most women expressed a desire to lose a few kilos but did not consider themselves unattractive, overweight or unhealthy. Changes in body image result from increased exposure to Western media and ideals, but lack of family or community support does not support any weight-loss attempts. Informal interviews with husbands revealed that they do not mind having overweight wives and may find it attractive; though most admit that a slimmer body size would be a healthier option.

Blood Pressure

Hypertension was significantly more prevalent in Koki compared to Kalo, but interestingly not to Wanigela. Several dietary factors, including the amount and type of dietary fat, cholesterol, fiber and minerals, affect blood pressure (Vogt *et al.*, 1999) as demonstrated by the DASH (Dietary Approaches to Stop Hypertension) combination diet where a 55% carbohydrate, 27% fat, 31g fiber diet lowered blood pressure compared to a control 48% carbohydrate, 37% fat, 9 g fiber diet (Svetkey *et al.*, 1999). The Wanigela diet may be considered high carbohydrate and low-fat, a regime that has long shown to alter lipoprotein, glucose and insulin metabolism as to increase the risk of cardiovascular disease (Ducimetre *et al.*, 1980). However, if carbohydrates are mostly complex and not simple (i.e. sugars), as is the case with the traditional Wanigela diet, an inverse association between carbohydrate intake and BMI, with no effect on blood pressure, is usually observed (Yang *et al.*, 2002). Of the other known hypertension risk factors,

including sodium, weight gain, diabetes and alcohol, the one that is most relevant to Wanigela is diabetes. Diastolic blood pressure has been associated with steady-state plasma glucose concentrations (Abbasi *et al.*, 2002), a parameter with significant difference in relation to Kalo. Changing dietary patterns and greater consumption of polished white rice are certainly agents that aggravate glucose intolerance.

Energy Expenditure

Increased sedentarianism is a major factor in the global prevalence of obesity and those who maintain an adequately active lifestyle have a decreased risk of developing noncommunicable disease. In the present study, EE was estimated using crude equations that were not meant to be precise, but rather for comparative purposes. Energy expenditure was similar in all villages, although Kalo men tended to expend slightly more energy than their urban and rural counterparts. This is likely due to the presence of a cleared and maintained sports field located close to the center of the village. Consequently, the community was able to participate in such sports as soccer, rugby, volleyball, netball, and cricket more often.

Diabetes Prevalence

The similarity between Koki and Wanigela is once more discerned in relation to diabetes prevalence. A genetic susceptibility to diabetes was suspected ever since Wanigelans were first identified as a high-risk group. The earliest survey was conducted in 1962 in the Hula district of Central Province, in which Kalo is located, and no cases of diabetes were detected among 407 adults (Hingston & Price, 1964). A survey in 1977 highlighted the susceptibility of Koki residents, where 15.6% of adults were diabetic, compared to 1% in Kalo and 0% in rural Wanigela (Martin *et al.*, 1981). A 1986 study recorded a prevalence increase in rural Wanigela up to 8.9% (Patel *et al.*, 1986). The most recent survey, conducted in 1991, found a two-fold increase in diabetes prevalence among adults in Koki and Wanigela, while rates remained unchanged in Kalo (Dowse *et al.*, 1994). Rates of 37.5% for Koki placed this group as the second highest in the world after the Pima Indians and even higher than Micronesian Nauruans, despite higher rates of obesity in the latter. After adjusting for obesity, the data suggested that urban Wanigelans were more susceptible to diabetes than the Pima Indians (Dowse *et al.*, 1994).

Our results are based on a smaller sample size and thus likely underestimate diabetes rates. However, our results concur with the pattern of higher rates among the urban and rural Wanigelans compared to Kalo. It was noted in the 1991 survey that Kalo and rural Wanigela shared similar lifestyles, and that higher diabetes rates in the later community indicated genetic susceptibility (Dowse *et al.*, 1994). Using an ecosystems approach, the present study found substantial environmental, social and cultural reasons that may rationalize enhanced susceptibility rather than a purely genetic one.

Consuming a single meal per day is not unusual in Wanigela, and dietary diversity is rather low. Such dietary behaviour over generations may have amplified the "thrifty genotype" whereby efficient processing of energy predisposes an individual to obesity and diabetes once food becomes plentiful. A more likely explanation however, is that impaired intrauterine growth caused by poor maternal nutrition predisposes the fetus to diabetes later in life. This is supported by data that found Koki children shorter, lighter and with a lower BMI, but with greater percent body fat than age-and sex-matched controls from two low-prevalence communities in Port Moresby. Moreover, birthweights were on average 0.35 kg lower (Amini *et al.*, 1997).

The observation that women had a lower prevalence of DM2 despite having excess body fat and an increased prevelance of abdominal obesity may be due to differences in physical activity patterns, since women expended more energy than males in Koki and Wanigela. This highlights the importance of physical activity in regulating glycemia, even in the obese. In the last 50 years, global prevalence trends suggested that women were more susceptible to develop DM2, however current data show that it is now equally prevalent among males. The changing sex ratio is thought to be due to decreasing parity in women and a more sedentary lifestyle leading to increased obesity in men (Gale & Gillespie, 2001). In our study, DM2 prevalence

Food variety and Dietary Diversity

Staple Crops

Post-World War II economics and greater urban drift has enabled many Papua New Guineans to participate in the wage market in order to increase their purchasing

power to access processed or imported foods. In Koki and Kalo, imported white rice had replaced traditional root crops such as sweet potato, yam, taro and cassava as a staple food (**Figure 3.4.**). Wanigela, however, has continued to rely on their traditional staple, mangrove bean, which grows wild in the lagoon. The beans are boiled in lagoon salt water for about twenty minutes, peeled, grated, then soaked in salt water for more than two hours to remove tannins, then boiled again for about twenty minutes. Mangrove beans are rather bland alone, but take on a salty flavour if boiled in lagoon water or a sweet flavour if coconut cream is added. Other staple crops were consumed in smaller quantities compared to Koki and Kalo.

Scones and other flour products such as fried flour and donuts were very popular in Koki and Kalo. Such foods are deep fried, high in saturated fats, sugar and virtually devoid of fiber, vitamins and minerals.

Fruits

Fruits were not frequently eaten by adults in the communities investigated, but rather reserved for children, a custom that is not uncommon in the Pacific (Lako, 2001). Kalo ate more fruit compared to Koki and Wanigela, with guava and sugarcane as the most frequently consumed (**Figure 3.5.**). Seasonality and fruit availability during the study period meant that certain fruits such as mangoes were not included in the present analysis. Current dietary guidelines recommend five servings of fruits and vegetables per day. Fruit consumption patterns in all three communities fell short of this guideline, so much so that five portions were rarely met even over the course of a week. A decline in fruit consumption in response to a decline in small-scale fruit production has been noted in the Pacific since the 1960s. This has been attributed to lack of economic viability, migration of the work force, importation of non-Pacific fruits and problems in transportation and marketing (Secretariat of the Pacific Community, 2001). Nevertheless, some Pacific Island governments have recognized the health and economic value of some fruit trees and are promoting agricultural research and policies for development. In PNG, the National Agricultural Research Institute (NARI) had organized the Highlands Horticulture Workshop (1999) and emphasized fruit production in rural enterprises (Watson, 2000).

Vegetables

Like fruits, vegetable consumption falls far short of the recommended five portions per day guideline (**Figure 3.6.**). Although not significantly different, Kalo tends to consume more green leafy vegetables compared to Koki and Wanigela. Aibika (*Abelmoschus manihot* (L.) Medik.) was the most popular vegetable and is an excellent source of iron, calcium, protein and other nutrients.

Salt and Sugar

Sodium has been associated with increased blood pressure in some sensitive individuals and current dietary guidelines suggest that salt intake should be restricted. Salt is regularly added to meals in PNG, but not in any excessive amounts (**Figure 3.7.**).

Sucrose consumption has been associated with increased body weight (Vermunt *et al.*, 2003). Sugar added to tea is the most common source, along with sweetened flour products such as scones and donuts. The form of sugar considered here does not include the hidden sugar that is in process foods such as biscuits, softdrinks (local name: lolly-water), scones and juices (local name: cordial). So although Kalo seems to consume significantly more sugar compared to Koki and Wanigela, it is likely that the amount is much greater in Koki. Most sugar in Kalo is ingested in their tea, of which they have between 3-6 cups daily (**Figure 3.8.**).

Overall Food Variety

The number of distinct food items included in this study was determined subjectively by the author. Food variety was highest in Koki since it had access to an open-air market and a grocery store and hence access to frozen foods, sweets, fried food and treats otherwise unavailable in Kalo and Wanigela (**Figure 3.9.**). Wanigela consumed significantly less variety of foods compared to Koki. Considering the low variety of fruits and vegetables consumed in Koki, variety in this case is not indicative of nutrient adequacy or diet quality. Most processed foods are usually high in fat and sugar energy and contribute to obesity and related complications. One must consider from which of the food groups most of the variety comes from and how many of the food groups have been included in one's diet.

Dietary Diversity

Of the twelve food groups considered in this study, Kalo residents consumed more than Koki and Wanigela. Whereas a large portion of Kalo's sample (38%) consumed 11 food groups per week, no fewer than 5% of the population ate less than 8 food groups. Several of the participants in Koki ate less than 9 food groups, but dietary diversity remained skewed towards 11 food groups. The average number of different food groups consumed by Wanigelans was between 7 and 8, with several people eating far fewer. None of the participants consumed items that belonged to more than 9 food groups during the week (**Figure 3.10.**).

Of the food groups most often consumed in Koki, Kalo and Wanigela, coconut products were the most popular (**Table 3.3.**). The importance of coconut to Pacific nations is illustrated in the fact that the Pacific Islands Food Composition Tables list it as a separate food group, as it is treated here (Dignan *et al.*, 2004). Tubers, rice, and cooked vegetables are usually cooked in coconut cream, and the juice of young nuts are popular refreshments.

In Kalo, masticants refer exclusively to betelnut (*Areca catechu*), mustard (*Piper betel*) and lime, a concoction with mild narcotic and antidepressant effects (Dar & Khatoon, 1997). Betelnut chewing is a social norm and is an important component of bride-prices, trade, and in forming relationships. The habit has been associated with impaired glucose tolerance in animal studies (Boucher *et al.*, 1994) and in humans (Benjamin, 2001). Although most Papua New Guineans are avid betelnut chewers, Koki and Wanigela were restricted from chewing due to Seventh-Day Adventist practices that forbid the use of stimulants, including coffee, tea and cigarettes. Despite this, several SDA followers in Koki were a little more liberal and chewed more than occasionally.

Cereals, comprised mostly of rice, wheat flour and products derived from them, and starchy crops including cooking banana were commonly consumed in all villages. Fruits were eaten more frequently in Wanigela, although in lower amounts compared to Koki and Kalo. This is not surprising considering that sugarcane and bananas were the most commonly grown crops in Wanigela. Nuts and legumes were the least popular food items.

Food variety and dietary diversity as indicators of health

Simple regression analysis using food variety score and dietary diversity score as dependent variables shows significant positive relationships between BMI and triceps skinfold thickness, suggesting that one's weight increases with greater food variety (**Table 3.4.**). Conversely, diastolic blood pressure, percent body fat, waist: hip ratio and fasting blood glucose concentrations were negatively associated, indicating a favourable clinical outcome and body composition with increased dietary diversity. Herein lies a seemingly contradictive situation, where dietary diversity or food variety is associated with an increase in body mass and at the same time a decrease in subcutaneous and abdominal fat. The increase in body mass is likely due to greater muscle mass, with concomitant low fat mass. However, in urban environments, food variety to a large extent includes unhealthy foods, which may explain the correlation with increased weight and triceps skinfold.

Medicinal Plant Use

Medicinal Plant Diversity

The residents of Kalo periodically used more plants with medical applications compared to Koki and Wanigela. This reflects the village's proximity to diverse ecotypes that offer a variety of plants not readily available to the other communities. In addition, Kalo's settlement on dry land enabled plantings of fruit and medicinal plants around houses and gardens. Species commonly planted included guava (*Psidium guajava*), neem (*Azadirachta indica* A. Juss)), lemon (*Citrus limon* (L.) Burm. f.), noni (*Morinda citrifolia*), and Madagascar periwinkle (*Catharathus roseus*(L.) G. Don). In contrast, due to their restricted access to land or garden space, most of the medicinal plants used in Koki and Wanigela were potted plants such as aloe (*Aloe vera* (L.) Burm. f.), life plant (*Kalanchoe pinnata* (Lam.) Pers.) and Indian pennywort (*Centella asiatica* (L.) Urban).

Medicinal plant use frequency

Medicinal plant use was more common in Kalo than in Koki and Wanigela, a finding that was consistent with the total number of medicinal plants used (**Figure 3.11.**). There were no large differences in plant use between males and females in Koki and

Kalo; however Koki males tended to utilize medicinal plants more often than females. This pattern was more apparent in Wanigela, where the difference was two-fold (**Figure 3.12.**).

Several generations ago, longer than any present-day elder can recall, Wanigela had escaped from warfare with a neighbouring tribe, the Waramabo (Miagolo), by relocating their village into the mudbanks of Marshall Lagoon. In 1964, Australian patrol officer L.F. Nolan observed no females among the numerous Wanigela canoes returning from the gardens located across the lagoon along the Imila River. He was told that this was due to the casualties inflicted during (at least before 1940) the conflict with the Waramabo and that the women were afraid to work in the gardens. Consequently, males overtook the responsibility of garden work and often left in large groups to ensure their security, while females worked in nearby gardens situated by mangrove inlets. Despite decades of peace and an attempt by the Australian government to resettle the Wanigela on land during the 1960s, they continue to live on the mudbanks of Marshal Lagoon and the males tend to the gardens located on the fertile banks of the Imila River. According to Nolan (1964):

This fear [of the Warambo] is very much in evidence as no men are prepared to construct houses in this area, and the women will not visit it. On this point alone, [it is] doubtful of a speedy resettlement of the Wanigela people. These people have many other fears and superstitions [to overcome]... before any large scale resettlement programme has a chance of success.

Such customs likely had an effect on medicinal plant lore since the men had access to a greater biodiversity by frequenting the banks of the Imila River and hence a broader base to build a *materia medica*. Females were restricted to the botanically less diverse mangrove swamps and accordingly became familiarized with fewer species.

Commonly used medicinal plant species

Plants included in this analysis are those that have been mentioned by at least two participants; their indicated use is summarized in **Table 3.5**.

Residents of Koki preferred to use plants of established medicinal use such as aloe (*A.vera*), mint (*O. americanum*), lemongrass (C. *citratus*), peppermint (*M. piperita*), ginger (*Z. officinale*) and garlic (*A. sativum*). The most commonly used traditional plants were noni (*M. citrifolia*), coconut (*C. nucifera*) and guava (*P. guajava*) (**Figure 3.13.**). In most cases, medicinal plants were grown in pots as garden space was unavailable in the urban landscape. Some species such as coconut and noni were purchased at the nearby open-air market, while others such as lemongrass were brought in by relatives from Marshall Lagoon who grew the plant in a few places close to gardens.

The presence of the Seventh-Day Adventist Church has had an enormous impact on traditional medicine. The church advocates healthy eating and natural treatment with herbs, but promotes plants of European and North American origin. These were included in the SDA publication guide on medicinal plants, "Back to Eden" (Kloss, 1981), which was referred to regularly by residents of Koki and Wanigela. Periodic workshops for both the men's and women's fellowship were held especially to promote SDA natural medicines. Although noni, coconut and guava were not listed in the guide, they remain important plants because of their widespread and popular use across the Pacific.

Noni, a popular garden ornamental in the Pacific, was the most utilized medicinal plant in Kalo (**Figure 3.14.**). Unlike Koki and Wanigela, the most common species were cultivated trees, such as noni (*M. citrifolia*), lemon (*C. limon*), guava (*P. guajava*), papaya (*C. papaya*), neem (*A. indica*), lime (*C. aurtifolia*), and cherimoya (*A. cherimola*), or wild, such as milky pine (*A. scholaris*) and coconut (*C. nucifera*). Potted plants were rare in Kalo, as garden and village space for food, medicinal and ornamental gardens were abundant.

Similar to Koki and Wanigela, religion contributed to the deterioration of traditional medicine in Kalo. The United Church discouraged traditional medicine in favour of Western biomedicine. In addition, traditional medicine is intricately connected with sorcery and witchcraft, practices which are strongly discouraged by Christian missionaries. Illness was traditionally thought to be due to a curse or an angered spirit or ancestor. Identification of the spellcaster or spirit responsible would lead to improvement in the patient's health. Traditional medicines thus were concerned chiefly with divination

spells. The appearance of biomedicine and germ theory awareness contributed to the decline of divination practices, although beliefs in curses and charms are still widely held. The United Church maintains that any person involved in any customs related to sorcery, including traditional medicines, would be banned from the church. This excludes herbal treatments that are clearly disassociated from witchcraft through popular use such as noni and guava.

Medicinal plants used in Wanigela were much the same as those used in Koki (**Figure 3.15.**), where potted plants and small herbs were relied on more frequently than trees and wild plants. Life expectancy in Wanigela seemed lower than the rest of PNG (PNG: males: 61.39 years; females: 65.64 years), due to infectious disease related to mudbank living. Consequently, only a few elders in their 60s remained, and of these only one was familiar with the traditional uses of plants. With no apparent practicality in the shadow of biomedicine, only a fraction of his plant knowledge is passed on to his kin.

Proximity to Public Health Centres (PHC) has had a clear impact on traditional medicine. Wanigela had its own PHC situated 500 m away on shore, and a short 40 minute boat ride to the clinic at Kupiano, the District Headquarters. Malaria, respiratory and gastrointestinal disorders were the most common complaints for which the PHC distributed medicines freely.

The PHC at Kalo was frequented rather often, the most common complaints being malaria, gastrointestinal disorders and skin infections. A significant portion of the village relied on relatives who practiced medicine in private clinics in Port Moresby. In this way, Kalo residents had ready access to biomedicine.

Table 3.1. Food groups and number of individual food items per group included in the survey.

Food group	Number of Items
1. Cereals	11
2. Starchy foods	12
3. Legumes	6
4. Vegetables	19
5. Fruits	17
6. Nuts	6
7. Coconut products	5
8. Spices	9
9. Masticants (betelnut)	4
10. Beverages	9
11. Meats	10
12. Other	46

Table 3.2. Age-adjusted characteristics of men and women 16+ years old of the coastal communities Koki, Kalo and Wanigela, Papua New Guinea. Results expressed as mean ± SEM. Population figures obtained from the 2000 National Census.

	KOKI Urban		**KALO** Semi-rural		**WANIGELA** Rural	
	Male	Female	Male	Female	Male	Female
Population	2,655	2,295	745	714	1,676	1,646
Sample number (n)	60	60	60	61	60	65
Modernity Index Score	26.4 ± 5.7[abc]	22.0 ± 3.8[bc]	23.2 ± 6.4[ad]	17.6 ± 4.6[d]	16.8 ± 5.3[a]	11.2 ± 2.2
Body Mass Index (kg/m^2)	25.4 ± 4.5[c]	26.0 ± 5.4[bc]	24.1 ± 3.6	23.0 ± 3.8[d]	22.3 ± 2.6[a]	21.0 ± 2.9
Overweight (%)	48.3[bc]	46.6[bc]	26.6[d]	26.2[d]	6.7	8.5
Waist: Hip Ratio (cm)	0.96 ± 0.06[c]	0.88 ± 0.07[bc]	0.95 ± 0.07	0.92 ± 0.10[d]	0.93 ± 0.05	0.90 ± 0.05
Abdominal Obesity (%)	48.3[abc]	73.3[bc]	36.7[d]	83.5[d]	31.7	69.6
% Body Fat	25.2 ± 5.5[ac]	37.4 ± 6.4[bc]	24.2 ± 5.5[ad]	33.0 ± 5.6[d]	19.1 ± 3.9[a]	29.7 ± 5.2
Excess Body Fat (%)	66[abc]	80[bc]	53[ad]	64[d]	33[a]	47
Systolic Blood Pressure (mm Hg)	129.3 ± 15	132.8 ± 17	133.6 ± 16	131.5 ± 13	130.3 ± 15	136.0 ± 15
Diastolic Blood Pressure (mm Hg)	80.9 ± 7[b]	81.7 ± 11[b]	77.5 ± 7	75.1 ± 10[d]	80.8 ± 7	80.8 ± 8
Hypertension (%)	23.3[a]	25.0[b]	16.7[a]	13.1[d]	18.3	23.1
Energy Expenditure (kcal/d)	2106.8 ± 52.1[ab]	2277.1 ± 65.4[b]	2586.9 ± 53.3[ad]	2125.8 ± 37.2[d]	2165.5 ± 34.8[a]	2241.2 ± 38.9
Diabetes (%)	13.3[ab]	3.3	3.3[ad]	1.7	13.3[a]	2.8

[a]Significance $p<0.05$ between genders of same village; [b]Significance $p<0.05$ between same gender of Koki vs. Kalo; [c]Significance $p<0.05$ between same gender of Koki vs. Wanigela; [d]Significance $p<0.05$ between same gender of Kalo vs. Wanigela

Table 3.3. Food groups ranked according to their frequency of use in Koki, Kalo and Wanigela, PNG.

Koki		**Kalo**		**Wanigela**	
1	Coconut products	1	Masticants	1	Coconut products
2	Cereals	2	Coconut products	2	Starchy foods
3	Starchy foods	3	Cereals	3	Cereals
4	Meats	4	Starchy foods	4	Fruits
5	Spices	5	Meats	5	Spices
6	Beverages	6	Beverages	6	Meats
7	Masticants	7	Fruits	7	Vegetables
8	Vegetables	8	Spices	8	Beverages
9	Fruits	9	Vegetables	9	Legumes
10	Legumes	10	Nuts	10	Masticants
11	Nuts	11	Legumes	11	Nuts

Table 3.4. Pearson's regression coefficients of various health parameters in relation to food variety score (FVS) and dietary diversity score (DDS).

	FVS		**DDS**	
	r	*p*	*r*	*p*
BMI (kg/m^2)	0.185	0.028*	0.267	0.001*
Systolic blood pressure (mmHg)	-0.035	0.556	0.036	0.540
Diastolic blood pressure (mmHg)	-0.140	0.021*	-0.214	<0.001*
Triceps skinfold (cm)	0.211	0.05*	0.346	0.001*
% Body Fat (%)	-0.042	0.636	-0.306	<0.001*
Waist: hip ratio (cm)	-0.152	0.007*	-0.097	0.079
Blood glucose (mmol/L)	-0.073	0.150	-0.114	0.021*

*$P<0.05$

Table 3.5. Medicinal plants of Central Province, PNG mentioned in the text.

Plant	English / Local name	Use	Voucher #
AGAVACEAE *Aloe vera* L.	Aloe	Leaf rubbed on sores and burns	Wan-68-PO04
AMARYLLIDACEAE *Allium sativum* L.	Garlic	Juice squeezed into ear for hearing loss or ear ache.	Wan-72-PO04
ANNONACEAE *Annona cherimola* Mill.	Cherimoya		
ARECACEAE *Cocos nucifera* L.	Coconut / Kokonas	Oil rubbed on any skin disorder or sore.	Wan-73-PO04
ASTERACEAE *Tridax procumbens* L.	Gawa	Crushed leaves applied to open sores.	Kal-38-PO04
CARICAEAE *Carica papaya* L.	Papaya / Popo	Seeds eaten for malaria.	Kal-53-PO04
CRASSULACEAE *Kalanchoe pinnata* (Lam.) Pers.	Life plant / Imoaita	Warmed leaf applied to sores; Leaves eaten for chest pain or constipation.	Wan-67-PO04
LAMIACEAE *Ocimum americanum* L.	Mint / Loka	Leaves crushed and rubbed on site of inflammation	Kal-31-PO04
MELIACEAE *Azadirachta indica* A.H.L. Juss.	Neem	Tea of leaves used as an tonic or diabetes.	Kal-25-PO04
POACEAE *Cymbopogon citratus* (DC) Stan.	Lemongrass / Tulau	Tea made of leaves used for colds and flu.	Wan-58-PO04
RUBIACEAE *Morinda citrifolia* L.	Noni / Nono	Fruit juice drunk for asthma and tuberculosis; crushed leaves or scraped roots are mixed with coconut oil and massaged on swollen joints	Kal-03-PO04
RUTACEAE *Citrus aurantifolia* (Christm.) Swing.	Lime / Tsiporo	Five unripe fruits eaten immediately after snakebite as an anti-venom; tea of leaves used for colds and sore throats.	Kal-27-PO04
Citrus limon (L.) Burn.f.	Lemon	Leaves and fruit juice in black tea for colds and sore throats.	

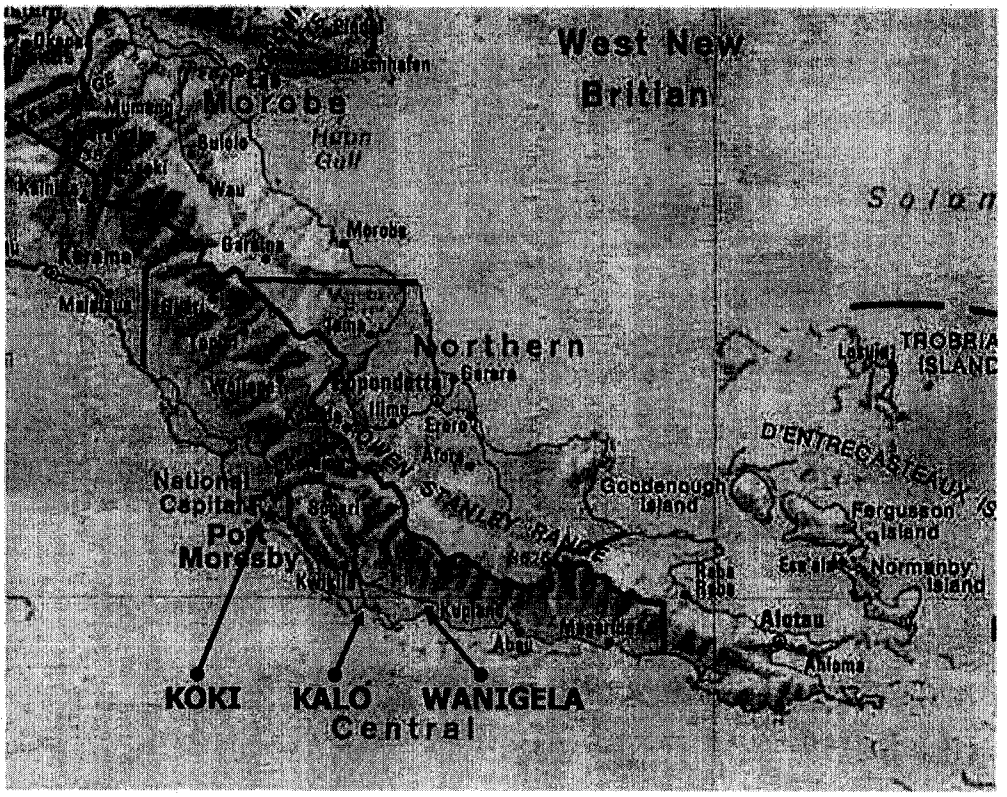

Figure 3.1. National Capitol District (NCD) and Central Province of coastal Papua New Guinea indicating the location of Koki (urban), Kalo (semi-rural) and Wanigela (rural). Map produced by the U.S. Central Intelligence Agency, original scale 1:6,500,000. (https://www.cia.gov/library/publications/cia-maps-publications/index.html).

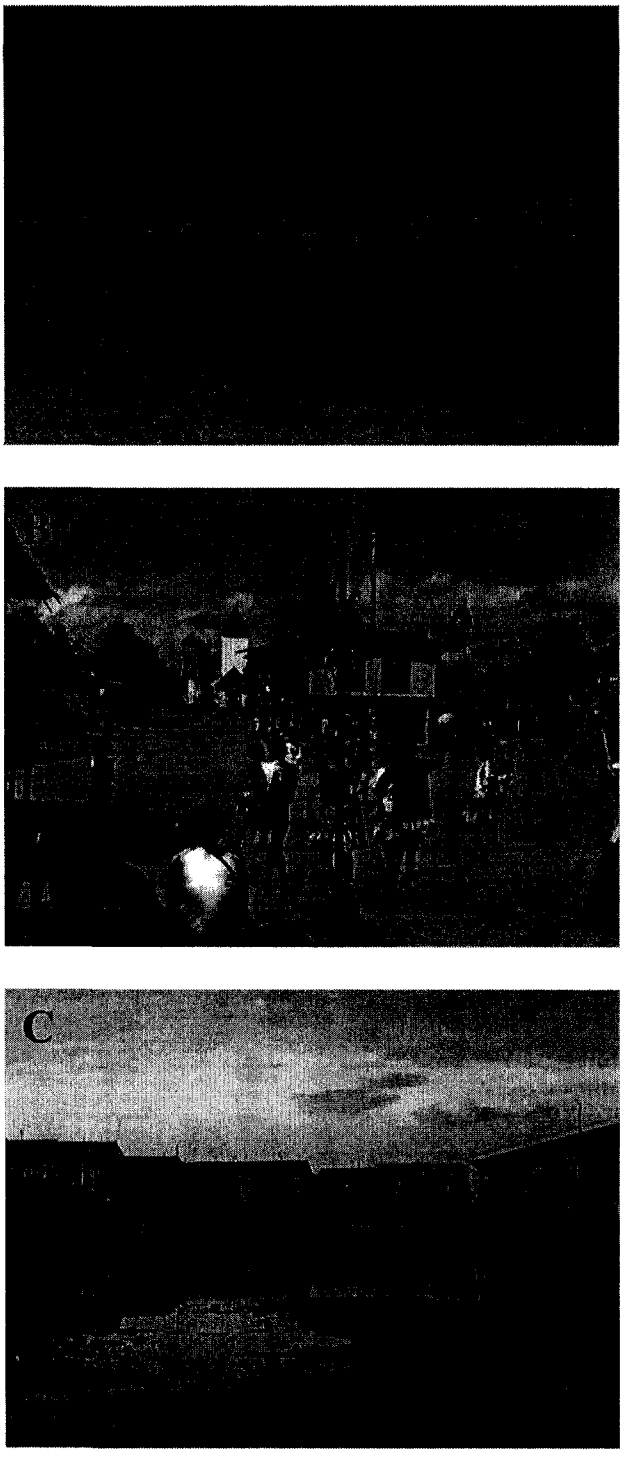

Figure 3.2. Villages included in the present study. A) Koki, an urban Wanigelan settlement, National Capitol District; B) Kalo, Central Province; and C) Wanigela, Central province.

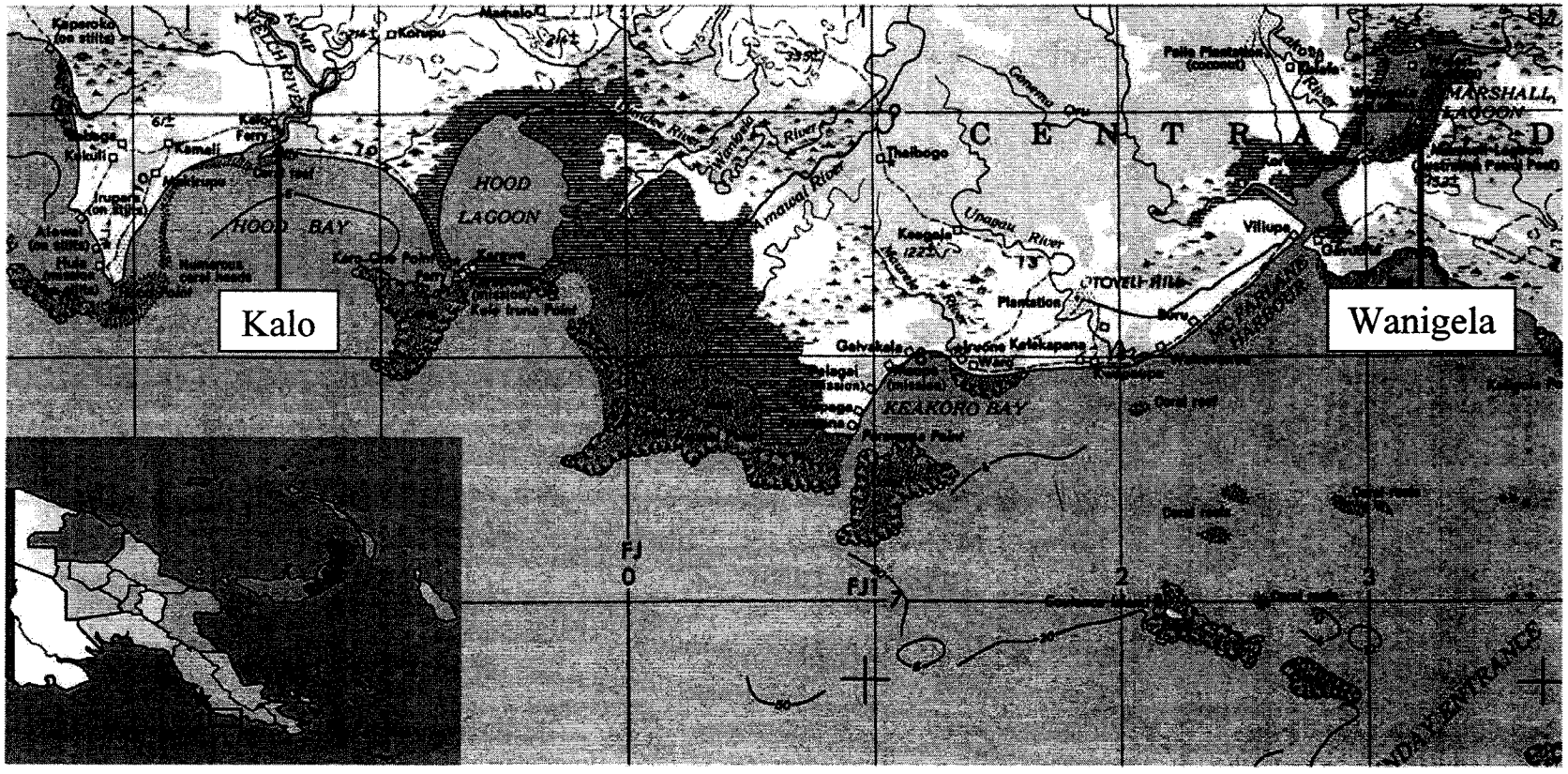

Figure 3.3. Location of Kalo, situated at the mouth of the Kemp Welch River and Wanigela, situated on the mud banks of Marshall Lagoon. Inset map indicates the capital (and Koki) in relation to the larger map. Obtained form the U.S. Army Map Service, Series T504, original scale 1:250,000. (http://www.lib.berkeley.edu/EART/ams.html).

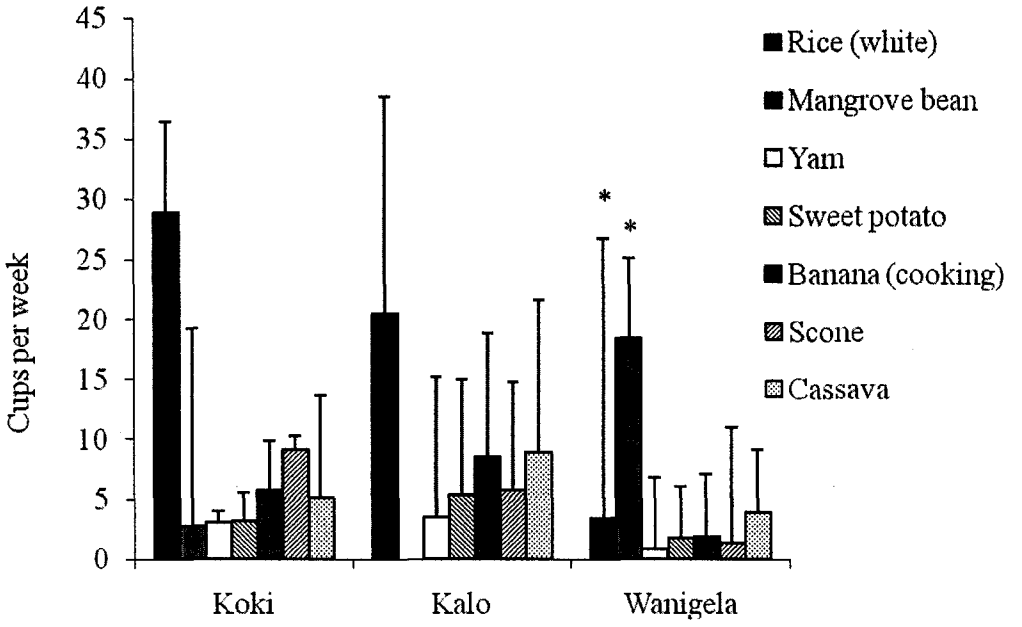

Figure 3.4. Amount (cups) of staples, starchy crops and cereals eaten in Wanigela, Kalo and Koki, PNG in seven days. Bars represent mean ± SD; *p<0.05

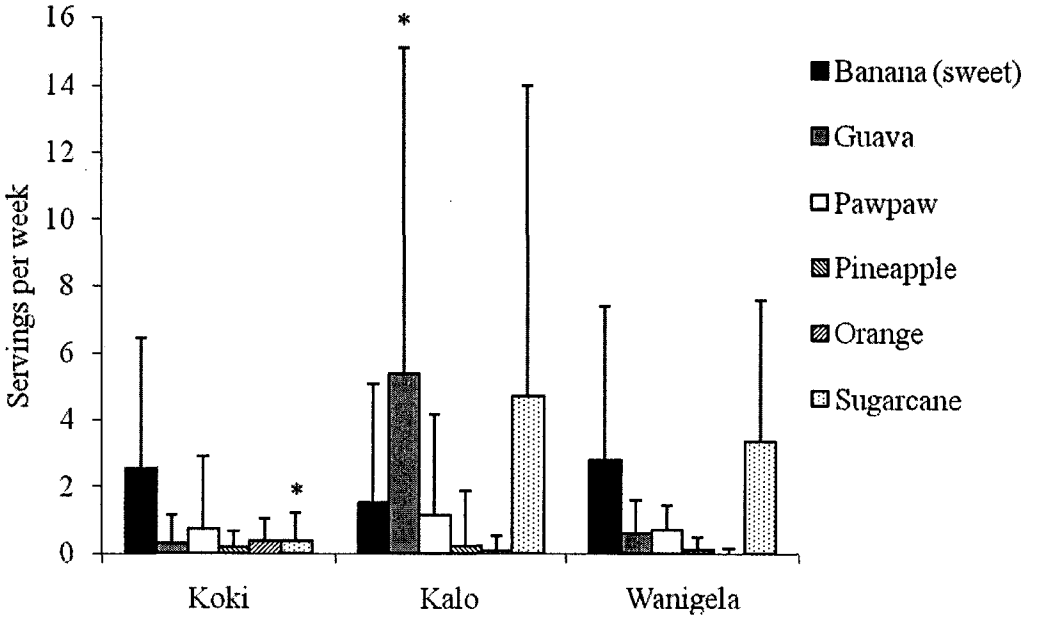

Figure 3.5. Amount of fruit eaten in Koki, Kalo and Wanigela, PNG over seven days. Bars represent mean ± SD; *$p<0.05$

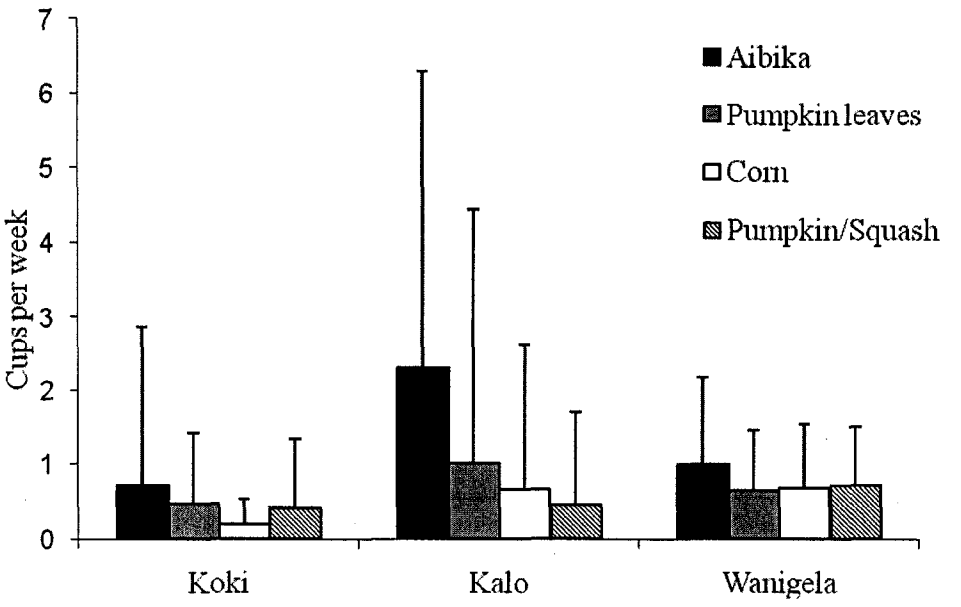

Figure 3.6. Amount (cups) of selected vegetables eaten in Koki, Kalo and Wanigela over seven days. Bars represent mean ± SD.

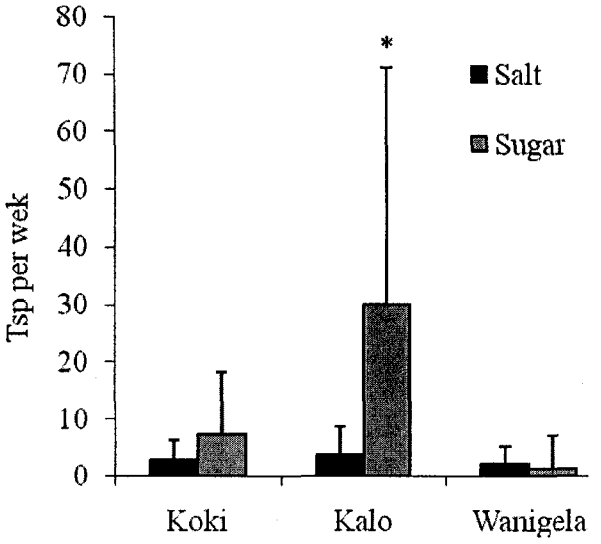

Figure 3.7. Amount of salt and sugar eaten in Koki, Kalo and Wanigela, PNG over seven days. Bars represent mean ± SD; *p<0.05

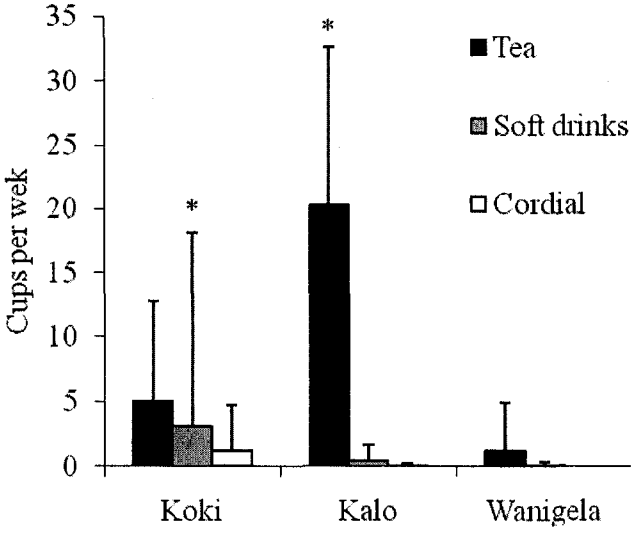

Figure 3.8. Amount of tea, soft drinks and cordial consumed in Koki, Kalo and Wanigela, PNG over seven days. Bars represent mean ± SD ; *p<0.05

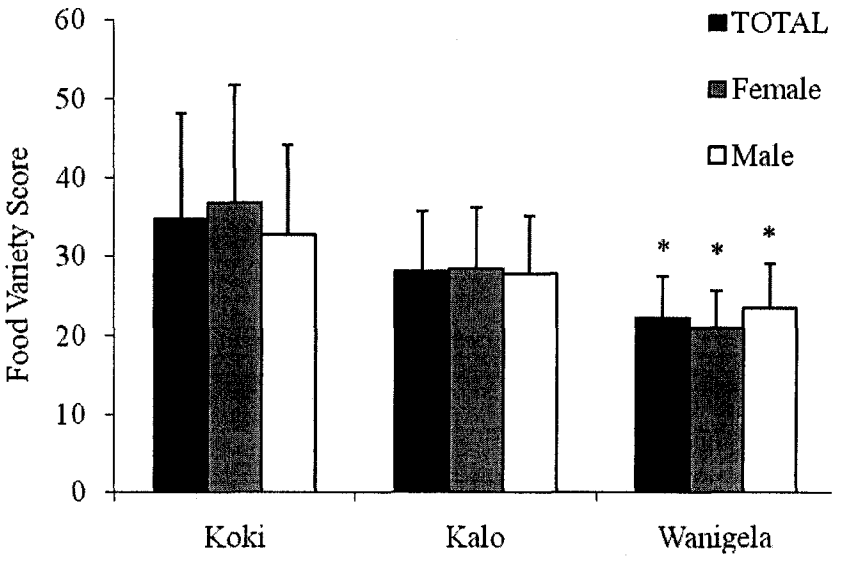

Figure 3.9. The total number of food items eaten in Koki, Kalo and Wanigela, PNG over seven days. Male and female food variety scores are also included. *$p<0.05$

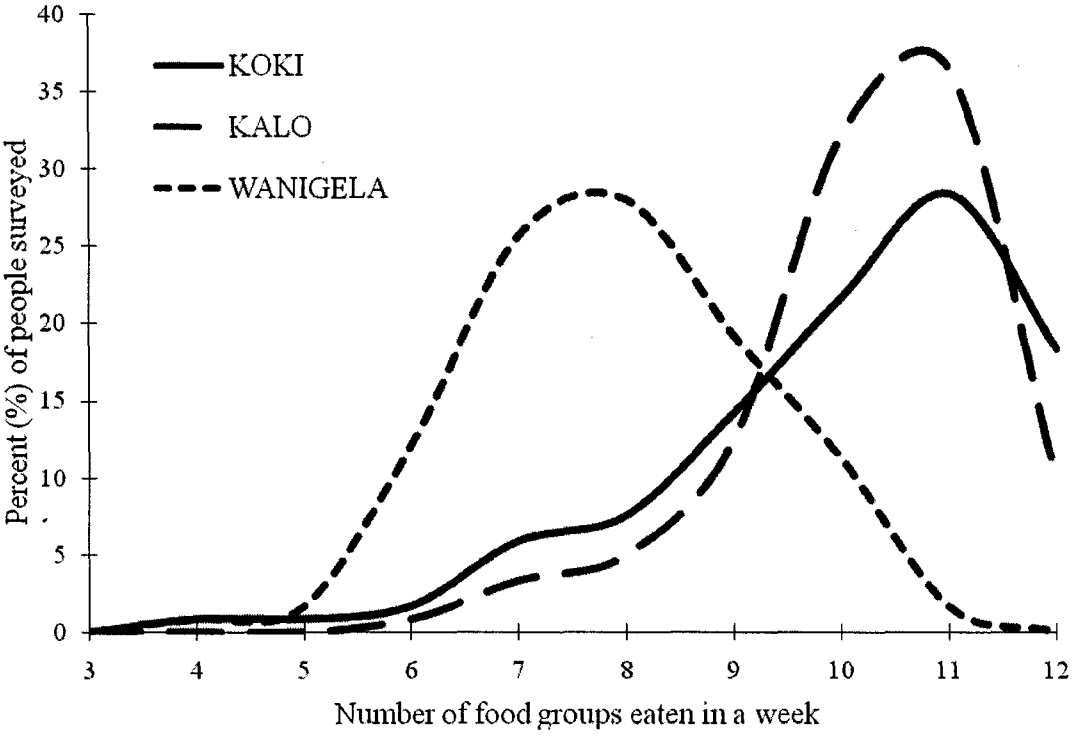

Figure 3.10. Distribution curve of the number of food groups consumed by Koki, Kalo and Wanigela, PNG, over seven days.

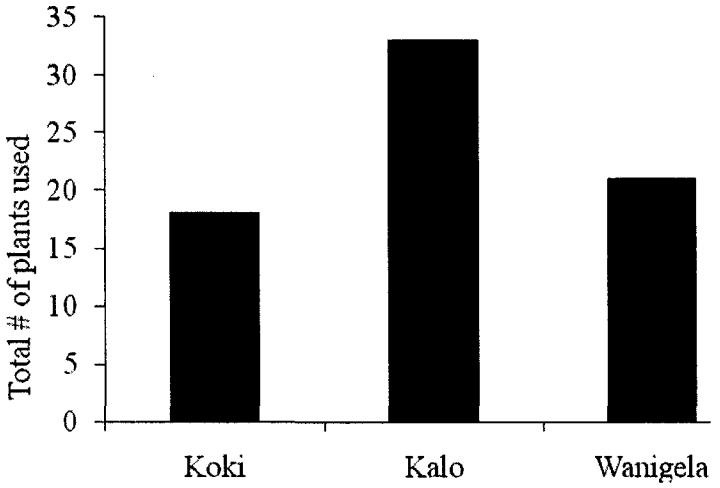

Figure 3.11. Total number of plants used as medicines in Koki, Kalo and Wanigela, PNG

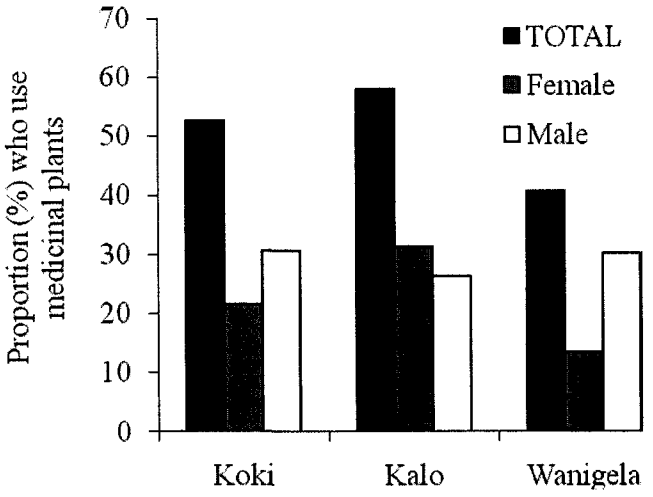

Figure 3.12. Percent of the total sample population that uses medicinal plants in Koki, Kalo and Wanigela, PNG, divided according to gender.

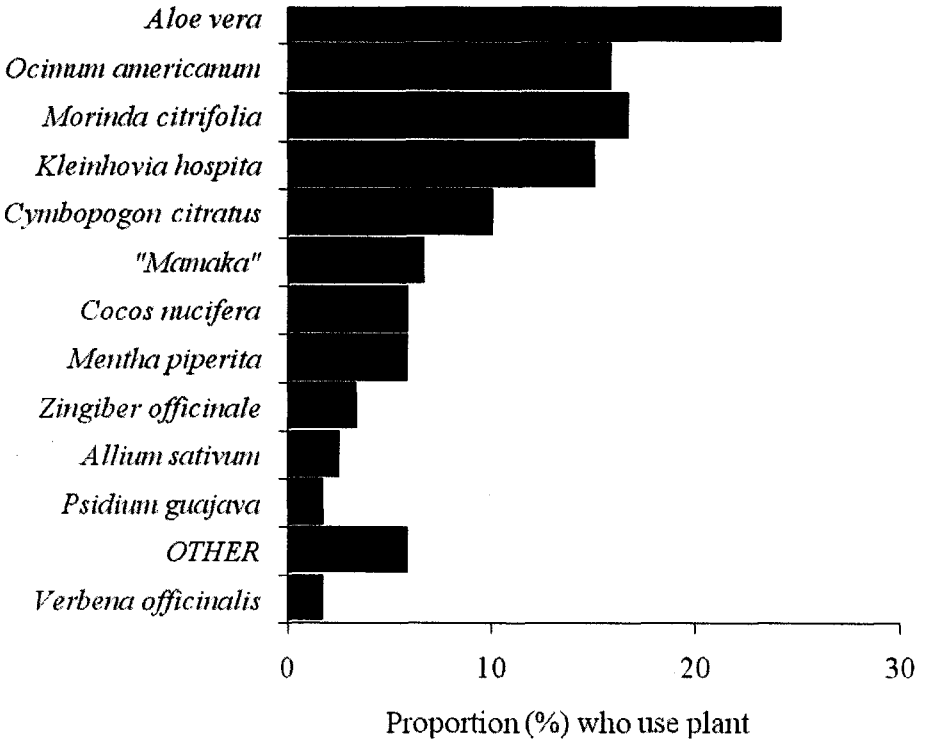

Figure 3.13. Common medicinal plants used in Koki, PNG according to percent of the sample population (n=120).

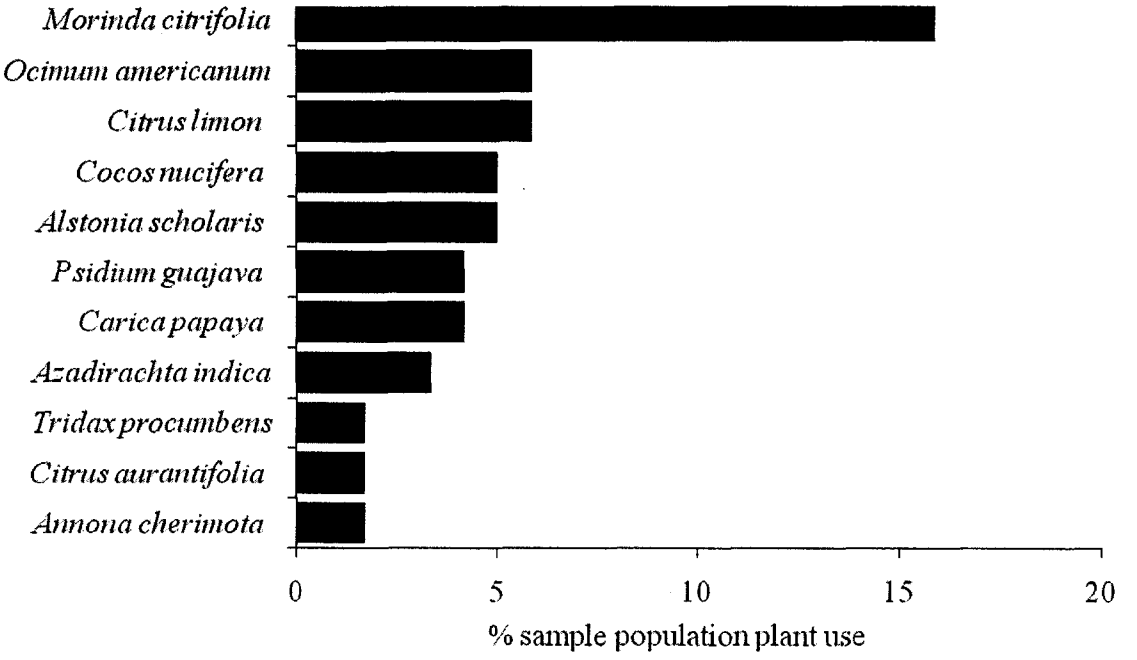

Figure 3.14. Common medicinal plants used in Kalo, PNG according to percent of the sample population (n=121).

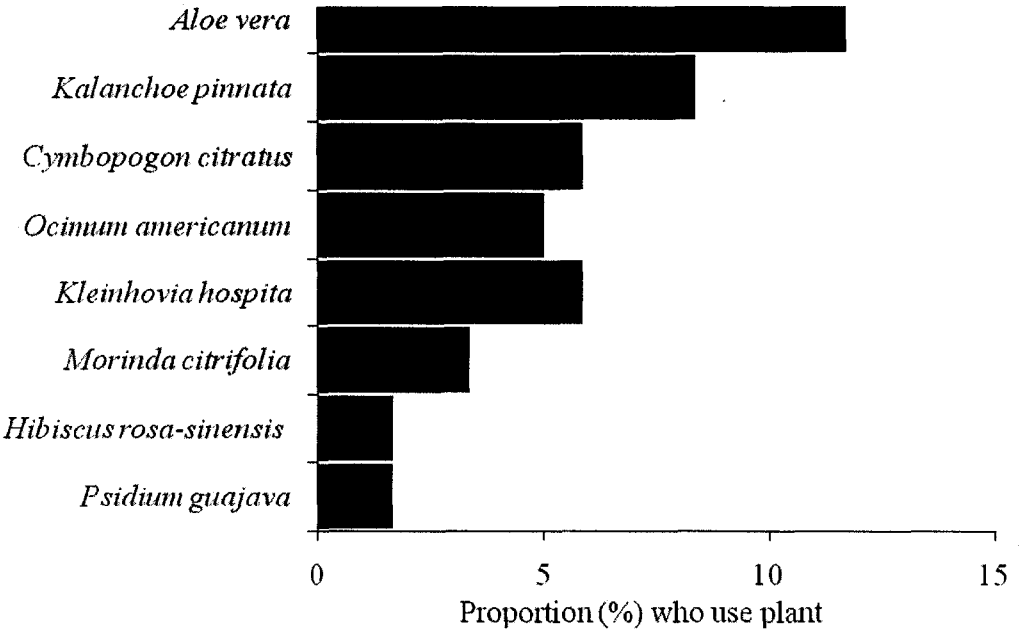

Figure 3.15. Common medicinal plants used in Kalo, PNG according to percent of the sample population (n=121).

BRIDGE 1

The previous manuscript broadly described differences in population characteristics, food variety and medicinal plant use in relation to area of residence. Such differences were rationalized within cultural constructs, religious contexts and ecological variables, providing a sociological perspective on the nutrition transition. Migration and settlement patterns of the Wanigelans and residents of Kalo help explain the development of their contemporary social systems that lead to difference in economic position, diet diversity and ethnobotanical knowledge. With this in mind, ensuing manuscripts can be conceptualized within the complex social factors outlined so far.

In the following manuscript, the use of food composition data enables a more detailed inspection of the association between dietary diversity and health. With nutrient data available, anaylsis suggested that two additional food groups (dairy; fats andoils) be included based on their contribution to total energy intake. The emphasis thus moves away from intercommunity differences towards comparative applications of methodologies for measuring dietary diversity. One of the common criticisms of the FVS and DDS is its lack of generalizability across cultures and subjectivity as to what is considered a discrete food item or food group. To remedy this, Katanoda et al. (2006) had recently developed the quantitative index for dietary diversity (QUANTIDD) and applied it to longitudinal nutrition data of Japan's caloric and nutrient intake. We consider the QUANTIDD a useful tool that should be incorporated into all future food diversity studies in order to allow objective comparisons. However, the QUANTIDD had never been applied to a population outside Japan, and has never been tested for its capacity to predict nutrient adequacy or metabolic health.

Manuscript 2 begins with the question, "what sociodemographic variables determine food variety?" and follows with a comparative analysis of QUANTIDD, FVS and DDS as indicators of nutrient adequacy. Although each indicator displays a significant association with one or more NCD risk health parameters, we demonstrate that the QUANIDD exposes food use patterns that cannot be detected by FVS or DDS.

CHAPTER 4
MANUSCRIPT 2

NUTRIENT ADEQUACY, HEALTH AND DIETARY DIVERSITY IN PAPUA NEW GUINEAN ADULTS:

COMPARATIVE ANALYSIS OF THE QUANTITATIVE INDEX FOR DIETARY DIVERSITY (QUANTIDD)

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ABSTRACT

Counts of food items or groups such as the food variety (FVS) and dietary diversity (DDS) scores are used in developing countries for their simplicity, but are highly context specific. The quantitative index for dietary diversity (QUANTIDD) which compares the proportional distribution of consumed foods is used in this study alongside FVS and DDS to characterize the relationship of dietary patterns of coastal Papua New Guineans to nutritional adequacy and health status. A total of 365 randomly-selected participants from rural, semi-rural and urban areas stratified by age and gender were administered a quantitative 7-d food frequency questionnaire (QFFQ). Nutrient intake, anthropometry, blood pressure and fasting glucose concentrations were compared between the lowest and highest tertiles for each index. Area of residence had the greatest influence on dietary diversity and QUANTIDD with urban populations consuming more diverse diets, followed by religious persuasion and marital status. Those with greater food variety had higher absolute and energy-adjusted intakes of carbohydrates, protein, total fat, cholesterol, iron, zinc, thiamin, riboflavin, niacin, folate and vitamin C. As a result of consumption of coconut and select traditional crops, the low variety group had higher intakes of fiber, % energy from fat, saturated fat and vitamin A. No association was observed with calcium. High food variety was associated with increased weight, stature, body mass index (BMI) and tricep skinfold thickness. DDS was the only index that correlated with systolic ($\beta \pm$ SE = -0.94 ± 0.43, $p<0.05$) and diastolic ($\beta \pm$ SE = -0.82 ± 0.27, $p<0.005$) blood pressure and QUANTIDD the only one to correlate with fasting glucose ($\beta \pm$ SE = -1.66 ± 0.59, $p<0.005$) after adjusting for age, gender and socioeconomic status. Of the indices, QUANTIDD was a better indicator of nutrient adequacy, reaching a specificity of 91% and a specificity of 71% for a cut-off point of 0.3 (range 0-1). Our study demonstrates that the QUANTIDD can contribute important dietary information not detectable by FVS and DDS and, due to its objectivity, be used for comparative analyses.

INTRODUCTION

"Eat a variety of foods" is a fundamental dietary recommendation, with the underlying principle of assuring nutrient adequacy and maintaining optimal health. In its simplest form of measurement, dietary diversity has been characterized as the number of single foods (Food Variety Score; FVS) or food groups (Dietary Diversity Score; DDS) consumed over a reference period of time (ranging from 1 to 15 days). Both indices associate positively with achieving the RDA for individual nutrients, improved cognitive and physical functions (Clausen *et al.*, 2005) and reduced risk of chronic disease (La Vecchia *et al.*, 1997) and all-cause mortality (Kant *et al.*, 1993). In order to address concerns related to diet-related chronic disease, a variety of additional quantitative methods to measure dietary quality have been developed such as the Healthy Eating Index (HEI) (Kennedy *et al.*, 1995), the Recommended Food Scores (RFS) (Kant *et al.*, 1993) and the Diet Quality Index (DQI) (Haines *et al.*, 1999)

In developing countries, experiences in measuring dietary quality are scarcer. The simplest methods remain the most popular (Ruel, 2003): Food Variety Score and Dietary Diversity Score are generally positively associated with nutrient intake and improved health/nutritional status indicators such as anthropometry, serum hemoglobin, retinol, C-reactive protein, ferritin, blood pressure, plasma lipids and fasting glucose levels (Hsu-Hage & Wahlqvist, 1996; Ogle *et al.*, 2001).

Such dietary indices, as employed in Botswana (Clausen *et al.*, 2005), Kenya (Onyango *et al.*, 1998), Ghana, Malawi (Ferguson *et al.*, 1993), Mali, Mozambique (Rose *et al.*, 2002), Burkina Faso (Savy *et al.*, 2005), Iran (Mirmiran *et al.*, 2004) and Vietnam (Ogle *et al.*, 2001) typically include foods that were endemic or traditional to that region or cultural group, preempting any attempt to standardize the number of food items or groups for comparative cross-national analyses. Differences in approaches, indicators and validation further limit comparability and generalizability between indices. This notwithstanding, these studies have consistently demonstrated that dietary variety is positively associated with increased intake for most nutrients and in some cases, improved nutritional status, especially when traditional staples are included.

In order to objectify dietary diversity measurements, Katanoda et al. (2006) developed an index that measures the quantitative distribution of consumed foods. The Quantitative Index for Dietary Diversity (QUANTIDD) measures the proportional contribution of energy or intake weight of food items that have been consumed within each food group irrespective of the timeframe of data collection, and the number of food items or food groups considered. Thus, while FVS and DDS are useful as indicators of variety in a diet, the QUANTIDD assesses the variety within food groups. Quantification of the amount of food consumed using the QUANTIDD adds an additional dimension to FVS and DDS since these do not consider portion sizes. The authors applied the index to data obtained from the Japan National Health and Nutrition Survey 1957-2000 and demonstrated agreement with the rise in food variety associated with economic prosperity.

The promotion of indigenous food systems as a vehicle to maintain health and prevent noncommunicable disease is an earnest strategy that recognizes the nutritional density of traditional staples over introduced staples. The WHO Global Strategy on Diet, Physical Activity and Health (WHO, 2004), although not directly addressing the importance of dietary variety, has recognized that health policy strategies must be culturally appropriate and able to challenge cultural influences and to respond to changes over time.

Papua New Guinea (PNG) is a Melanesian country undergoing rapid socioeconomic transition. Tuber-based diets are rapidly being replaced by grains, namely polished rice, leading to energy and nutrient imbalance-based disorders such as obesity, type 2 diabetes and cardiovascular disease (Dowse *et al.*, 1994; Natsuhara *et al.*, 2000). Previous work has demonstrated a strong positive association between socioeconomic status and dietary diversity in developing countries (Torheim *et al.*, 2004; Ponce *et al.*, 2006). For populations undergoing nutritional transition such as PNG, a simple count of food items or groups does not provide a complete picture of dietary diversity and its effect on health. We propose that quantification of the energy and intake distribution patterns of diets using the QUANTIDD index will function as a comparatively superior indicator of nutrient adequacy and health status than FVS and DDS.

In this study, QUANTIDD scores for energy and food intake are used alongside the more familiar FVS and DDS to determine the association between dietary diversity, demographic factors, nutrient adequacy and selected health indicators in coastal Papua New Guinea. Sociodemographic variables that potentially influence dietary diversity were also examined. This is the first known dietary diversity study to be conducted in a developing country in the Pacific region, and the first to apply QUANTIDD techniques outside Japan.

MATERIALS AND METHODS

Study Area

The study was conducted in Central Province, Papua New Guinea (PNG) from January to August, 2004 in three areas representing disparate socioeconomic conditions. The rural sample, Wanigela, is a stilt village located approximately 400 km east of the capital. The diet consists primarily of mangrove bean (*Bruguiera gymnorrhiza*), coconut, bananas and fish. As Seventh-Day Adventists, villagers are discouraged to consume stimulants such as alcohol, cigarettes, coffee, betelnut, and certain animal products such as pork and shellfish. Fish is often traded for fresh produce or market items. The urban sample, Koki, is an urban coastal settlement in Port Moresby populated primarily with Wanigela migrants. Situated close to the city centre, residents rely on outdoor markets and grocery stores for their food. Rice, tubers, bananas and street food form the bulk of the diet. The semi-rural sample, Kalo, is situated equidistant between Koki and Wanigela at the mouth of the Kemp Welsh River. Residents have access to fresh water and marine resources and cultivate a diversity of traditional crops on nearby fertile riverine soils. Although purchased rice and instant noodles are regular fare, the diet is dominated by locally grown tubers, vegetables and fruits. United Church forms the primary religion, and a smaller proportion follows the Salvation Army.

Subjects

Participants were selected by stratified random sampling according to gender and age. All individuals above 16 years were eligible to participate except pregnant and lactating women and in circumstances where communication or cognitive difficulties made it impossible for the participant to provide the necessary information. Questionnaires were administered at the individual level. The survey covered a final sample of 365 participants roughly divided between the three villages. Sample size was calculated for multiple regression with 5 independent variables at a significance level of $\alpha = 0.05$ and a power of 0.80 in order to detect a minimum R^2 value of 12% and greater. Approval from the head of each local level government was obtained, followed by a public information session and individual prior informed consent before initiation of the study. Ethics approval was obtained from the McGill University Ethics Committee and the Papua New Guinea Medical Advisory Board.

Dietary Consumption

A modified quantitative seven-day food frequency questionnaire (QFFQ) based on that developed and validated by the International Diabetes Institute for Papua New Guinea (Hodge *et al.*, 1996b) included additional food items such as masticants, spices and medicinal plants. Interviews were conducted in the local language by locally trained surveyors. Participants were asked to estimate their intake and portion sizes using measuring cups and spoons as visual aids. The QFFQ included 102 items divided into 15 food groups, modified from Kant *et al.* (1993; 1995): grains/cereals, baked/ processed carbohydrates, starchy foods/tubers, legumes, vegetables, fruits, nuts, herbs/spices/medicinal plants, masticants, beverages, meat, fish/seafood, sweets/sugar/confectionnaries, fats/oils, and dairy. Food groupings were based on those of Masticants were considered a separate group since betelnut (*Areca catechu*) chewing, a widespread and popular practice, is known to contribute energy and nutrients. To accentuate certain food items associated with the nutrition transition, a refined carbohydrate group was formed seperate from the grain group. Although herbs, spices and medicinal plants do not contribute quantitatively to calories or nutrient intake, their cultural importance in indigenous systems and the potential health benefits derived from bioactive phytochemicals warrant a separate food grouping.

Nutrient composition was analyzed using Nutribase 5 Clinical Edition (Cybersoft, Inc. 2004) customized to include PNG foods with additional information taken from the Pacific Island Food Composition Tables (Dignan *et al.*, 2004). Nutritional composition of mangrove bean was performed by Certispec Food Laboratory (Montreal, Canada).

Dietary pattern indices were calculated as follows:

Food Variety Score (FVS): the number of different dietary items consumed during the week (range of 1 - 102).

Dietary Diversity Score (DDS): the number of different food groups consumed during the week (range of 1 – 15).

Quantitative Index for Dietary Diversity (QUANTIDD): calculates the total proportion of energy/intake from the food groups. The index ranges from 0 to 1 where a higher score indicates that the proportion of energy/intake was distributed more evenly across food groups. Both energy (QUANTIDD-E) and intake in grams (QUANTIDD-I) was calculated using the equation $QUANTIDD = (1 - \Sigma_j^n \text{ prop}[j]^2)/(1 - 1/n)$, where *prop[j]* is the proportion of food groups *j* that contribute to total energy or intake, *n* is the number of food groups, and $j = 1,2,\ldots n$.

Dietary quality was assessed in terms of nutrient adequacy, measured as the Mean Adequacy Ratio (MAR), a truncated index of the proportion of recommended intakes for 12 nutrients (Nutrient Adequacy Ratio; NAR): energy, protein, fiber, vitamin A, thiamine, riboflavin, niacin, folate, vitamin C, calcium, iron and zinc (Krebs-Smith *et al.*, 1987).

Anthropometric measurements and blood glucose determination

Participants were weighed without shoes and in light clothing to the nearest 100g on a Seca Dial Scale (Vogel & Halke, Germany). Standing height was measured using wooden boards with a measuring tape (0.1 cm precision) that were built locally based on the UNICEF model (Programme, 1986). Waist and hip circumference was measured using a fiberglass measuring tape. Skin-fold thickness was obtained using Lange calipers and body fat % calculated according to formulae supplied by the manufacturer for four sites: tricep, bicep, subscapular and suprailiac.

Blood pressure was taken in duplicate after a participant was asked to sit quietly for five minutes. In cases where hypertension was detected, another reading was taken after ten minutes. If still hypertensive, the participant was referred to the Community Health Worker. Participants were encouraged to retest their blood pressure at any time during the study.

Blood glucose concentration was determined using a portable glucometer (CardioChek^{TM} Analyzer, Polymer Technology Systems Inc., Indiana) after an overnight 12-hour fast. Readings were obtained within 2 minutes. Participants with glucose concentrations above 7 mmol/L were retested to confirm hyperglycemia and referred to the Port Moresby General Hospital Diabetes Clinic for further testing and treatment. Participants with known diabetes were excluded from the analyses since this may have influenced dietary and lifestyle patterns.

Socioeconomic status

To determine the degree of acculturation or socioeconomic status, a modernity index score (MIS), developed and validated for PNG (Hodge *et al.*, 1995) was administered. The score is based on seven questions pertaining to area of origin, father's employment, type and duration of individual's employment, education, etc., giving a maximum of 40 points. A high score indicated a more modernized participant.

Statistical analysis

Data were analyzed with SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL, USA). The indices QUANTIDD-E and QUANTIDD-I were logit-transformed (log[p/1-p] in order to normalize the data. For descriptive statistics, dietary pattern indices were divided into tertiles and the odds ratio calculated between the lowest and highest tertiles for categorical variables. For continuous variables, differences between two groups were analyzed with an independent *t* test.

To determine the sociodemographic variables that contributed significantly to explain the dietary pattern indices, stepwise selection and exclusion-based multiple linear regression was used where the criteria to include was a probability of $p \leq 0.05$ and exclude $p \geq 0.10$ for F.

To explore the associations between dietary pattern indices and anthropometric measures, blood glucose concentration, and nutrient adequacy, simple (Pearson's correlation) and multivariate regression (with age, gender and MIS as covariates) were used. Data are expressed as regression coefficients and β–weights with a 95% confidence interval.

To evaluate FVS, DDS, QUANTIDD-E and QUANTIDD-I as a diagnostic tool for nutrient adequacy, sensitivity and specificity were calculated for MAR values ranging from 0.70 to 0.85, using various index cut-off points. Sensitivity was defined as nutrient inadequate diets with a MAR value below a cut-off point and below a dietary index cut-off point. Specificity was defined as nutrient adequate diets with a MAR and dietary index value above a certain cut-off point. An ideal diagnostic test would have a high specificity and sensitivity in order to correctly detect most nutritionally inadequate diets without sacrificing its ability to correctly identify adequate ones.

RESULTS

Sociodemographic characteristics

The four dietary pattern indices differed in their capacity to describe and differentiate subjects who ate a low variety or amount of food from those who ate more. When divided into tertiles, FVS and DDS decreased significantly with increasing age such that those above 60 years had an odds ratio (OR) of 5.2 and 2.7 of eating a low variety diet, respectively, when compared to those in the16-20 year range (**Table 4.1.**). This may explain differences for marital status where younger unmarried individuals had greater diet variety than their older married or widowed counterparts. Unlike FVS and DDS, the QUANTIDD values did not decrease with age, indicating that older subjects obtained the same proportion of energy and food intake from the various food groups as younger ones, albeit from a smaller variety of total foods.

Food variety increased as one moved from rural to urban settings, concomitant with an increase in socioeconomic status as represented by MIS. A similar trend was apparent when measuring the contribution of each food group to total energy or intake.

Females consumed a lower quantity of foods, as indicated by the significantly lower QUANTIDD-I score, although the variety of consumed foods equaled that of males. Religious differences had a significant effect on DDS (OR=0.3), QUANTIDD-E (OR=0.2) and QUANTIDD-I (OR=0.3) due to the exclusion of certain food groups by Seventh-day Adventists. Adherents abstained from food items within the meat, masticants, beverages, seafood and dairy groups.

Sociodemographic determinants of dietary diversity

Potential sociodemographic determinants for each dietary pattern index were assessed using stepwise multiple linear regression (**Table 4.2.**). Rural residence had the strongest negative influence on determining dietary diversity, explaining 11.7%, 41.2%, 34.1% and 6.3% of FVS, DDS, QUANTIDD-E and –I, respectively. Religion explained a significant portion of the variation for DDS and QUANTIDD-I, and along with widowhood, explained 66.5% and 29.3% of the model, respectively. Of the indices, DDS was the most strongly influenced by the sociodemographic variables considered here, while 70% of the variation for QUANTIDD-I was affected by other factors not included in the model.

Nutrient adequacy

Those in the highest tier of each dietary pattern index obtained significantly more energy and nutrients, both in absolute and energy-adjusted quantities, than those in the lowest tertile except for % energy from fat, saturated fat, fiber, calcium, and retinol equivalents (**Table 4.3.**). Increased dietary diversity was positively associated with nutrient adequacy for all nutrients except fiber and retinol equivalents, which had a negative correlation, and calcium, in which no association was observed except for QUANTIDD-I (**Table 4.4.**). A difference in QUANTIDD-E scores for the % E from fat between low and high variety groups indicated that the former derived their dietary fat energy from fewer food groups, a pattern that could not be measured with the food count indices FVS and DDS. This and the observation that saturated fat intake was higher

among the 1st tertile group without a concomitant rise in cholesterol, which would have typified increased animal product intake, suggested that coconuts were the responsible food. Indeed, the nut group was the only food group that was consumed in greater quantity by the low variety group (**Table 4.5.**).

Differences in QUANTIDD scores for calcium intake indicated that those with lower dietary diversity relied on fewer food groups to obtain comparable amounts to those with higher variety (**Table 4.3.**). Further analyses showed that mangrove bean, part of the 'starchy food' group eaten primarily by the rural sample, supplied up to half (47%) of the total calcium consumed by those in the low variety group (DDS) (data not shown). The starchy hypocotyl contains 144 mg calcium / 100g, with a nutrient density of 41 g / 100 kcal. Green leafy vegetables were also a significant contributor (23%). Despite this and irrespective of food diversity, calcium intake was insufficient to meet RDA recommendations for 94.5% of the sample population (**Table 4.4.**).

In addition to calcium, mangrove bean was also the primary source of fiber (23.1g / 100g) for the lowest DDS tier, providing 59% of total dietary fiber, versus 9% for those in the highest tier. Greater intake of refined carbohydrates, classified in the 'baked goods' group, was the likely reason why fiber was significantly lower in the higher variety group (**Table 4.5.**).

Similar to fiber, vitamin A was negatively associated with FVS and DDS, but this was not significant for both QUANTIDDs. This suggests that although the nutrient was obtained from a variety of food groups, the 1st tertile group consumed more choice vitamin A-rich foods from these groups. Analysis of individual foods showed that the 3rd DDS tertile group obtained 62% of their total dietary vitamin A from fortified rice, while the 1st tertile group obtained theirs from carotene-rich sweet potatoes (37%) and green leafy vegetables (10%), in addition to rice (45%) (data not shown).

Dietary diversity indices

Mean MAR was 0.83 for the total sample. Nutrients for which more than half of the population had inadequate intake were vitamin A (54.5%), riboflavin (59.2%), zinc (86.6%), and as mentioned, calcium (95.5%). All four indices were positively associated

with MAR, although DDS (r=0.70, p<0.001) and QUANTIDD-E (r=0.68, p<0.001) had higher correlation coefficients relative to FVS (r=0.50, p<0.001) and QUANTIDD-I (r=0.44, p<0.001).

A MAR of 1.0 was achieved in only 1.9% of the sample population, whereas 66.8% had a MAR of 0.90; 46.8% a MAR of 0.85; 37.3% a MAR of 0.80; 29.6% a MAR of 0.75; and 20% a MAR of 0.70. Using a MAR of 0.75 as a point of comparison, a FVS cut-off of 26 could correctly identify 90% of nutrient inadequate diets (sensitivity) while correctly identifying only 57% as adequate (specificity) (**Figure 4.1.**). A similar sensitivity (93%) and specificity (53%) resulted from using DDS with a cut-off of 11 (**Figure 4.2.**). A slightly better specificity (71%) resulted from using a QUANTIDD-E cut-off of 0.3 while keeping a similar sensitivity (91%) to that of DDS and FVS (**Figure 4.3.**). The index QUANTIDD-I had the weakest diagnostic potential if 0.5 was used as a cut-off, resulting in an ideal sensitivity of 94%, but a low specificity of 28% (**Figure 4.4.**). From these results, we can conclude that from the tested indices, QUANTIDD-E has the greatest potential as a diagnostic tool to identify inadequate diets.

Health/nutritional status outcome

Generally, those with a higher score for all dietary pattern indices tended to be heavier, taller, and wider, with more muscle mass and subcutaneous fat deposition than those with a lower score (**Table 4.6.**). Even when adjusted for age, gender and socioeconomic status, all indices were positive independent predictors of weight, height, BMI, tricep skinfold thickness and mid-upper arm circumference (**Table 4.7.**). The association with waist circumference and total percent body fat, however, was lost when adjusting for covariables. No difference in blood pressure was observed between groups in the highest and lowest tertile except for DDS which was the only index to display a significant negative correlation after adjustment, explaining 1.4% and 3.4% of the variation in systolic and diastolic blood pressure, respectively. Groups in the highest tertile of each index also had lower fasting blood glucose levels (**Table 4.4.**), indicating that increased variety is associated with glycemic control. However, after adjusting for cofactors, the relation was found to be due to the proportion of energy and total food

intake from each food group rather than the absolute number of individual food items or food groups consumed (**Table 4.7.**).

DISCUSSION

This study has highlighted the importance of energy and intake distribution across food groups as an indicator of nutrient adequacy and selected health status indicators. Of particular interest is the diagnostic power that QUANTIDD-E provided relative to FVS and DDS to correctly identify nutrient inadequate diets. The underlying presumption is that energy is related to nutrient intake, so measurement of energy distribution across food groups thereby makes a suitable proxy indicator of nutrient adequacy, especially if variety is high in groups such as fruits, vegetables and nuts. More importantly, the use of QUANTIDD as a diagnostic tool has greater application for comparability across cultures. The sensitivity-specificity analyses carried out by Hatløy et al. (1998) in Mali used a MAR cut-off point of 0.75 and found that a score of 23 and 6 for FVS and DDS, respectively, gave a sensitivity above 75% and a specificity above 29%. In their study, a theoretical maximum of 75 food items and 8 food groups resulted in a correlation coefficient of 0.33 and 0.39 for FVS and DDS, respectively, in respect to MAR. Our study categorized a theoretical maximum of 102 food items into 15 groups, resulting in a stronger association (r=0.496 and r=0.696 for FVS and DDS, respectively) with MAR, as well as a better sensitivity (> 90%) and specificity (> 53%) combination to identify the same MAR cut-off point.

Whether these findings were due to differences in methodology or sociocultural patterns is difficult to ascertain since both QUANTIDDs were influenced by the same variables as FVS and DDS. In most developing countries, the nutritional status of urban residents is usually better than that of rural due to a more diversified diet from market foods (Popkin, 2001; Clausen *et al.*, 2005). This was observed in our study, where rural residence was the primary determinant of dietary diversity and for QUANTIDD. Religion was another major variable, considering that Seventh-Day Adventism emphasizes health and diet by advocating vegetarianism. Consequently, several animal

product food groups were excluded, reducing DDS and QUANTIDD-I scores. The cardiovascular health benefits of SDA diets have been demonstrated in several countries (Fraser, 1988), and was associated with glycemic control in Papua New Guinea (King *et al.*, 1989), possibly due to higher intakes of fiber. Analysis of our results show that fiber intake was correlated with SDA religious adherence (r=0.46, p<0.001), but this did not translate into lower fasting blood glucose levels (r=0.12, p<0.027). This is supported by the observations of Hodge et al. (1996c) who did work in the same communities and proposed that the effect was more likely due to patterns of physical activity.

Age and SES are known powerful determinants of dietary diversity. Elderly persons in developing countries are particularly susceptible to nutritional deficiencies due to poverty, deprivation, poor access to health care and a poor quality diet (Charlton & Rose, 2001; Clausen *et al.*, 2005). In our study, the risk of a lower FVS and DDS score increased as one got older or less modernized. However, age and SES were not as significant as widowhood in explaining variation for all indices used except FVS. Representing only 6% of our sample population, widowers had significantly lower intakes of energy, protein, fat, vitamin A, vitamin E, and sodium (p<0.05) compared to married participants. Widowers also weighed less, were of shorter stature and had lower levels of physical activity. This finding supports existing evidence that changes in marital status have profound effects on diet and exercise (Lee *et al.*, 2004; Bennett, 2006).

Similar to previous studies (Ferguson *et al.*, 1993; Hatløy *et al.*, 1998; Ogle *et al.*, 2001), a strong correlation between dietary diversity and nutrient adequacy was demonstrated. This was also observed with the QUANTIDD indices, which themselves correlated strongly with FVS and DDS (**Table 4.4.**). When used alongside FVS and DDS, QUANTIDD score provided a fuller picture of dietary patterns. Examination of the QUANTIDD-E tertile scores for % energy from fat, saturated fat, and cholesterol lead to the conclusion that coconut was a major energy source for rural areas in which there was generally poorer dietary diversity. This has implications for cardiovascular health since coconut meat contains roughly 60 g saturated fat per 100 grams. Experimental and clinical studies have suggested that because of this, coconut consumption can cause hypercholesterolemia, hyperlipidemia and atherosclerosis, leading to CHD mortality

(Barr *et al.*, 1992; Kromhout *et al.*, 2000). Observational and population studies, however, generally report that such risk factors are uncommon among high coconut-consuming populations (Lindeberg & Lundh, 1993; Kumar, 1997; Lipeto *et al.*, 2004), and that the association between saturated fat intake and serum cholesterol is not significant (Samuelson *et al.*, 2001).

Relevant to this study, Aro et al. (1998) reported that dietary fatty acid composition, including saturated fat, did not seem to affect blood pressure. The only index associated with blood pressure in the present study was DDS, even after correcting for age, gender and SES. In a comparison of three dietary pattern indices, Kant and Graubard (2005) reported that DDS was a stronger predictor of blood pressure than HEI and RFS in Americans. A similar negative correlation between FVS and SBP was reported by Hsu-Hage and Wahlqvist (1996) in Melbourne Chinese immigrants. The affect of dietary diversity on blood pressure was eloquently demonstrated in the Dietary Approaches to Stop Hypertension (DASH) trial (Appel *et al.*, 1997). When two variants of the DASH diet were compared, the one that included greater variety from fruit, vegetables, and low-fat dairy products was more effective in reducing blood pressure than the diet emphasizing only fruits and vegetables.

Of note, vitamin A was negatively associated with FVS and DDS yet lacked a significant relationship with QUANTIDD. Several studies have found that increased food variety leads to improved vitamin A intake (Hsu-Hage & Wahlqvist, 1996; Hatløy *et al.*, 1998; Ogle *et al.*, 2001; Mirmiran *et al.*, 2004), while others found no association (Randall *et al.*, 1985; Rose *et al.*, 2002). In their attempt to develop a diet quality index for Mozambique, Rose et al. (2002) observed that the vitamin A content in food correlated inversely with energy, protein and other nutrients and that intake actually improved during periods of food scarcity. This was due to greater reliance on pumpkin squash, leaves and other fruits and vegetables when staple grains and beans were in short supply. A similar pattern in our population would mean greater variety within certain food groups concomitant with a decrease in others, resulting in no overall difference in QUANTIDD scores. Although FVS and DDS detected significant intake differences in our study, Randall et al. (Randall *et al.*, 1985) have stressed that the total number of foods

does not adequately reflect vitamin A intake, but rather the different mixture of foods providing that nutrient, and that nutrient density values would be a more appropriate measure. The authors also found this to be the case with calcium, a nutrient with an opposite pattern as that of vitamin A in our study; that is, no association with FVS and DDS but more so with QUANTIDD.

Inadequate calcium intake is a common observation in many countries (Hatløy *et al.*, 1998; Kim *et al.*, 2003b; Torheim *et al.*, 2004). In developing regions, this may be alleviated if wild foods were incorporated into traditional food systems. Wild greens and famine foods are often high in calcium and other nutrients (Freiberger *et al.*, 1998; Sena *et al.*, 1998) and may make important contributions to diet (Ogle *et al.*, 2001). In our study, wild mangrove bean was the major source of calcium for rural residents and provided 20% of their recommended requirements. Coastal Papua New Guineans are one of the few remaining populations in the world to rely on this common halophytic intertidal woody tree as a staple. The starchy hypocotyls are harvested from wild mangrove stands, boiled, peeled, sliced, soaked in salt water and boiled again to remove unpalatable tannins. The resulting product is a sustaining, bland pasta-like food that is flavoured with coconut cream. As the primary source of energy and fiber, this wild food clearly deserves greater scrutiny in future nutrition research.

Wild foods have received greater recognition as purveyors of nutrients and phytochemicals, and make essential contributions to the nutritional adequacy of many developing regions. Unfortunately, limited and uneven compositional data preclude their consideration in many dietary assessment studies (Grivetti & Ogle, 2000). In this study, we purposefully included spices, herbs, medicinal plants and condiments in a separate group in order to emphasize their nutritional contribution. Counting these as individual food items would give a falsely favourable impression of the quality of the diet by furnishing a high FVS score. In contrast, this had little effect on the DDS, which could explain why DDS, in our study and that of Hatloy et al. (1998), was a better predictor of MAR.

A similar argument could be made for including betelnut in its own food group. This decision was based on the finding that habitual consumption was associated with

increased weight, waist size, type 2 diabetes (Mannan *et al.*, 2000; Benjamin, 2001) and possibly cardiovascular disease (Trivedy *et al.*, 1999). Papua New Guineans are avid betelnut chewers, some consuming up to 50 nuts a day. This would have a significant impact on nutrition, given that each nut (5 g) provides roughly 70 kJ and 20 mg of calcium. Further analysis of our results support the finding that betelnut chewing was associated with weight (r=0.143, p<0.01), waist circumference (r=0.135, p<0.01) and energy intake (r=0.145, p<0.01), but not with fasting blood glucose concentrations (r=-0.031, p=0.557). Because the habit is practiced by virtually all Papua New Guineans, regardless of gender, SES or sector, there were no differences between the lowest and highest tertile for all dietary diversity indices examined here (**Table 4.5.**).

The role of dietary diversity in the nutritional transition of modernizing regions has implication for policy regarding body weight regulation and metabolic health. Animal and human studies confirm that food consumption increases when there is more variety in the diet, thus leading to increased body weight and body fat (Raynor & Epstein, 2001). Our research supports this finding, where weight, stature, BMI, waist circumference and tricep skinfold thickness were significantly higher in the third tertile relative to the first for all dietary pattern indices (**Table 4.6.**). With the exception of QUANTIDD-I, the indices were also independent predictors of % body fat (**Table 4.7.**). In concordance with previous studies (McCrory *et al.*, 1999; Ponce *et al.*, 2006), the major contributor to increased BMI and % body fat was the grain (r=0.286, p<0.001; r=0.121, p<0.05, respectively), refined carbohydrates (r=0.257, p<0.001; r=0.155, p<0.005) and edible oils groups (r=0.193, p<0.001; r=0.112, p<0.05). In a 10-country study, researchers found a positive association between dietary variety, household food expenditures per capita and household energy availability per capita (Hoddinott & Yohannes, 2002), indicating that households with limited incomes were trying to maximize energy per dollar spent (Kennedy, 2004), resulting in eating patterns that are higher in fat and processed carbohydrates. Such scenarios are why it is important to properly define a dietary recommendation statement encouraging increased variety to refer to specific food groups known to be nutrient dense and protective against noncommunicable disease. Increased fruit and vegetable consumption for example, has long been associated with reduced incidence of type 2 diabetes and CVD (Ford &

Mokdad, 2001; Bazzano *et al.*, 2002). In this study, the fruit group was the only group to have a negative association with both systolic (r=-0.105, p<0.05) and diastolic (r=-0.129, p<0.01) blood pressure.

Although QUANTIDD could be calculated for any nutrient, the decision to use energy and gram intake was to facilitate data collection and analysis. One of the most often mentioned advantages of using FVS and DDS is its simplicity for use in survey field conditions. Also, a straightforward count of foods consumed over a standard reference period is much less biased by dietary recall. QUANTIDD calculations require that quantitative data be included in dietary assessment questionnaires, so in this regard is not as simple as FVS or DDS, nor free from inaccurate reporting. The QFFQ used in this study was pre-validated for this population (Hodge *et al.*, 1996b) and employed at the individual level in a culture where meals are consumed from plates and not communal bowls. As a result, we believe our data to be a reliable assessment of true intake. In this population, portion sizes were fairly easily recorded and, in contrast to some micronutrients, the calorie content of foods was not difficult to find in food composition tables, making QUANTIDD calculations possible. One limitation to the QUANTIDD is that it quantifies the distribution of constituent food groups, and does not consider what the food groups are (Katanoda *et al.*, 2006). Much like FVS and DDS, greater insight into dietary patterns are achieved with the QUANTIDD if individual components within food groups are examined. Although the QUANTIDD provided greater diagnostic potential to identify nutrient adequate diets and other dietary trends not picked up by FVS or DDS, it should not be used independently, but rather as a component of multiple indices that more accurately capture the complexity of dietary diversity.

CONCLUSION

The QUANTIDD has practical applications for diet quality research that can be used alongside the more common indices FVS and DDS, especially in regions undergoing nutrition transition. The additional information obtained from the QUANTIDD scores facilitated the interpretation of patterns that were particular to this population.

Specifically, the QUANTIDD-E score for saturated fat and % energy from fat for low variety groups suggested that the source was a single food group or item, in this case, coconut. As a predictor of glycemic control, the QUANTIDD was superior to FVS and DDS in its relationship with fasting blood glucose levels. Most importantly, given that the QUANTIDD was meant to objectively summarize a quantitative aspect of dietary diversity, its strength as a diagnostic tool may allow cross-cultural comparisons.

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Table 4.1. Proportion of study population that fall into the lowest (1st) and highest (3rd) tertile of dietary pattern indices according to sociodemographic variables. Odds ratio (95% Confidence Interval) are calculated for the lowest tertile.

	n	Food Variety Score (%)			Dietary Diversity Score (%)			QUANTIDD-Energy (%)			QUANTIDD-Intake (%)		
		1st tertile	3rd tertile	OR (95% CI)	1st tertile	3rd tertile	OR (95% CI)	1st tertile	3rd tertile	OR (95% CI)	1st tertile	3rd tertile	OR (95% CI)
Age (years)													
<20[1]	58	22.4	32.8	1.0	25.9	34.5	1.0	37.9	31.0	1.0	32.8	36.2	1.0
20 to 40	135	27.4	36.3	1.2 [ns] (0.5-2.6)	31.9	38.5	1.1 [ns] (0.5-2.4)	25.9	37.8	0.6 [ns] (0.3-1.22)	23.7	39.2	0.6 [ns] (0.3-1.4)
41-60	113	39.8	23.0	2.5 [a] (1.1-5.7)	41.6	24.8	2.2 [a] (1.0-5.1)	35.4	31.0	1.0 [ns] (0.5-2.2)	40.7	31.0	1.5 [ns] (0.7-3.2)
>60	59	57.6	18.6	5.2 [d] (2.0-13.4)	54.2	27.1	2.7 [a] (1.1-6.6)	40.7	28.8	1.2 [ns] (0.5-2.9)	45.8	22.0	2.0 [ns] (0.8-4.9)
Gender													
Male	179	33.5	31.3	1.0	36.3	30.7	1.0	29.6	36.3	1.0	30.2	38.5	1.0
Female	186	37.1	26.9	1.3 [ns] (0.8-2.2)	38.7	32.8	1.0 [ns] (0.6-1.6)	36.6	31.2	1.4 [ns] (0.8-2.3)	36.6	70.4	1.6 [a] (1.0-2.7)
Modernity Index Score													
Highest[1]	130	23.8	43.1	1.0	20.8	57.7	1.0	14.6	48.5	1.0	30.8	36.9	1.0
Middle	108	27.8	32.4	1.5 [ns] (0.8-2.9)	25.0	38.0	1.5 [ns] (0.8-2.9)	30.6	64.8	2.8 [b] (1.4-5.6)	78.7	34.3	1.1 [ns] (0.6-2.1)
Lowest	126	54.0	11.1	7.1 [d] (3.7-13.8)	65.9	9.5	15.9 [d] (7.5-33.8)	54.8	15.1	12.0 [d] (5.9-24.7)	40.5	29.4	1.7 [a] (1.0-3.1)
Area of residence													
Urban[1]	119	16.0	55.5	1.0	14.3	63.0	1.0	12.6	48.7	1.0	31.9	37.8	1.0

	n	Food Variety Score (%)			Dietary Diversity Score (%)			QUANTIDD-Energy (%)			QUANTIDD-Intake (%)		
		1st tertile	3rd tertile	OR (95% CI)	1st tertile	3rd tertile	OR (95% CI)	1st tertile	3rd tertile	OR (95% CI)	1st tertile	3rd tertile	OR (95% CI)
Semi-rural	121	33.9	24.8	4.5 [d] (2.3-8.8)	15.7	33.1	2.1 [a] (1.0-4.5)	16.5	50.4	1.3 [ns] (0.6-2.8)	21.5	46.3	0.6 [ns] (0.3-1.1)
Rural	125	55.2	8.0	16.9 [d] (8.1-35.7)	80.8	0.8	445.0 [d] (58.0-3422.7)	68.8	1.6	166.7 [d] (37.3-767.3)	46.4	17.6	3.0 [c] (1.6-5.8)
Religion													
SDA	243	36.2	30.9	1.0	48.6	31.3	1.0	41.6	24.7	1.0	39.5	27.6	1.0
United	111	33.3	26.1	1.2 [ns] (0.7-2.0)	14.4	34.2	0.3 [d] (0.2-0.6)	17.1	51.4	0.2 [d] (0.1-0.4)	21.6	45.9	0.3 [d] (0.2-0.6)
Marital status													
Married[1]	286	38.1	27.6	1.0	42.7	29.4	1.0	36.0	31.8	1.0	35.3	31.5	1.0
Single	58	19.0	34.5	0.4 [a] (0.2-0.9)	12.1	41.4	0.2 [d] (0.1-0.5)	22.4	39.7	0.5 [a] (0.2-1.0)	20.7	46.6	0.4 [a] (0.2-0.8)
Widowed	21	42.9	23.8	1.1 [ns] (0.4-3.2)	38.1	38.1	0.7 [ns] (0.2-1.9)	23.8	33.3	0.5 [a] (0.2-1.7)	42.9	28.6	1.6 [ns] (0.5-5.0)

[a] $p<0.05$; [b] $p<0.005$; [c] $p<0.001$; [d] $p<0.0001$; [ns] non-significant; [1] Comparison group for odds ratio calculation

QUANTIDD: quantitative index for dietary diversity; Lowest tertile: FVS<21; DDS<9; QUANTIDD-E<0.22; QUANTIDD-I<0.34. Highest tertile: FVS>28; DDS>11; QUANTIDD-E>0.43; QUANTIDD-I>0.49.

Table 4.2. Relation between dietary pattern indices (dependent continuous variable) and explanatory sociodemographic independent predictors as assessed by stepwise linear regression.

Variables	$\beta \pm$ SE	95% CI	β weights	p
Food Variety Score				
Area of residence				
Semi-rural	-8.485 ± 1.153	-10.752 – -6.217	-0.394	<0.001
Rural	-11.568 ± 1.142	-13.813 – -9.323	-0.542	<0.001
Model R^2	0.482			
Dietary Diversity Score				
Area of residence				
Rural	-3.530 ± 0.220	-3.962 – -3.098	-0.743	<0.001
Religion	0.795 ± 0.219	0.364 – 1.226	0.166	<0.001
Marital status				
Widowed	-1.039 ± 0.385	-1.796 – -0.281	-0.107	0.007
Model R^2	0.665			
QUANTIDD-Energy				
Area of residence				
Rural	-0.274 ± 0.020	-0.312 – -0.235	-0.600	<0.001
Marital status				
Widowed	-0.097 ± 0.040	-0.175 – -0.018	-0.104	0.016
Model R^2	0.593			
QUANTIDD-Intake				
Area of residence				
Rural	-0.077 ± 0.022	-0.121 – -0.033	-0.203	0.001
Religion	-0.048 ± 0.022	-0.092 – -0.004	-0.126	0.033
Marital status				
Widowed	-0.079 ± 0.039	-0.156 – -0.002	-0.103	0.045
Model R^2	0.293			

Variables entered in stepwise multiple linear regression: Continuous: age, modernity index score Dichotomous: gender (male=0, female=1); religion (United church=0, Seventh Day Adventist=1); area of residence (urban=reference category), marital status (married=reference category)

Table 4.3. Comparison of mean absolute (mean ± SD) and energy-adjusted (mean ± SE) daily nutrient intake in those within the lowest (1st) and highest (3rd) tertile of diversity indices.

Nutrient	Food Variety Score		Dietary Diversity Score		QUANTIDD-Energy		QUANTIDD-Intake	
	1st tertile	3rd tertile	1st tertile	3rd tertile	1st tertile	3rd tertile	1st tertile	3rd tertile
Energy (kcal/d)	2250.2 ± 45.5	2718.6 ± 42.5[d]	2225.6 ± 37.9	2714.3 ± 44.0[d]	2262.9 ± 41.6	2660.2 ± 47.3[d]	2350.3 ± 48.2	2604.8 ± 42.7[d]
CHO (g)	373.4 ± 101.1	435.2 ± 83.9[d]	367.2 ± 90.0	431.7 ± 84.3[d]	380.6 ± 93.5	419.8 ± 90.2[d]	390.3 ± 96.0	414.6 ± 86.3[a]
CHO (%E)	65.6 ± 0.7	63.1 ± 0.6[a]	65.2 ± 0.6	62.6 ± 0.62[b]	66.6 ± 0.7	62.2 ± 0.6[c]	65.9 ± 0.6	62.6 ± 0.6[d]
Fiber (g)	56.2 ± 32.7	39.5 ± 18.3[d]	66.4 ± 30.1	32.9 ± 11.6[d]	66.6 ± 31.1	33.8 ± 12.2[d]	54.2 ± 33.8	43.1 ± 19.7[b]
/1000 kcal	25.8 ± 1.3	14.9 ± 0.7[d]	30.4 ± 1.1	12.2 ± 0.4[d]	30.4 ± 1.2	12.8 ± 0.4[d]	24.1 ± 1.4	17.1 ± 0.8[b]
Protein (g)	53.3 ± 19.7	75.2 ± 21.0[d]	53.6 ± 16.4	73.8 ± 21.7[d]	52.8 ± 14.0	73.6 ± 25.4[d]	54.2 ± 17.1	72.5 ± 23.3[d]
Protein (%E)	9.3 ± 0.2	10.9 ± 0.2[d]	9.5 ± 0.2	10.7 ± 0.2[d]	9.3 ± 0.2	10.8 ± 0.3[d]	9.1 ± 0.2	11.0 ± 0.3[d]
Fat (g)	63.2 ± 24.0	78.8 ± 22.3[d]	63.1 ± 21.8	81.0 ± 22.2[d]	61.5 ± 23.4	79.6 ± 21.7[d]	66.6 ± 25.7	76.4 ± 20.9[c]
Fat (%E)	25.0 ± 0.7	25.6 ± 0.5[ns]	25.2 ± 0.6	26.4 ± 0.5 [ns]	24.1 ± 0.7	26.6 ± 0.5[b]	24.9 ± 0.6	26.1 ± 0.5[ns]
Saturated Fat (g)	34.4 ± 20.8	28.8 ± 16.7[a]	36.9 ± 20.4	27.7 ± 16.9[d]	34.3 ± 20.4	31.1 ± 17.6[ns]	32.1 ± 20.8	31.7 ± 17.8[ns]
/1000 kcal	15.6 ± 0.8	10.8 ± 0.6[d]	16.9 ± 0.8	10.4 ± 0.6[d]	15.5 ± 0.81	12.0 ± 0.7[c]	13.9 ± 0.8	12.6 ± 0.7[d]
Cholesterol (mg)	50.1 ± 41.2	119.9 ± 86.7[d]	53.5 ± 47.5	113.5 ± 82.5[d]	47.7 ± 32.8	115.1 ± 92.0[d]	31.7 ± 17.7	104.2 ± 82.5[d]
/1000 kcal	22.2 ± 1.6	43.7 ± 2.5[d]	23.9 ± 1.7	41.4 ± 2.6[d]	21.3 ± 1.3	42.0 ± 2.7[d]	22.7 ± 1.9	39.3 ± 2.5[d]
Calcium (mg)	585.2 ± 339.1	622.4 ± 249.4[ns]	597.2 ± 221.6	571.2 ± 244.1[ns]	610.0 ± 224.8	671.7 ± 422.4[ns]	546.8 ± 219.7	697.6 ± 409.1[d]
/1000 kcal	261.9 ± 10.6	229 ± 7.3[a]	273.6 ± 8.8	209.6 ± 7.4[d]	275.9 ± 9.3	250.3 ± 12.8[ns]	237.9 ± 8.2	265.8 ± 12.3[ns]

Nutrient	Food Variety Score		Dietary Diversity Score		QUANTIDD-Energy		QUANTIDD-Intake	
	1st tertile	3rd tertile	1st tertile	3rd tertile	1st tertile	3rd tertile	1st tertile	3rd tertile
Iron (mg)	14.9 ± 6.3	20.8 ± 5.5[d]	13.8 ± 4.4	20.9 ± 4.8[d]	14.1 ± 5.2	21.1 ± 6.3[d]	15.7 ± 6.1	20.3 ± 6.3[d]
/1000 kcal	6.5 ± 0.2	7.6 ± 0.1[d]	6.2 ± 0.1	7.6 ± 0.1[d]	6.2 ± 0.1	7.9 ± 0.1[d]	6.6 ± 0.1	7.7 ± 0.1[d]
Zinc (mg)	4.6 ± 2.0	7.6 ± 2.5[d]	4.1 ± 1.7	7.7 ± 1.9[d]	4.2 ± 1.9	7.8 ± 2.4[d]	5.0 ± 2.3	7.1 ± 2.5[d]
/1000 kcal	2.0 ± 0.1	2.8 ± 0.1[d]	1.8 ± 0.1	2.8 ± 0.1[d]	1.8 ± 0.1	2.9 ± 0.1[d]	2.1 ± 0.1	2.7 ± 0.1[d]
Vitamin A (μg RE)	1036.8 ± 818.8	740.0 ± 553.1[b]	973.8 ± 806.3	802.1 ± 588.9[a]	958.1 ± 756.5	921.1 ± 908.2[ns]	976.4 ± 789.6	964.6 ± 909.7[ns]
/1000 kcal	460.8 ± 140.5	272.2 ± 101.4[d]	437.5 ± 116.5	295.5 ± 102.2[d]	423.4 ± 140.8	346.25 ± 99.6[d]	415.4 ± 145.8	370.8 ± 112.1[d]
Thiamin (mg)	0.94 ± 0.59	1.59 ± 0.55[d]	0.78 ±0.42	1.65 ± 0.45[d]	0.82 ± 0.52	1.64 ± 0.53[d]	1.00 ± 0.57	1.56 ± 0.62[d]
/1000 kcal	0.41 ± 0.02	0.58 ± 0.01[d]	0.35 ± 0.01	0.61 ± 0.01[d]	0.35 ± 0.02	0.61 ± 0.01[d]	0.41 ± 0.02	0.59 ± 0.02[d]
Riboflavin (mg)	0.81 ± 0.57	1.60 ± 0.55[d]	0.65 ± 0.35	1.46 ± 0.48[d]	0.68 ± 0.41	1.56 ± 0.59[d]	0.82 ± 0.49	1.46 ± 0.65[d]
/1000 kcal	0.35 ± 0.02	0.53 ± 0.01[d]	0.29 ± 0.01	0.54 ± 0.01[d]	0.29 ± 0.01	0.58 ± 0.01[d]	0.34 ± 0.02	0.55 ± 0.02 [d]
Niacin (mg)	12.8 ± 6.8	23.1 ± 7.4[d]	11.5 ± 5.4	23.5 ± 6.7[d]	11.7 ± 5.5	23.3 ± 7.7[d]	13.6 ± 6.7	22.3 ± 8.2[d]
/1000 kcal	5.6 ± 0.2	8.5 ± 0.2[d]	5.1 ± 0.2	8.7 ± 0.2[d]	5.1 ± 0.17	8.7 ± 0.2[d]	5.6 ± 0.2	8.5 ± 0.2[d]
Folate (DFE mg)	400.4 ± 364.9	669.2 ± 324.7[d]	323.2 ± 320.7	735.2 ± 301.5[d]	356.2 ± 405.5	627.3 ± 260.9[d]	504.7 ± 436.3	561.4 ± 291.7[ns]
/1000 kcal	170.1 ± 12.1	240.9 ± 8.9[d]	139.9 ± 10.7	267.8 ± 8.8[d]	147.6 ± 13.9	232.2 ± 6.7[d]	204.4 ±14.7	209.7 ± 8.3[ns]
Vitamin C (mg)	127.7 ± 131.5	198.5 ± 158.4[d]	110.1 ± 76.9	199.9 ± 170.3[d]	118.2 ± 103.3	235.8 ± 180.0[d]	108.0 ± 81.2	247.7 ± 201.6[d]
/1000 kcal	56.1 ± 4.5	73.8 ± 5.5[a]	49.9 ± 2.9	75.1 ± 6.3[d]	52.18 ± 3.7	88.9 ± 5.9[d]	46.4 ±2.9	93.7 ± 6.9[d]

[a]$p<0.05$; [b]$p<0.005$; [c]$p<0.001$; [d]$p<0.0001$;[ns]non-significant. FVS: food variety score; DDS: dietary diversity score; QUANTIDD-E: quantitative index for dietary diversity based on energy contribution; QUANTIDD-I: index based on intake contribution in grams. Lowest tertile: FVS<21; DDS<9; QUANTIDD-E<0.22; QUANTIDD-I<0.34. Highest tertile: FVS>28; DDS>11; QUANTIDD-E>0.43; QUANTIDD-I>0.49

Table 4.4. Prevalence of inadequate intake of nutrients and Pearson's correlations between continuous dietary pattern variables and individual nutrient adequacy ratios and the mean adequacy ratio

Nutrient Adequacy Ratio			Food Variety Score		Dietary Diversity Score		QUANTIDD-Energy		QUANTIDD-Intake	
	Median NAR	Proportion with intake below RDA	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Total energy	1.09	29.6%	0.356	<0.001	0.393	<0.001	0.243	<0.001	0.104	0.024
Protein	1.07	41.4%	0.432	<0.001	0.409	<0.001	0.347	<0.001	0.231	<0.001
Fiber	1.77	18.1%	-0.175	<0.001	-0.511	<0.001	-0.507	<0.001	-0.193	<0.001
Vitamin A	0.93	54.5%	-0.119	0.011	-0.099	0.029	-0.020	0.351	-0.044	0.201
Thiamin	1.10	43.8%	0.454	<0.001	0.633	<0.001	0.558	<0.001	0.354	<0.001
Riboflavin	0.86	59.2%	0.482	<0.001	0.616	<0.001	0.595	<0.001	0.409	<0.001
Niacin	1.10	41.9%	0.532	<0.001	0.653	<0.001	0.599	<0.001	0.391	<0.001
Folate	1.20	43.8%	0.232	<0.001	0.485	<0.001	0.290	<0.001	0.025	0.316
Vitamin C	2.78	9.3%	0.188	<0.001	0.272	<0.001	0.316	<0.001	0.337	<0.001
Calcium	0.53	94.5%	0.057	0.139	0.014	0.397	0.078	0.070	0.191	<0.001
Iron	0.58	20.0%	0.151	0.002	0.300	<0.001	0.335	<0.001	0.179	<0.001
Zinc	0.51	86.6%	0.268	<0.001	0.404	<0.001	0.458	<0.001	0.223	<0.001
MAR	0.86	98.1%	0.496	<0.001	0.696	<0.001	0.681	<0.001	0.438	<0.001
FVS			-	-	0.729	<0.001	0.549	<0.001	0.503	<0.001
DDS			-	-	-	-	0.654	<0.001	0.502	<0.001
QUANTIDD-E			-	-	-	-	-	-	0.783	<0.001

QUANTIDD-Energy: quantitative index for dietary diversity based on energy contribution; QUANTIDD-Intake: index based on intake contribution in grams.

Table 4.5. Comparison of mean ± SD daily intake (g) of food groups between those in the lowest (1st) and highest (3rd) tertile of dietary pattern indices.

	Food Variety Score		Dietary Diversity Score		QUANTIDD-Energy		QUANTIDD-Intake	
	1st tertile	3rd tertile	1st tertile	3rd tertile	1st tertile	3rd tertile	1st tertile	3rd tertile
Grains	364 ± 36	527 ± 30 [d]	281 ± 31	602 ± 30 [d]	321 ± 44	473 ± 24 [c]	486 ± 45	408 ± 25 [ns]
Baked goods	84 ± 11	143 ± 10 [d]	57 ± 7	145 ± 8 [d]	63 ± 11	138 ± 7 [d]	85 ± 10	134 ± 10 [d]
Starchy foods	497 ± 23	506 ± 21 [ns]	489 ± 19	471 ± 23 [ns]	515 ± 27	497 ± 18 [ns]	504 ± 30	488 ± 17 [ns]
Legume	40 ± 14	27 ± 3 [ns]	30 ± 9	23 ± 3 [ns]	30 ± 10	33 ± 6 [ns]	28 ± 10	33 ± 5 [ns]
Vegetables	88 ± 14	149 ± 11 [d]	77 ± 5	130 ± 12 [d]	81 ± 6	159 ± 16 [d]	73 ± 6	174 ± 16 [d]
Fruit	111 ± 13	177 ± 14 [d]	94 ± 9	187 ± 16 [d]	91 ± 11	205 ± 17 [d]	83 ± 8	217 ± 16 [d]
Nuts	165 ± 8 [b]	130 ± 10	176 ± 8 [d]	111 ± 9	161 ± 8	145 ± 10 [ns]	142 ± 9	167 ± 11 [ns]
Herbs & spices	3 ± 0	8 ± 1 [d]	4 ± 1	7 ± 1 [a]	5 ± 1	8 ± 1 [ns]	4 ± 1	7 ± 1 [b]
Masticants	37 ± 17	29 ± 5 [ns]	46 ± 32	21 ± 3 [ns]	11 ± 2	40 ± 9 [ns]	19 ± 4	37 ± 10 [ns]
Beverages	471 ± 54	502 ± 39 [ns]	265 ± 49	483 ± 37 [d]	299 ± 50	598 ± 39 [d]	433 ± 70	468 ± 28 [ns]
Meat	28 ± 6	67 ± 9 [d]	41 ± 8	59 ± 9 [ns]	29 ± 6	72 ± 11 [d]	39 ± 6	64 ± 12 [ns]
Fish	78 ± 5	96 ± 5 [a]	82 ± 5	89 ± 5 [ns]	72 ± 4	100 ± 6 [d]	69 ± 5	99 ± 6 [d]
Sweets	19 ± 2	20 ± 3 [ns]	12 ± 2	22 ± 3 [ns]	18 ± 7	24 ± 3 [ns]	20 ± 5	21 ± 3 [ns]
Fats & oils	4 ± 1	4 ± 1 [ns]	4 ± 1	3 ± 0 [ns]	1 ± 0	5 ± 1 [ns]	3 ± 1	4 ± 1 [ns]
Dairy	13 ± 4	22 ± 3 [ns]	10 ± 3	22 ± 3 [ns]	10 ± 2	25 ± 4 [ns]	12 ± 2	22 ± 5 [ns]

[a] $p< 0.05$; [b] $p<0.01$; [c] $p<0.005$; [d] $p<0.001$; QUANTIDD-Energy: quantitative index for dietary diversity based on energy contribution; QUANTIDD-Intake: index based on intake contribution in grams.

Table 4.6. Comparison of mean daily intake of food groups between those in the lowest and highest tertile of food variety and dietary diversity scores.

	Food Variety Score		Dietary Diversity Score		QUANTIDD-Energy		QUANTIDD-Intake	
Health outcomes	1st tertile	3rd tertile	1st tertile	3rd tertile	1st tertile	3rd tertile	1st tertile	3rd tertile
Weight (kg)	56.1 ± 12.5	65.6 ± 17.3[d]	54.2 ± 10.6	64.5 ± 18.0[d]	54.2 ± 10.6	64.5 ± 18.0[d]	56.4 ± 12.8	64.0 ± 1.5[d]
Stature (m)	1.58 ± 0.09	1.61 ± 0.09[b]	1.57 ± 0.08	1.61 ± 0.09[d]	1.57 ± 0.08	1.61 ± 0.09[d]	1.58 ± 0.08	1.62 ± 0.09[c]
BMI (kg/m^2)	22.3 ± 3.8	25.2 ± 5.6[d]	21.8 ± 3.3	24.6 ± 5.6[d]	21.8 ± 3.3	24.6 ± 5.6[d]	22.4 ± 4.0	24.4 ± 5.3[c]
Waist circumference	82.1 ± 11.5	86.0 ± 14.4[a]	79.8 ± 10.0	86.0 ± 14.7[d]	79.8 ± 10.0	86.0 ± 14.7[d]	81.5 ± 11.5	85.0 ± 14.3[a]
Waist-to-hip ratio	0.92 ± 0.11	0.90 ± 0.10	0.91 ± 0.11	0.92 ± 0.11	0.91 ± 0.11	0.92 ± 0.11)	0.91 ± 0.11	0.91 ± 0.10
Tricep skinfold (mm)	13.2 ± 6.2	17.4 ± 9.4[d]	12.6 ± 6.3	16.5 ± 8.8[d]	12.6 ± 6.3	16.5 ± 8.8[d]	13.6 ± 6.7	15.9 ± 8.9[a]
Body fat (%)	26.5 ± 8.3	28.2 ± 10.2	25.7 ± 8.6	28.0 ± 9.5	25.7 ± 8.6	28.0 ± 9.5	26.9 ± 8.7	26.6 ± 9.9
MUAC (cm)	26.1 ± 3.4	28.0 ± 4.3[d]	25.5 ± 3.2	27.9 ± 4.3[d]	25.5 ± 3.2	27.9 ± 4.3[d]	25.9 ± 3.5	27.9 ± 4.1[d]
SBP (mm Hg)	131.0 ± 16.6	127.3 ± 17.3	131.3 ± 18.8	129.2 ± 16.4	131.3 ± 18.8	129.2 ± 16.4	131.7 ± 19.8	128.2 ± 15.0
DBP (mmHg)	80.4 ± 9.4	77.9 ± 12.0	79.5 ± 10.2	78.9 ± 11.9	79.5 ± 10.2	78.9 ± 11.9	80.1 ± 10.8	78.8 ± 11.4
Fasting glucose (mmol/L)	4.3 ± 2.8	3.7 ± 1.7	4.4 ± 3.2	3.7 ± 1.6[a]	4.4 ± 3.2	3.7 ± 1.6[a]	4.4 ± 3.2	3.6 ± 0.9[b]

[a] $p< 0.05$; [b] $p<0.01$; [c] $p<0.005$; [d] $p<0.001$; [ns]non-significant; QUANTIDD-Energy: quantitative index for dietary diversity based on energy contribution; QUANTIDD-Intake: index based on intake contribution in grams.

Table 4.7. Unadjusted and adjusted correlations between continuous dietary pattern variables and anthropometric and health parameters

	Unadjusted[a]		Adjusted[b]			
Health parameters	*r*	*p*	$\beta \pm$ SE	*p*	95% CI	Model R^{2c}
Weight (kg)						
FVS	0.231	<0.001	0.171 ± 0.071	0.017*	0.031 – 0.311	0.284
DDS	0.309	<0.001	0.924 ± 0.338	0.007*	0.259 – 1.589	0.287
QUANTIDD-E	0.304	<0.001	9.729 ± 3.435	0.005*	2.975 – 16.484	0.288
QUANTIDD- I	0.198	<0.001	11.888 ± 3.842	0.002*	4.332 – 19.443	0.291
Stature (m)						
FVS	0.023	0.328	0.000 ± 0.000	0.664	-0.001 – 0.001	0.374
DDS	0.207	<0.001	0.006 ± 0.002	0.001*	0.002 – 0.009	0.391
QUANTIDD-E	0.222	<0.001	0.052 ± 0.018	0.004*	0.016 – 0.087	0.387
QUANTIDD- I	0.177	<0.001	0.056 ± 0.020	0.006*	0.016 – 0.096	0.387
BMI (kg/m^2)						
FVS	0.278	<0.001	0.075 ± 0.023	0.001*	0.029 – 0.121	0.226
DDS	0.279	<0.001	0.219 ± 0.112	0.050	-0.001 – 0.440	0.212
QUANTIDD-E	0.263	<0.001	2.427 ± 1.139	0.034	0.187 – 4.667	0.214
QUANTIDD- I	0.158	0.001	3.188 ± 1.274	0.013	0.683 – 5.693	0.218
Waist circumference (cm)						
FVS	0.116	0.014	0.002 ± 0.064	0.978	-0.124 – 0.128	0.154
DDS	0.163	0.001	-0.038 ± 0.304	0.902	-1.789 – -0.087	0.154
QUANTIDD-E	0.195	<0.001	3.158 ± 3.090	0.307	-2.919 – 9.236	0.156
QUANTIDD- I	0.114	0.015	5.291 ± 3.458	0.127	-1.509 – 12.091	0.159
Waist-to-hip ratio						
FVS	-0.14	0.004	-0.002 ± 0.001	0.001*	-0.003 – -0.001	0.087
DDS	-0.108	0.020	-0.008 ± 0.002	0.001*	-0.013 – -0.003	0.087
QUANTIDD-E	-0.016	0.384	-0.037 ± 0.025	0.145	-0.086 – 0.013	0.064
QUANTIDD- I	0.012	0.412	-0.008 ± 0.028	0.767	-0.064 – 0.047	0.059
Tricep skinfold (mm)						
FVS	0.274	<0.001	0.124 ± 0.035	<0.001*	0.056 – 0.192	0.351
DDS	0.304	<0.001	0.600 ± 0.165	<0.001*	0.275 – 0.926	0.351
QUANTIDD-E	0.221	<0.001	4.694 ± 1.694	0.006*	1.361 – 8.026	0.342
QUANTIDD- I	0.117	0.013	5.176 ± 1.900	0.007*	1.440 – 8.913	0.341

Health parameters	Unadjusted[a]		Adjusted[b]			
	r	*p*	$\beta \pm$ SE	*p*	95% CI	Model R^{2c}
Body fat (%)						
FVS	0.155	0.002	0.037 ± 0.035	0.286	-0.031 – 0.105	0.521
DDS	0.140	0.004	0.015 ± 0.165	0.927	-0.309 – 0.339	0.519
QUANTIDD-E	0.092	0.040	0.873 ± 1.675	0.603	-2.422 – 4.168	0.520
QUANTIDD-I	0.007	0.450	1.423 ± 1.875	0.448	-2.265 – 5.112	0.520
Mid-upper arm circumference (cm)						
FVS	0.212	<0.001	0.042 ± 0.019	0.031	0.004 – 0.080	0.197
DDS	0.285	<0.001	0.242 ± 0.092	0.009*	0.060 – 0.423	0.201
QUANTIDD-E	0.269	<0.001	2.348 ± 0.939	0.013*	0.502 – 4.194	0.200
QUANTIDD-I	0.204	<0.001	3.482 ± 1.045	0.001*	1.427 – 5.538	0.210
Systolic blood pressure (mm Hg)						
FVS	-0.059	0.130	-0.107 ± 0.091	0.243	-0.287 – 0.073	0.005
DDS	-0.104	0.024	-0.938 ± 0.433	0.031*	-1.789 – -0.087	0.014
QUANTIDD-E	-0.070	0.092	-6.102 ± 4.417	0.168	-14.789 – 2.586	0.006
QUANTIDD-I	-0.058	0.136	-5.278 ± 4.957	0.288	-15.027 – 4.470	0.004
Diastolic blood pressure (mmHg)						
FVS	-0.011	0.414	-0.047 ± 0.058	0.421	-0.160 – 0.067	0.011
DDS	-0.098	0.030	-0.817 ± 0.272	0.003*	-1.352 – -0.282	0.034
QUANTIDD-E	-0.024	0.321	-3.508 ± 2.793	0.210	-9.001 – 1.986	0.014
QUANTIDD-I	-0.045	0.198	-3.194 ± 3.133	0.309	-9.356 – 2.968	0.013
Fasting glucose (mmol/L)						
FVS	-0.057	0.139	-0.014 ± 0.012	0.273	-0.038 – 0.110	0.014
DDS	-0.087	0.049	-0.111 ± 0.058	0.057	-0.226 – 0.004	0.021
QUANTIDD-E	-0.125	0.009	-1.663 ± 0.590	0.005*	-2.824 – -0.502	0.032
QUANTIDD-I	-0.153	0.002	-2.070 ± 0.660	0.002*	-3.368 – -0.772	0.037

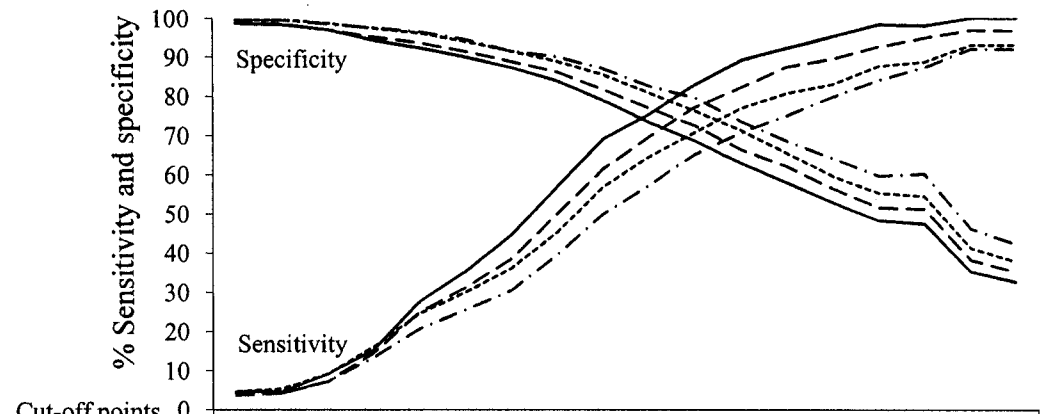
[a]Pearson's correlation

[b]Adjusted for age, gender and modernity index score (MIS).

[c]Proportion of variation due to multivariate linear regression that is explained by age, gender, modernity index score and the various dietary pattern variables

* Correlation still significant when energy expenditure included in model.

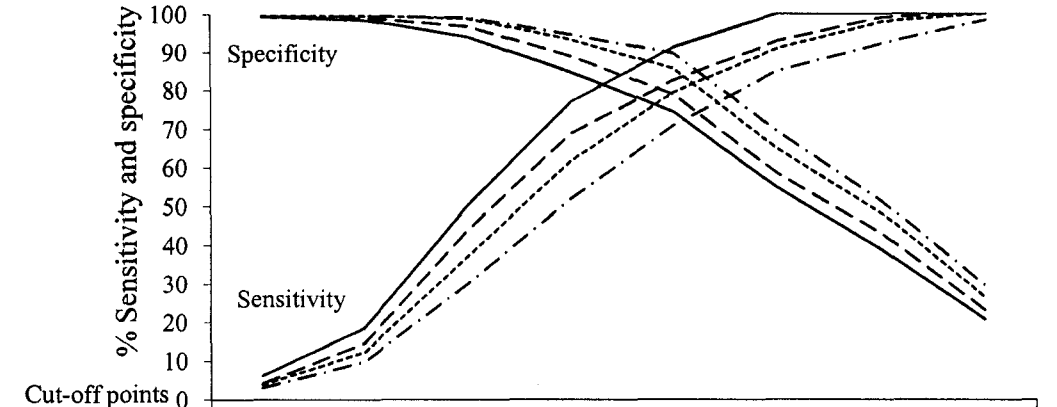
FVS: Food Variety Score; DDS: Dietary Diversity Score; QUANTIDD-Energy: quantitative index for dietary diversity based on energy contribution; QUANTIDD-Intake: index based on intake contribution in grams.



	Cut-off points for FVS	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Sensitivity	MAR 0.70	5	5	9	15	28	35	45	57	69	75	83	89	92	95	98	98	100	100
	MAR 0.75	4	4	7	15	25	31	39	50	61	70	77	82	88	90	93	95	97	97
	MAR 0.80	5	5	9	16	25	30	36	45	57	65	71	77	81	83	88	89	93	93
	MAR 0.85	4	4	7	13	21	26	30	40	50	57	65	71	75	80	84	87	92	92
Specificity	MAR 0.70	99	98	97	94	92	90	87	84	79	73	69	63	58	53	48	47	35	33
	MAR 0.75	99	99	97	95	94	91	89	86	82	77	72	67	62	57	52	51	38	35
	MAR 0.80	100	100	99	97	96	94	91	89	86	81	76	71	66	60	55	55	41	38
	MAR 0.85	100	100	99	98	97	95	92	90	87	83	80	74	69	64	60	60	46	42

% Sensitivity and specificity for various cut-off points of FVS

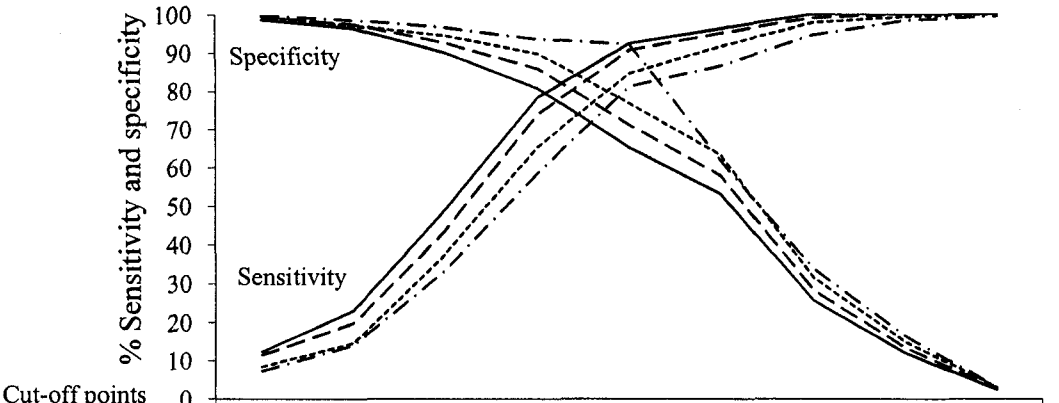
Figure 4.1. Sensitivity and specificity of different cut-offs for FVS with MAR changing from 0.70 to 0.85. Sensitivity = identify nutritionally inadequate diets as inadequate; Specificity = identify nutritionally adequate diets as adequate.



	Cut-off points for DDS	6	7	8	9	10	11	12	13
Sensitivity	MAR 0.70	6	18	50	77	91	100	100	100
	MAR 0.75	4	15	44	69	82	93	99	100
	MAR 0.80	4	12	37	62	79	91	98	100
	MAR 0.85	3	10	30	52	71	85	92	98
Specificity	MAR 0.70	99	98	94	85	74	55	39	21
	MAR 0.75	99	99	97	89	79	58	43	23
	MAR 0.80	100	100	99	93	86	65	48	26
	MAR 0.85	100	100	99	95	90	69	51	29

% Sensitivity and specificity for various cut-off points of DDS

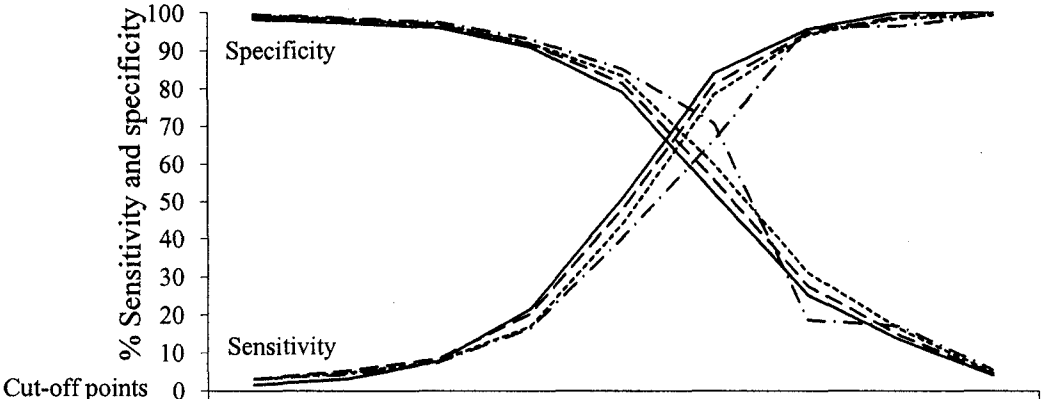
Figure 4.2. Sensitivity and specificity of different cut-offs for DDS with MAR changing from 0.70 to 0.85. Sensitivity = identify nutritionally inadequate diets as inadequate; Specificity = identify nutritionally adequate diets as adequate.



	Cut-off points for QUANTIDD-E	-0.1	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Sensitivity	MAR 0.70	12	23	49	78	92	96	100	100	100
	MAR 0.75	11	20	44	74	91	95	99	100	100
	MAR 0.80	8	15	38	65	85	92	98	99	100
	MAR 0.85	7	14	34	59	81	87	95	98	99
Specificity	MAR 0.70	99	96	90	81	65	53	26	12	2
	MAR 0.75	100	97	93	86	71	58	28	13	3
	MAR 0.80	100	97	94	90	77	63	31	15	3
	MAR 0.85	100	99	97	94	92	62	34	16	3

% Sensitivity and specificity for various cut-off points of QUANTIDD-E

Figure 4.3. Sensitivity and specificity of different cut-offs for QUANTIDD-E with MAR changing from 0.70 to 0.85. Sensitivity = identify nutritionally inadequate diets as inadequate; Specificity = identify nutritionally adequate diets as adequate.



Cut-off points for QUANTIDD-I		-0.1	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Sensitivity	MAR 0.70	2	3	8	22	51	84	95	100	100
	MAR 0.75	3	5	8	20	48	81	94	99	100
	MAR 0.80	3	5	8	17	44	78	94	98	99
	MAR 0.85	3	4	7	16	40	66	96	96	99
Specificity	MAR 0.70	98	97	96	91	79	52	25	13	4
	MAR 0.75	99	98	97	92	81	56	28	14	4
	MAR 0.80	99	98	97	92	83	60	31	16	5
	MAR 0.85	100	99	98	93	85	71	19	17	5

% Sensitivity and specificity for various cut-off points of QUANTIDD-I

Figure 4.4. Sensitivity and specificity of different cut-offs for QUANTIDD-I with MAR changing from 0.70 to 0.85. Sensitivity = identify nutritionally inadequate diets as inadequate; Specificity = identify nutritionally adequate diets as adequate.

BRIDGE 2

Despite the well-recognized importance of dietary diversity, there is still a lack of consensus on how it should be conceptualized, operationalized and measured, particularly in developing countries (Ruel, 2003). The previous manuscript makes a significant contribution to this field by incorporating and evaluating the QUANTIDD and validating its use as a predictor of nutrient adequacy. The next step is to consider dietary diversity in terms of functionality.

The driving hypothesis of this dissertation is that dietary diversity enables greater exposure to bioactive elements which in turn reduce NCD risk. To test this, a quantitative measure of dietary functionality was required. The phytochemical index (PI), proposed by McCarty (2004) was the only measure, to our knowledge, that had been developed for this purpose. The PI is simply the proportion (%) of dietary calories derived from foods rich in phytochemicals. The index suffers from some evident weaknesses, most notably the assumption of a relationship between phytochemical composition and calories. Phytochemical-rich items such as teas, masticants, spices and condiments that contribute little energy are not accounted for. Moreover, the utility of some phytochemicals over others towards specific pathologies is not considered.

The dietary functionality index (DFI) was conceived to address these limitations and provide a score that is evidence-based. The following manuscript describes theory and calculations, and assesses its association with NCD risk proxy-indicators. Importantly, it emphasizes the concept that long term health outcome is not due to the functional properties of one particular food or nutrient, but rather the synergisms between diverse whole and plant-based foods.

CHAPTER 5
MANUSCRIPT 3

THE "DIETARY FUNCTIONALITY INDEX":

A NEW QUANTITATIVE
MEASURE OF FUNCTIONAL FOOD INTAKE AND ITS APPLICATION TO COASTAL
PAPUA NEW GUINEAN METABOLIC HEALTH

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ABSTRACT

A dietary functionality index (DFI), developed as a composite quantitative indicator of intake of foods that have a positive effect on health maintenance and chronic disease prevention beyond their role as simple purveyors of nutrients, is calculated from the mean sum of scores allotted for each food item that had displayed evidence-based pharmacological activity. Two variations of the DFI, one expressed as the ratio of functional food items to total food variety (DFIv) and the other as weight (DFIw), were tested in a coastal region of Papua New Guinea comprised of an urban, semi-rural and rural village (n=365) in order to explore sociodemographic differences in functional food intake, and possible associations with obesity, blood pressure and fasting blood glucose concentration (FBG). Both indices displayed normal frequency distributions, and comparative analyses among population subgroups separated into the lowest and highest DFI tertile was performed using independent t-test and odds ratio analysis. Pearson's correlation and multiple regression analysis using age and gender as covariates were used to test for associations. This study demonstrates that rural residence and low socioeconomic position was associated with a higher intake of functional foods, while Seventh-Day religious persuasion, which advocates vegetarianism, was associated with a greater variety of ingested functional foods, but not with a greater quantity or frequency of intake. Both DFIs were favourably associated with weight, body mass index (BMI), tricep skinfold thickness and body fat percentage, however stature (r=-0.096, p=0.033), systolic blood pressure (r=0.175, p<0.0001) and FBG (r=0.108, p=0.020) were adversely associated with DFIv. In contrast, DFIw was significantly correlated to low diastolic blood pressure (r=-0.090, p=0.029) and a tendency towards lower FBG (r=-0.036, p=0.245). Despite some inherent limitations, we conclude that the DFIw is the better indicator of functional food intake, and could be successfully employed by epidemiologists exploring the health consequences of functionality-poor diets.

INTRODUCTION

Over the last half century interest in non-nutrient dietary elements has risen exponentially. Phytochemicals in particular are recognized for their potential role in maintaining optimal health and reducing the risk of developing chronic metabolic diseases associated with modernized lifestyles. As more is understood about the bioavailability, metabolism and pharmacokinetics of functional dietary compounds, the question arises as to whether sufficient information exists to develop quantitative methods to measure intake in human populations. McCarty (2004) theorized that the ratio of calories derived from plant sources relative to total calories, termed the "phytochemical index" (PI), may serve as a rough estimate of phytochemical intake. Dreosti (2000) goes so far as to recommend that certain phytochemicals be allocated a recommended dietary intake (RDI) or a reference value (RV). Prospective studies often focus on the association between functional compounds such as phenolics, and chronic disease development, but have yielded mixed results (Hertog *et al.*, 1993; Hollman *et al.*, 1996; Rimm *et al.*, 1996; Hertog *et al.*, 1997; Yochum *et al.*, 1999; Sesso *et al.*, 2003). A primary limitation has been lack of reliable food compositional data (Sampson *et al.*, 2002), compounded by inherently large variation in non-nutrient concentration.

Given our current level of knowledge of food non-nutrient composition, a food-based approach may be an effective alternative to measure diet functionality. Various food pattern indices that quantify dietary diversity, such as the food variety score (FVS) and dietary diversity score (DDS) (Hatløy *et al.*, 1998; Ogle *et al.*, 2001) or diet quality, such as the diet quality index-international (DQI-I) (Kim *et al.*, 2003b) have yielded positive results, especially in developing nations where noncommunicable disease prevalence rise with changes in nutrition and activity levels. The role of functional elements found in traditional food systems as mediators of health has been demonstrated in diverse areas of the world (O'Dea, 1984; Johns & Chapman, 1995; Krishnaswamy & Ragharamulu, 1998; Cambie & Ferguson, 2003), although the consequences of their reduced intake or outright elimination is unknown. We therefore developed a food-based quantitative index modeled after the FVS that reflects the functionality of a diet. The dietary functionality index (DFI), a composite indicator of functional food intake, was

constructed from the current store of evidence-based knowledge pertaining to foods and other ingestibles that have been scientifically proven to have a beneficial effect on biological systems.

To test whether the DFI could detect differences in population subgroups sharing common sociodemographic characteristics, the index was calculated from dietary and ethnobotanical data obtained from a survey conducted in a coastal Papua New Guinean (PNG) region encompassing an urban, semi-rural and rural area. These regions are situated in close geographical proximity to each other, thus reducing the likelihood of cultural, environmental and genetic influences. The DFI was also analyzed for its association with biomarkers of obesity, hypertension and type 2 diabetes mellitus (DM2), considered the first pathologies to arise from acculturation. We postulate that groups with sociodemographic characteristics prone to increased risk factors for noncommunicable disease, such as acculturation, will have lower DFI scores. Likewise, the DFI is hypothesized to be inversely correlated with symptoms of obesity, hypertension and diabetes. Two variants of the DFI were examined: one that quantifies the number of functional food items (DFI_V), and the other, the food's weight in grams (DFI_W). For comparative purposes, the PI was also tested in this study.

MATERIALS AND METHODS

Calculation of the Dietary Functionality Index (DFI)

The DFI is defined as the ratio of evidence-based pharmacologically active foods consumed by an individual relative to the total amount of food over a defined time period. Two variations of the formula were developed: One that defines the ratio in terms of number (variety) of food items and one in weight (grams), denoted as DFI_V and DFI_W, respectively.

1. The DFI is a composite index comprised of individual *Food Functionality Indices* (FFI), defined as the number of foods in a person's diet that possesses a distinct pharmacological activity. For example, each food possessing

hypoglycemic activity will be included in the $FFI_{GLYCEMIC\ CONTROL}$ category and given a food score of "1". Otherwise, it is given a score of "0". This particular FFI would include all other biological activities that mediate glucose metabolism such as insulin release stimulation, insulin receptor increase, glucose transport stimulation, α-glucosidase inhibition and so forth. The $FFI_{GLYCEMIC\ CONTROL}$ is therefore the sum of all foods that had been given a score of 1.0 in a person's weekly intake. This sum will be averaged along with other summations from related FFIs such as $FFI_{SERUM\ LIPID\ AMELIORATION}$, $FFI_{VASCULAR\ TENSION}$ and so forth, to give the final DFI_V score. The mathematical model is thus:

$$DFI_V = \frac{\frac{1}{n}\sum_{i}^{n} FFI_V(i)}{FVS}$$

where: DFI_V = Dietary Functionality Index – Variety

n = number of FFI_V categories.

$i = 1,2,3,\ldots,n$

FVS = Food Variety Score; the number of discrete food items in the diet.

FFI_V = Score for individual Food Functionality Index – Variety category or categories i (range 0.0 → 1.0).

$$FFI_V = \sum_{h}^{m} f(h)$$

f = Score for food item h (1.0 or 0.0).

m = number of foods that possess the biological activity.

$h = 1,2,3,\ldots,m$

Thus: $$DFI_V = \frac{\frac{1}{n}\left(FFI_{V1} + FFI_{V2} + FFI_{V3} + \ldots + FFI_{Vn}\right)}{FVS}$$

2. To calculate the DFI_W, the portion size in weight (grams) is multiplied by the number of portion servings for each food to give the total grams of food consumed within the survey period. This is then multiplied by its f-score (either 1.0 or 0.0) for every FFI category. The FFI_W will therefore be the sum of grams of food that possesses the pharmacological activity. As above, the DFI_W is obtained from the average of the FFI_W sums, divided by the total grams of foods consumed. Thus:

$$DFI_W = \frac{\frac{1}{n}\sum_{i}^{n} FFI_W(i)}{\sum_{h}^{p} j(h)}$$

where: DFI_W = Dietary Functionality Index – Weight

n = number of FFI_W categories

$i = 1,2,3,\ldots,n$

j = Weight in grams (g) of food item h consumed

p = number of foods item(s) consumed (maximum p = FVS)

$h = 1,2,3,\ldots,p$

FFI_W = Score of Food Functionality Index – Weight category or categories i (range 0.0 → 1.0).

$$FFI_W = \sum_{h}^{m} jf(h)$$

f = Score for food item h (1.0 or 0.0)

m = number of foods that possess the biological activity

$h = 1,2,3,\ldots,m$

Thus: $$DFI_W = \frac{\frac{1}{n}\left(FFI_{W1} + FFI_{W2} + FFI_{W3} + \ldots + FFI_{Wn}\right)}{j_1 + j_2 + j_3 + \ldots + j_p}$$

3. The total number of FFIs that is to be included is ultimately subjective to the researcher but needs to be based on parameters that have direct relvance to the

pharmacological activity. Caution should be exercised in including a FFI category in which few foods are allocated. The result will be a small sum which will pull the DFI towards zero. **Appendix VIII** shows the pharmacological categories and the biological parameters included in this study.

4. To determine the functionality of a plant, a comprehensive review the literature is required. Since the majority of functional dietary items are plants, the most useful resource currently available is the NAPRALERT^{SM} database (University of Chicago in Illinois; www.napralert.org) which continuously incorporates the latest pharmacological findings concerning natural products and marine organisms. Queries could be conducted using a plant's name (common or scientific) and a specified biological activity. Since the database is continuously expanding, it is important to state when the database was accessed. Consequently, the DFI is likely to change over time with advances in functional food and phytochemical research. Pharmacological activity for a plant can only be included in the DFI if:
    a. the activity has been observed in the plant part normally consumed;
    b. activity was observed in experimental animal or human studies after ingestion (not injection) of the plant, and;
    c. the activity was observed from scientifically sound experimental protocols (based on subjective assessment). The bioactive constituent of the plant need not be identified to be included in the DFI as the index is designed to roughly estimate exposure to functional elements in the diet rather than their bioavailablity and pharmacokinetics.

    Appendix IX summarizes the bibliography compiled for the foods included in this study.

5. Food items that constitute the FVS should be distinct species or if biochemical/ pharmacological information is available, varieties or cultivars. Thus, each spice, wild vegetable, adjunct, and condiment is considered a separate food item. In contrast, processed foods with similar macronutrient

composition such as chocolate bar brands or varieties of doughnuts, can be lumped as a single item.

6. Functional foods that are non-botanical are also included in the DFI. These include, probiotics, Ω-3 PUFA-rich fish, and wine, which are well-documented purveyors of health maintainance and disease prevention (Yosefy *et al.*, 1999).

A sample layout for the calculation of DFI_V and DFI_W is shown in **Table 5.1.a** and **Table 5.1.b** respectively. Note that an individual food item can be included in more than one FFI category. For example, ginger (*Zingiber officinale*) can be included in the FFIs for antithrombotic (Bordia *et al.*, 1996), antioxidant (Durak *et al.*, 2002), hypotensive (Silagy & Neil, 1994), hypolipidemic (Ismail *et al.*, 1999), and hypoglycemic (Jelodar *et al.*, 2005) activity. Conversely, a plant can only be included once in a specified FFI. As a ratio, the DFI has a possible score range of 0.0 to 1.0 where a higher score indicates a diet rich in functional foods. A maximum score of 1.0 would indicate that every food in a person's diet was functional.

Application of the DFI

A modified quantitative seven-day food frequency questionnaire (QFFQ) based on that developed and validated by the International Diabetes Institute for Papua New Guinea (Hodge *et al.*, 1996b) included additional food items such as masticants, spices and medicinal plants. The number of dietary items (FVS) totaled 102, divided into 15 food categories: grains and cereals (2 items), refined and processed baked goods (7 items), tubers and starchy foods (10 items), legumes (5 items), vegetables (16 items), fruits (13 items), nuts (6 items), herbs, spices and medicinal plants (8 items), masticants (3 items), beverages (6 items), meat (11 items), fish and seafood (4 items), confectionaries and sweets (6 items), fats and oils (2 items), and eggs and dairy (3 items). From these, 64 items were plants (62.7%), distributed among 9 food groups (60%). Fish and seafood contributed an additional two items considered functional. In the present study, fresh and canned fish constituted separate food items based on nutrient content (canned fish was packed in vegetable oil), both of which were included in the DFI,

whereas deep-fried battered fish was excluded because of the increased content of unhealthy saturated fats that outweigh any benefits that could be obtained from the fish oil.

Thus the maximum DFI_V score that could be attained in this population was 0.66, while the DFI_W depended on consumption quantities, resulting in a possibly higher score. Besides the evident plant-based groups, items such as tea (*Camillia sinensis*) and coffee (*Coffea arabica*) from the beverage group, betelnut (*Areca catechu*) and piper inflorescence (*Piper betle*) from the masticant group and sugarcane (*Saccharum officinale*) from the sweets groups were included in DFI calculations. In contrast, refined grains such as white rice and white flour were excluded, as well as the white European potato, a poor source of phytochemicals notable for its high glycemic index.

Plant items were searched in the NAPRALERT[SM] database for all known biological activity, accessed 16 / 05 / 2006. MEDLINE (U.S. National Library of Medicine; www.pubmed.gov) was used to search for the pharmacological properties of fish and seafood. Probiotics and wine were not consumed in this population.

Parameters of interest included metabolic- and lifestyle-related chronic disorders that affect energy metabolism, glycemic control, cardiovascular health, serum lipid levels, adiposity, oxidative stress, inflammation and related conditions (**Appendix VIII**). Information was compiled into a database that included literature references (**Appendix IX**), research design, phytochemical and nutritional composition, ethnobotanical use and pharmacological activity. FFI_V and FFI_W were calculated for each of the 7 pharmacological parameters and then averaged to obtain a final DFI_V and DFI_W score.

Phytochemical Index

The phytochemical index (PI) is defined as the percentage of dietary calories supplied by foods typically high in phytochemicals (McCarty, 2004). This includes wine, beer, and cider, but excludes distilled hard liquors. Soy protein and olive oil are also included, but these items were not consumed in PNG. The same 64 plant items that were included in the calculation of DFI were used to calculate the PI.

Study Area

A quantitative ethnobotanical survey of medicinal plants and a semi-quantitative food frequency questionnaire was administered in Koki (National Capital District, 9.483° S, 147.167° E), Kalo (Central Province, 10.050° S, 148.200° E) and Wanigela (Central Province, 10.050° S, 147.783° E) from January to August, 2004. The rural sample, Wanigela, is a lagoon stilt village where some residents engage in subsistence fishing and farming, while others rely on city relatives to provide trade store goods. The urban sample, Koki, is a permanent Wanigela settlement located in a relatively affluent suburb of the capitol Port Moresby and has direct access to grocery stores and open air markets. Both Wanigela and Koki adhere to the Seventh-Day Adventist denomination of Christianity which emphasizes health and diet by advocating vegetarianism and abstinence from stimulants such as betel quid, alcohol, caffeine and tobacco. Residents of Kalo, a road-connected semi-rural village located roughly equidistant between Koki and Wanigela, are ethnically similar and are predominantly subsistence farmers and fishermen.

Participants were selected by stratified random sampling according to gender and age. All individuals above 16 years were eligible to participate except pregnant and lactating women. The survey covered a final sample of 365 participants roughly divided between the three villages. Approval from the head of each local level of government was obtained, followed by a public information session and individual prior informed consent before initiation of the study. Permission and ethics approval was obtained from the McGill University Ethics Committee, the Papua New Guinea Medical Advisory Board and the Papua New Guinea Department of Environment and Conservation.

Anthropometric measurements and blood glucose determination

Participants were weighed without shoes and in light clothing to the nearest 100g on a Seca Dial Scale (Vogel & Halke, Germany). Standing height was measured using wooden boards with a measuring tape (0.1 cm precision) that were built locally based on the UNICEF model (Programme, 1986). Waist, hip and mid-upper arm (MUAC) circumference was measured using a fiberglass measuring tape. Skin-fold thickness was obtained using Lange calipers and body fat % calculated according to formulae supplied

by the manufacturer for four sites: tricep (TSF), bicep, subscapular and suprailiac. Mid-arm muscle circumference (MMC), a measure of lean tissue mass, was derived from the equation MMC (cm) = MUAC (cm) – [0.314 x TSF (mm)].

Blood pressure was measured in duplicate after a participant was asked to sit quietly for five minutes. In cases where hypertension was detected, another reading was taken after ten minutes. If still hypertensive, the participant was referred to the Community Health Worker (CHW). Participants were encouraged to retest their blood pressure at any time during the study.

Fasting blood glucose (FBG) concentration was determined using a portable glucometer (CardioChek™ Analyzer, Polymer Technology Systems Inc., Indiana) after an overnight 12-hour fast. Readings were obtained within 2 minutes. Participants with glucose concentrations above 7 mmol/L were retested to confirm hyperglycemia and referred to the Port Moresby General Hospital Diabetes Clinic for further testing and treatment. Participants with known diabetes were excluded from the analyses since this may have influenced dietary and lifestyle patterns.

Socioeconomic position

To determine the degree of acculturation or socioeconomic position, a modernity index score (MIS), developed and validated for PNG (Hodge *et al.*, 1995) was administered. The score is based on seven questions pertaining to area of origin, father's employment, type and duration of individual's employment, education, etc., giving a maximum of 40 points. A high score indicated a more modernized participant.

Statistical analysis

Data was analyzed with SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL, USA). All continuous variables, including DFI_V, DFI_W and PI, were normally distributed. Fasting blood glucose was normalized by inverse-transformation ($1-1/x$).

Independent t-tests were used to compare both DFIs and PI between sociodemographic categories. To assess population distribution relative to dietary functionality scores, the DFIs and PI were divided into tertiles and differences in the

proportion of the population occurring in the lowest and highest tertile were tested using chi-square and expressed as odds ratio.

Similarly, anthropometric parameters, blood pressure and FBG were divided according to the lowest and highest tertiles for the DFIs and PI and independent t-tests performed to assess statistical differences. To explore the associations between the DFIs, PI and anthropometric measures, simple (Pearson's correlation) and multivariate regression (with age and gender as covariates) were used. Data are expressed as regression coefficients and β–weights ± SE with 95% confidence interval.

RESULTS

Application of the DFI to our sample population resulted in a frequency distribution closely resembling a normal Gaussian curve, although the DFI_V had a relatively high kurtosis (3.36 ± 0.25) (**Figure 5.1.**).

The population mean ± SD for the dietary functionality indices was 0.32 ± 0.06 (range 0.01 – 0.46), 0.30 ± 0.09 (range 0.00 – 0.53), 0.42 ± 0.16 (range 0.01 – 1.00) for DFI_V, DFI_W and PI, respectively (**Table 5.2.**). These values suggest that only a third of the sample population's diet contained known functional dietary elements, whereas the PI indicated that close to 45 % of calories came from plants. To determine whether these scores differed from the total number of dietary plant items or their weight, independent t-tests were performed and Pearson's correlation coefficients calculated. Approximately 65 % of the diet, both in the number of items and their weight, was comprised of plant foods, half of which possessed known pharmacological activity. Despite their smaller score range, both DFIs were strongly correlated with each other as well as with the number and weight of total dietary plants ($p<0.0001$). The inclusion of fish and seafood in the DFI had little influence on modifying these results, indicating that the majority of bioactive compounds were from botanical sources.

Dietary Functionality Index and sociodemographic characteristics

Mean index scores between age and gender categories were not significantly different. However, when comparing the proportion of the population who fell into the lowest and highest tertile of the indices, it was observed that significantly fewer individuals in the 41-60 year age group were in the lowest tertile for DFI_V compared to the <20 year group (odds ratio = 4, $p<0.001$), as was the DFI_W for those above 60 years (**Table 5.3.**). This suggests that older individuals consumed a greater variety and quantity of functional foods, a trend that is not detected when considering plant caloric contribution expressed as PI. A much stronger sociodemographic determinant of dietary patterns was socioeconomic position (modernity index score) reflected in the area of residence. In general, consumption of functional foods increased as one moved from an economically prosperous urban environment towards a less affluent rural area, supporting existing evidence that traditionally fare, consumed more frequently in rural regions, contain more pharmacologically active elements than modern introduced foods (Johns & Chapman, 1995).

Religious persuasion also contributed to significant differences in DFI_W and PI, since Seventh-Day Adventism advocates vegetarianism. This did not translate into higher consumption of functional foods, however, but merely into higher consumption of plant foods as an energy source, indicated by the higher mean for PI relative to those belonging to United Church. Although the number of functional food items consumed by followers of United Church was slightly less than for Adventists, the quantity consumed was significantly greater, indicated by the larger proportion of the population classified in the highest tertile for DFI_W and PI (**Table 5.3.**).

Marital status had little effect on all three functionality indices. However when divided according to DFI_V tertiles, a significant proportion of the unmarried population fell into the lowest third (43.1%) compared to the highest (17.2%). This is likely an effect of age since younger individuals are more apt to consume more imported processed foods and soft drinks.

Dietary Functionality Index and its association to health parameters

When population health parameters are divided into DFI and PI tertiles, weight is the only parameter shared by all three indices in which the proportion of the population in the highest tertile weighed significantly less than those in the lowest (**Table 5.4.**). This suggests that those who consume more functional foods or obtain a higher proportion of their calories from plants weigh less than those who do not. This was also observed for body mass index, although statistical significance was only attained with differences in the quantity of functional food intake (DFI_W: p=0.004). Inter-tertile differences in subcutaneous adiposity measured as TSF thickness was significant for both DFIs (DFI_V; p=0.006; DFI_W: p=0.010) but not for PI (p=0.449), although this did not translate equally for body fat percentage (DFI_V; p=0.555; DFI_W: p=0.073). Of interest is the finding that systolic blood pressure was higher in the 3rd tertile for both DFI_V and PI, which is seemingly contradictory to established health associations between plant food consumption and cardiovascular health (Appel *et al.*, 1997).

Simple and multiple regression analyses confirm such a relationship between DFI_V and systolic blood pressure (r=0.175; p<0.0001) (**Table 5.5.**). Of the three indices, DFI_W was the only one to suggest an inverse relationship with SBP, although this did not attain statistical significance in our adjusted regression analysis (β=-0.47 ± 9.76; p=0.962). More importantly, DFI_W was the only index associated with reduced diastolic blood pressure in both simple (r=-0.090, p=0.029) and adjusted analyses (β=-14.12 ± 6.70; p=0.036), a parameter with greater health implications than SBP. The R^2 value for blood pressure informs us that DFI_W, adjusted for age and gender, only explained 9.6 % and 1.9 % of the variation in systolic and diastolic pressure, respectively. Likewise, DFI_W was alone in suggesting a trend towards lower FBG (r=-0.036; p=0.245). In contrast, DFI_V showed a significant association with higher blood glucose concentrations (r=0.108; p=0.020), a finding that conflicts with the accepted view that fruit and vegetable intake reduces type-2 diabetes risk (Ford & Mokdad, 2001). A similar relationship was observed with short stature (r=-0.096; p=0.033). Despite this, DFI_V, as well as DFI_W, were favourably associated with other anthropometric measures, including weight, BMI, TSF thickness and percent body fat, the latter only reaching significance when adjusted for age and weight. From these observations, it can be concluded that of the three indices

compared here, the quantitative measure of functional food consumption in weight (DFI_W) is the better overall indicator of metabolic health.

DISCUSSION

The present study demonstrates that quantification of functional food intake expressed in weight (DFI_W) is a better indicator of general metabolic health in this transitional coastal Papua New Guinean population, relative to measures expressed as the number of discrete food items (DFI_V), or as calories (PI). This is not surprising considering that the amount or frequency of an ingested functional food would more likely exert a measurable long-term biological effect. The disadvantage with simple food counts is that a subject receives a score of 1.0 for having consumed a food during the survey period regardless of whether it was just once or habitual. Nevertheless, food diversity counts such as the food variety score (FVS) and the dietary diversity score (DDS) have demonstrated adequate diagnostic sensitivity and specificity to predict nutrient deficiencies in a variety of cultural settings (Hatløy *et al.*, 1998; Ogle *et al.*, 2001; Torheim *et al.*, 2004; Savy *et al.*, 2005). In a few studies, the FVS and DDS were reported to have a beneficial relationship with such metabolic biomarkers as serum cholesterol (Kant & Graubard, 2005), insulin sensitivity (Azadbakht *et al.*, 2005), and blood pressure (Wahlqvist *et al.*, 1989).

This was not the case for DFI_V in our population for the reason that the index does not have a wide enough score range to effectively detect population differences in blood pressure or FBG values. Furthermore, the significant associations that were detected were in a direction that implied an increased risk of systolic hypertension (r=0.175, p<0.0001) and hyperglycemia (r=-0.108, p=0.020). Given what is known concerning the health benefits of functional foods, it can be assumed that a simple count of functional foods is an inadequate strategy for the quantification of functionality.

Similarly, PI is not an ideal index since it displayed no significant relationships with any of the anthropometric measures in our study, except for height (β=0.05 ± 0.02; p=0.015) and mid-arm muscle circumference (β=3.06 ± 1.59; p=0.050), compounded

with its tendency to be associated with higher systolic blood pressure (r=0.046; p=0.377) and FBG concentrations (r=0.017; p=0.745). The lack of an association between the functionality of a food and its caloric content is not an unexpected finding given the fact that phytochemicals in themselves do not contribute any calories. The primary limitation of the PI, as noted by McCarty (2004), is that functional foods such as teas, spices and masticants are not included since they contribute few calories, yet are associated with numerous health benefits. Conversely, plant items that have few allelochemicals would inadvertently inflate the PI and give an inaccurate estimate of phytochemical intake. This was the reason why starchy plants, most notably the European potato, were excluded from PI calculations. In this study, propagules of the common mangrove (*Bruguiera gymnorrhiza* (L.) Lam) presented a similar dilemma. The hypocotyls are traditionally soaked, cooked in two changes of salt water and peeled to remove unpalatable tannins, resulting in a bland, energy-rich starchy food that forms the staple of the rural sample population. For this reason, mangrove bean was considered much like the potato and excluded from the PI. Lack of phytochemical or biological research on this plant also meant that it was not included in the DFI. Inclusion would have rendered all associations between PI and health parameters skewed towards the characteristics of the rural populace, producing a bimodal frequency distribution.

The mathematical design of the DFI closely mirrors that of the mean adequacy ratio (MAR), a composite indicator of nutrient intake calculated from the average of separate nutrient adequacy ratios (NAR). A NAR is the ratio of the observed intake of an essential nutrient divided by its recommended nutrient intake (RNI), and the average of nine individual NARs (energy, protein, iron, riboflavin, niacin, vitamin C, calcium, folic acid and vitamin A), truncated to 1.0, is used to calculate the MAR. The MAR identifies individuals who are deficient in one of its component nutrients but does not specify which one. Similarly, the DFI is a composite index comprised of individual FFIs added together and averaged, producing a single score designed to identifying groups that consume few functional foods. The decision to use a composite indicator of functional food intake arose from the inability of individual FFIs to detect significant associations with some of the health parameters measured here, as well as an inability to detect differences within population subgroups. For example, no association emerged between intake of

antidiabetic foods and fasting blood glucose, or of hypotensive foods and blood pressure. When individual FFIs were compounded, the strength of the correlations progressively ameliorated. In this study, seven pathophysiological categories (glycemic control, inflammation, lipid profile amelioration, coronary and peripheral cardiovascular protection, obesity, oxidative stress and vascular tension) encompassing more than 150 pharmacological activities was deemed adequate enough to construct a DFI capable of detecting inter-group differences and able to correspond to changes in chronic metabolic biomarkers. Any additional pharmacological categories would theoretically augment the predictive power of the DFI until a plateau is reached where, consistent with the law of diminishing returns, no new information would be gained. At this point, and undoubtedly throughout the process, the limiting factor remains the scope of valid scientific information available on which to build the DFI.

Functional food research remains in its infancy and the pharmacokinetics, toxicity and metabolic fate of phytochemicals and other functional elements remains unclear. This is particularly relevant for tropical foods, wild crops and medicinal plants, not to mention their varieties and cultivars. Emerging and future research will continuously expand the knowledge database and provide new insights into food functionality. Consequently, the DFI can be continuously improved upon and become more sensitive to changes in predictor variables. Currently, the NAPRALERT[SM] database remains the most comprehensive resource for plant and natural product pharmacology, biochemistry and ethnobotany (www.napralert.org). Also of note are HerbMed® (Alternative Medicine Foundation Inc., 2000), Dr. Duke's Phytochemical and Ethnobotanical Database (http://www.ars-grin.gov/duke/) and the USDA Agricultural Research Service (http://www.pl.barc.usda.gov/home.cfm). Broader in scope is the MEDLINE database (U.S. National Library of Medicine) which is the premier source for bibliographic coverage of the biomedical literature (http://pubmed.gov).

The rationale of building an indicator index based on a literature review recognizes that not all phytochemicals have the same health-promoting utility and should thus not be scored equally. For example, tea polyphenolic compounds have an established reputation as strong antioxidants (Frei & Higdon, 2003), but are also able to modulate

enzyme expression and hormone activity leading to reduced vascular tension (Yang *et al.*, 2004b), body fat (Wolfram & Thielecke, 2006) and improved cardiovascular health (Kris-Etherton & Keen, 2002), enabling it to be classified under more than one FFI pharmacological category according to the rules used in constructing the DFI. In contrast, little research may have been conducted on another plant where little on no biological activity had been observed. For example, sea almond (*Terminalia catappa* L.) has been reported to have moderate hypoglycemic activity, but little is known about the nut (Nagappa *et al.*, 2003). Tea would therefore inflate the DFI due to its inclusion in several FFIs. Thus, two diets identical in the number of functional foods consumed may have different DFI scores based on the food's particular functionality and potency. Weighting a plant's functionality would theoretically provide a better indicator of health outcomes by limiting the contribution of functional foods with weak potency.

In our study, a strong correlation between the total weight of plants in the diet and the DFI was observed (in weight: r=0.694, p>0.0001, data not shown), and both had similar associations with various health outcomes, suggesting no difference between the two. The former, however, was more strongly associated with measures of obesity (% plant foods in grams vs. BMI: r=-0.235, p<0.0001 compared to DFI_W vs. BMI: r=-0.142, p=0.003), but had no correlation with diastolic blood pressure (r=-0.004 p= 0.938). In this respect, DFI_W may be a better indicator of cardiovascular health, but not necessarily for other health outcomes. Both the weight ratio of plant food intake and DFI_W displayed a non-significant trend towards low FBG, but the former seemed a better potential indicator (% plant foods in grams vs. FBG: r=-0.056, p<0.144 compared to DFI_W vs. FBG: r=-0.036, p=0.245). These measures could potentially become significant indicators of FBG with larger sample sizes. A sample size of 1978 for % plant food weight and 4790 for DFI_W would have been needed to detect a significant correlation at α=0.05 and β=0.08.

A further limitation of the DFI is that the toxicity and carcinogenicity of certain foods is not considered. This is particular relevant for betel nut (*Areca catechu* L.), a popular masticant in Papua New Guinea. We have reported elsewhere that in this sample population, betel nut chewing was inversely correlated with diastolic blood pressure (r=-

0.184, $p<0.0001$) and FBG (r=-0.111, $p<0.05$) (38), and seemed to exert some health benefit. However, epidemiological studies strongly suggest that betel nut is diabetogenic (Benjamin, 2001), and it has been established as a known carcinogen due to its association with the occurrence of oral leukoplasia, and other oral cancers (Norton, 1998). Consequently, an attempt was made to catalogue the pharmacological antitheses of the FFI categories included in this study according to the literature and subtract these from the DFI, but oftentimes the very same natural compounds that had effectuated a positive health outcome were also responsible for the toxic effect at different concentration. This is commonly seen in phenolic antioxidants that become prooxidants at higher concentrations or in the presence of transition metals (Fijisawa *et al.*, 2002). When constructing the DFI, this results in the annulment of positive points and a reduced overall DFI score. We therefore opted to retain only the positive, protective pharmacological activities.

The DFI requires a thorough review of the literature and thus is not a straightforward calculation. The formula is subject to variations in the availability and accessibility of the bibliography and to the number and nature of the pharmacological categories included. Because of these limitations, the DFI is best used as a comparative tool between population subgroups to identify diets that are low in functionality. Nevertheless, given an adequate sample size, the DFI could aid epidemiologists in exploring the metabolic consequences of diets high in functional foods. Clinical nutritionists may also find the index useful in their efforts to improve the functionality of their client's diet.

CONCLUSION

The dietary functionality index was developed in an attempt to quantify the pharmacological properties of diets in order to explore differences in population subgroups and associations with noncommunicable disease risk factors. A version of the DFI expressed as the ratio of functional food items relative to total food items (DFI_V) was as successful in detecting differences between groups of dissimilar socioeconomic

position, area of residence and religious persuasion as the version expressed in weight (DFI_W) in a coastal region of Papua New Guinea. Both indices were also favourably associated with anthropometric indicators of obesity although DFI_V displayed a significant relationship with systolic hypertension and indicated a trend towards diastolic hypertension and hyperglycemia. In contrast, DFI_W was inversely correlated with diastolic blood pressure and implied a beneficial relationship with FBG. The DFI was compared to the phytochemical index, defined as the ratio of calories derived from plant foods known to be rich in phytochemicals to total calories (McCarty, 2004), which showed similar demographic subgroup differences in our sample population, but virtually no associations with health parameters. From the data presented in this study, we conclude that DFI_W was superior as a predictor of noncommunicable disease risk. As a composite index dependent on a comprehensive pharmacology bibliography, the DFI is subject to the investigative thoroughness of the researcher and the classification and designation of constituent pharmacological categories. Nevertheless, the DFI may be a useful tool for epidemiologists exploring social, economical, environmental and cultural determinants of functional food intake, as well as its consequence on health outcomes.

Table 5.1.a. Sample layout for the calculation of DFI_V for a hypothetical individual who had consumed 5 food items during the survey period. In this example, "food 1" had been consumed at least once and a review of the scientific literature showed that this food exerted a hypoglycemic and antiinflammatory effect in a biological system and thus receives a point of 1.0 for each category. The total number of categories included here is four, but more may be added provided that it is relevant to a shared metabolic condition. "Food 2" also has antiinflammatory activity and "food 3" contains bioactive antioxidants, and so forth. The sum for each FFI is calculated and averaged with the rest of the FFIs, and divided by the total number of foods.

	$FFI_{glycemic\ control}$	$FFI_{inflammation}$	$FFI_{obesity}$	$FFI_{oxidative\ stress}$
Food 1	1	1	0	0
Food 2	0	1	0	0
Food 3	0	1	0	1
Food 4	1	0	1	0
Food 5	0	0	0	1
Sum	2	3	1	2
DFI_V = ((2+3+1+2)/4)/5 = 0.4				

Table 5.1.b. Sample layout for the calculation of DFI_W. Following the same hypothetical individual as in Table 1a., portion sizes and frequency of intake is multiplied to obtain total food weight. This value is recorded in each FFI category where the food had displayed pharmacological activity. The average of all the FFIs is divided by the total weight of all foods.

	Number of portions	Portion weight (g)	Total food weight (g)	FFI glycemic control	FFI inflammation	FFI obesity	FFI oxidative stress
Food 1	1	100	100	100	100	0	0
Food 2	3	200	600	0	600	0	0
Food 3	1	300	300	0	300	0	300
Food 4	2	400	800	800	0	800	0
Food 5	3	500	1800	0	0	0	1500
Sum			3600	900	1000	800	1800
DFI_W = ((900+1000+800+1800)/4)/3600 = 0.31							

Table 5.2. Pearson's correlation coefficients (r) between the various dietary functionality indices and plant food intake. 'Total plant items' is the ratio of plant food items to all food items consumed during the survey period (7 d). 'Total plant grams' is the weight ratio of plant to total foods. PI: Phytochemical Index; DFI_V: Dietary Functionality Index – variety; DFI_W: – weight.

		r			
	Mean ± SD[1]	DFI_V	Total plant items	DFI_W	Total plant grams
PI	0.42 ± 0.16 [a]	0.313[2]	0.419	0.624	0.648
DFI_V	0.32 ± 0.06 [c]		0.726	0.425	0.562
Total plant items	0.65 ± 0.12 [b]			0.754	0.754
DFI_W	0.30 ± 0.09 [c]				0.792
Total plant grams	0.65 ± 0.21 [b]				

[1] Different lettered superscripts are significantly different at $p<0.05$.
[2]All correlations are significant at $p<0.0001$

Table 5.3. Mean ± SD of dietary functionality indices and the proportion of study population that fall into the lowest (1st) and highest (3rd) tertile according to sociodemographic variables. The odds ratio (OR) (95% Confidence Interval) is the ratio of the probability of low diversity scores, to the probability of higher scores according to the demographic category.

		Dietary Functionality Index – Variety (DFI_V) [2]				**Dietary Functionality Index – Weight (DFI_W)** [3]				**Phytochemical Index (PI)** [4]			
			Percent (%) of population				Percent (%) of population				Percent (%) of population		
	n	Mean ± SD[1]	1st tertile	3rd tertile	OR (95% CI)	Mean ± SD[1]	1st tertile	3rd tertile	OR (95% CI)	Mean ± SD[1]	1st tertile	3rd tertile	OR (95% CI)
Age (y)													
<20†	48	0.30 ± 0.06 [a]	45.8	18.7	1.0	0.30 ± 0.01 [a]	43.8	25.0	1.0	0.38 ± 0.11 [a]	39.6	14.6	1.0
20-40	139	0.31 ± 0.05 [a]	32.4	26.6	1.6 [ns] (0.7-3.6)	0.30 ± 0.09 [a]	34.5	21.6	1.1 [ns] (0.5-2.5)	0.40 ± 0.15 [ab]	35.3	28.8	1.8 [ns] (0.7-4.4)
41-60	115	0.33 ± 0.06 [a]	23.5	35.7	4.0*** (1.7-9.3)	0.31 ± 0.10 [a]	33.9	66.1	1.8 [ns] (0.8-4.0)	0.45 ± 0.19 [ab]	26.1	42.6	3.4 * (1.3-8.6)
>60	63	0.32 ± 0.06 [a]	27.0	27.0	2.0 [ns] (0.8-4.9)	0.32 ± 0.11 [a]	27.0	38.1	2.9* (1.1-7.4)	0.44 ± 0.17 [b]	22.2	31.7	3.3 * (1.2-9.6)
Gender													
Male	179	0.32 ± 0.06 [a]	31.3	25.7	1.0	0.30 ± 0.10 [a]	32.4	34.6	1.0	0.42 ± 0.17 [a]	29.6	32.4	1.0
Female	186	0.32 ± 0.05 [a]	28.5	32.3	1.2 [ns] (0.8-2.0)	0.31 ± 0.09 [a]	30.1	26.9	0.9 [ns] (0.6-1.5)	0.42 ± 0.16 [a]	31.2	32.3	1.0 [ns] (0.6-1.7)
Modernity Index Score													
High†	130	0.29 ± 0.05 [a]	46.9	16.9	1.0	0.27 ± 0.11 [a]	47.7	18.5	1.0	0.39 ± 0.17 [a]	60.0	27.7	1.0
Middle	108	0.32 ± 0.04[b]	30.6	25.0	2.8** (1.4-5.4)	0.31 ± 0.10 [b]	34.3	33.3	2.1* (1.1-3.8)	0.43 ± 0.19 [a]	62.0	35.2	1.4 [ns] (0.8-2.6)

	n	Dietary Functionality Index – Variety (DFI_V) [2]				Dietary Functionality Index – Weight (DFI_W) [3]				Phytochemical Index (PI) [4]			
			Percent (%) of population				Percent (%) of population				Percent (%) of population		
		Mean ± SD[1]	1st tertile	3rd tertile	OR (95% CI)	Mean ± SD[1]	1st tertile	3rd tertile	OR (95% CI)	Mean ± SD[1]	1st tertile	3rd tertile	OR (95% CI)
Low	126	0.35 ± 0.05 [c]	13.5	44.4	10.0 [ns] (5.1-19.7)	0.34 ± 0.06 [b]	16.7	34.9	6.1 [****] (3.1-12.3)	0.44 ± 0.14 [a]	15.1	33.3	2.7 [***] (1.4-5.4)
Area of residence													
Urban[†]	119	0.28 ± 0.10 [a]	57.1	8.4	1.0	0.23 ± 0.05 [a]	61.3	16.0	1.0	0.35 ± 0.16 [a]	55.5	20.2	1.0
Semi-rural	121	0.31 ± 0.09 [b]	28.9	26.4	2.4 [*] (1.1-4.9)	0.35 ± 0.04 [b]	20.7	46.3	13.3 [****] (6.6-26.9)	0.50 ± 0.18 [b]	18.2	53.7	7.7 [****] (4.0-14.8)
Rural	125	0.36 ± 0.05 [c]	6.4	49.6	50.6 [****] (20.5-124.9)	0.32 ± 0.04 [c]	13.6	28.8	10.6 [****] (5.1-22.1)	0.41 ± 0.10 [c]	18.4	22.4	2.7 [***] (1.4-5.4)
Religion													
SDA	246	0.32 ± 0.06 [a]	30.9	29.3	1.0	0.28 ± 0.09 [a]	36.2	23.6	1.0	0.38 ± 0.14 [a]	36.6	21.5	1.0
United	119	0.31 ± 0.04 [a]	28.6	27.7	0.4 [**] (0.2-0.8)	0.35 ± 0.09 [b]	21.0	45.4	4.4 [****] (2.4-8.0)	0.50 ± 0.18 [b]	17.6	55.5	5.4 [****] (3.0-9.6)
Marital Status													
Married[†]	286	0.32 ± 0.06 [a]	26.9	32.5	1.0	0.30 ± 0.09 [a]	29.4	68.9	1.0	0.42 ± 0.16 [a]	26.2	32.9	1.0
Single	58	0.30 ± 0.05 [a]	43.1	17.2	0.3 [***] (0.1-0.6)	0.30 ± 0.09 [a]	41.4	24.1	0.6 [ns] (0.3-1.2)	0.40 ± 0.16 [a]	37.9	24.1	0.6 [ns] (0.3-1.2)
Widowed	21	0.31 ± 0.05 [a]	38.1	4.8	0.2 [*] (1.0-7.4)	0.32 ± 0.11 [a]	33.3	23.8	1.5 [ns] (0.5-4.2)	0.46 ± 0.16 [a]	38.1	42.9	1.2 [ns] (0.5-3.2)

[*] $p<0.05$; [**] $p<0.005$; [***] $p<0.001$; [****] $p<0.0001$; [ns]non-significant; [†] Comparison group for odds ratio calculation; [1]Means with different letters are significantly different at $p<0.05$; [2] DFI_V lowest tertile : <0.30, highest tertile : >0.34; [3] DFI_W lowest tertile : <0.27, highest tertile : > 0.33; [4] PI lowest tertile : <0.35, highest tertile : >0.46

Table 5.4. Comparison (mean ± SD) of anthropometric parameters, blood pressure and fasting blood glucose between individuals who are in the lowest versus the highest tertile of the three dietary functionality indices. DFI: Dietary functionality index measures the ratio of functional foods to total foods expressed as number of food items (DFI_V) or their weight in grams (DFI_W). PI: Phytochemical index is the ratio of energy derived from plant sources.

	Dietary Functionality Index - Variety (DFI_V) [1]			**Dietary Functionality Index - Weight (DFI_W)** [2]			**Phytochemical Index (PI)** [3]		
Health outcomes	1st tertile (n=137)	3rd tertile (n=122)	*p*	1st tertile (n=112)	3rd tertile (=141)	*p*	1st tertile (n=121)	3rd tertile (n=122)	*p*
Weight (kg)	61.9 ± 16.7	57.9 ± 13.3	0.035	64.2 ± 18.7	59.7 ± 13.6	0.037	62.3 ± 18.0	61.9 ± 13.9	0.470
Stature (m)	1.68 ± 0.09	1.58 ± 0.08	0.054	1.60 ± 0.09	1.61 ± 0.08	0.389	1.59 ± 0.09	1.61 ± 0.08	0.616
BMI (kg/m^2)	23.9 ± 5.2	22.9 ± 4.5	0.122	24.9 ± 5.8	22.9 ± 4.4	0.004	24.3 ± 5.6	23.9 ± 4.7	0.063
Waist circumference	83.6 ± 13.4	82.1 ± 11.7	0.344	85.3 ± 14.7	82.7 ± 11.3	0.129	84.4 ± 14.5	85.3 ± 12.7	0.427
Waist-to-hip ratio	0.89 ± 0.08	0.91 ± 0.07	0.153	0.89 ± 0.08	0.91 ± 0.07	0.311	0.90 ± 0.08	0.92 ± 0.08	0.853
Tricep skinfold (mm)	16.3 ± 8.7	13.5 ± 7.2	0.006	17.1 ± 9.5	14.3 ± 7.0	0.010	16.3 ± 9.3	15.5 ± 7.8	0.440
Body fat (%)	27.2 ± 9.1	26.5 ± 8.9	0.555	28.5 ± 9.12	26.4 ± 9.1	0.073	27.2 ± 9.4	27.9 ± 9.3	0.939
MAMC (cm)	22.0 ± 3.2	22.2 ± 3.1	0.730	22.1 ± 3.1	22.4 ± 3.0	0.347	22.0 ± 3.0	22.6 ± 2.9	0.665
SBP (mm Hg)	127.3 ± 14.2	132.5 ± 19.2	0.016	128.9 ± 16.5	129.7 ± 17.2	0.687	128.2 ± 13.9	131.5 ± 18.9	0.011
DBP (mmHg)	79.2 ± 10.1	80.4 ± 10.8	0.330	80.1 ± 11.2	78.3 ± 10.6	0.170	79.4 ± 10.4	79.1 ± 11.8	0.652
Fasting glucose (mmol/L)	3.8 ± 2.4	4.2 ± 2.3	0.158	4.0 ± 2.6	4.0 ± 2.3	0.988	4.0 ± 2.5	3.9 ± 1.9	0.263

[1] DFI_V lowest tertile : <0.30, highest tertile : >0.34 ; [2] DFI_W lowest tertile : <0.27, highest tertile : > 0.33 ; [3] PI lowest tertile : <0.35, highest tertile : >0.46

Table 5.5. Unadjusted and adjusted (for age and gender) correlations between various health parameters and dietary functionality indices.

	Unadjusted[a]		Adjusted[b]			
Health parameters	r	*p*	$\beta \pm$ SE	*p*	95% CI	Model R^{2c}
Weight (kg)						
DFI_V	-0.123	0.009*	-30.81 ± 15.43	0.047*	-61.14 – -0.48	0.094
DFI_W	-0.110	0.018*	-17.38 ± 9.00	0.054	-35.08 – 0.32	0.094
PI	0.0045	0.389	2.26 ± 4.76	0.630	-7.11 – 11.62	0.085
Stature (m)						
DFI_V	-0.096	0.033†	-0.06 ± 0.07	0.372	-0.18 – 0.08	0.423
DFI_W	0.071	0.089	0.14 ± 0.04	0.001*	0.05 – 0.23	0.425
PI	0.076	0.149	0.05 ± 0.02	0.015*	0.01 – 0.09	0.431
BMI (kg/m^2)						
DFI_V	-0.101	0.027*	-9.94 ± 5.09	0.052	-19.95 – 0.07	0.012
DFI_W	-0.142	0.003*	-8.18 ± 2.95	0.006*	-13.99 – -2.58	0.022
PI	0.008	0.884	-0.46 ± 1.57	0.771	-3.55 – 2.63	0.002
Waist circumference (cm)						
DFI_V	-0.061	0.122	-20.26 ± 13.19	0.126	-46.20 – 5.69	0.042
DFI_W	-0.069	0.096	-12.64 ± 7.69	0.101	-27.72 – 2.48	0.043
PI	0.033	0.531	0.25 ± 4.06	0.951	-7.74 – 8.24	0.036
Waist-to-hip ratio						
DFI_V	0.044	0.203	0.01 ± 0.07	0.921	-0.13 – 0.15	0.223
DFI_W	0.070	0.093	0.036 ± 0.04	0.461	-0.05 – 0.11	0.224
PI	0.053	0.319	0.024 ± 0.02	0.286	-0.02 – 0.07	0.226
Tricep skinfold (mm)						
DFI_V	-0.137	0.004*	-22.77 ± 7.49	0.003*	-37.49 – -8.05	0.194
DFI_W	-0.143	0.003*	-12.97 ± 4.37	0.003*	-21.56 – 4.38	0.193
PI	-0.017	0.741	-0.76 ± 2.33	0.744	-5.34 – 3.82	0.174
Body fat (%)						
DFI_V	-0.034	0.256	-16.76 ± 7.10	0.019*	-30.73 – 2.79	0.456
DFI_W	-0.070	0.110	-9.88 ± 4.15	0.018*	-18.04 – -1.72	0.456
PI	0.027	0.605	0.119 ± 2.20	0.957	-4.21 – 4.45	0.448
Mid-arm muscle circumference (cm)						
DFI_V	0.024	0.322	2.25 ± 2.39	0.347	-2.45 – 6.95	0.572
DFI_W	0.060	0.128	3.06 ± 1.59	0.056	-0.08 – 6.20	0.576
PI	0.095	0.069	1.59 ± 0.81	0.050	0.00 – 3.17	0.333

	Unadjusted[a]		Adjusted[b]			
Health parameters	r	*p*	$\beta \pm$ SE	*p*	95% CI	Model R^{2c}
Systolic blood pressure (mm Hg)						
DFI_V	0.175	<0.001†	46.13 ± 16.56	0.006*	13.56 – 78.69	0.115
DFI_W	0.029	0.292	-0.47 ± 9.76	0.962	-19.67 – 18.74	0.096
PI	0.046	0.377	4.37 ± 5.14	0.395	-5.73 – 14.47	0.098
Diastolic blood pressure (mmHg)						
DFI_V	0.060	0.128	9.89 ± 10.08	0.327	-9.93 – 29.70	0.108
DFI_W	-0.090	0.029*	-14.12 ± 6.70	0.036*	-27.33 – -0.90	0.019
PI	-0.035	0.506	0.35 ± 3.42	0.919	-6.37 – 7.07	0.009
Fasting glucose (mmol/L)						
DFI_V	0.108	0.020†	0.16 ± 0.07	0.179	-0.05 – 0.25	0.120
DFI_W	-0.036	0.245	-0.01 ± 0.04	0.755	-0.09 – 0.07	0.116
PI	0.017	0.745	-0.03 ± 0.02	0.185	-0.08 – 0.01	0.120

[a] Pearson's correlation; [b] Adjusted for age and gender; [c] Proportion of variation due to multivariate linear regression that is explained by age, gender and the various functionality indices.

* Significant correlation in a direction favourable to health.

† Significant correlation in a direction unfavourable to health.

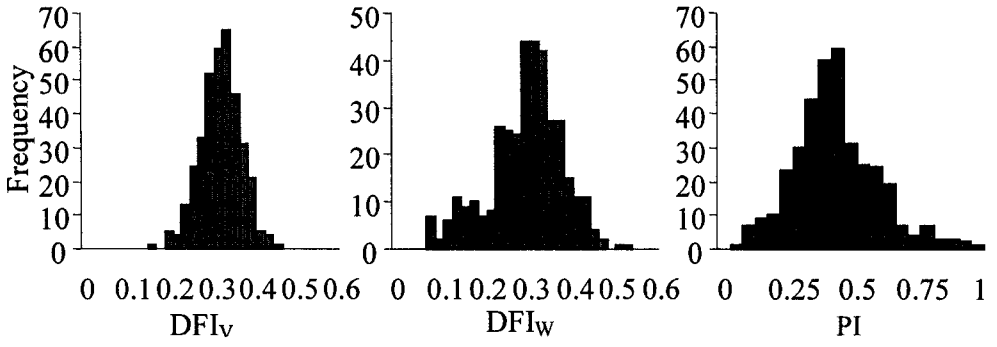

Figure 5.1. Frequency distribution patterns of functionality indices calculated from the diets of three coastal Papua New Guinean villages representing rural, semi-rural and urban conditions. DFI_V: dietary functionality index - variety depicts the ratio of functional foods to total food in terms of number of items, and DFI_W in terms of weight in grams. PI: phytochemical index is the ratio of energy derived from plants.

BRIDGE 3

Despite the difficulties and limitations in ascribing functionality to dietary patterns, we have demonstrated some significant correlations between our DFI score system and metabolic health parameters. With continuously emerging research in the field of food functionality and phytochemical composition, the DFI will undoubtedly become a more reliable tool in nutrition science. The next logical progression is to identify particular foods that impart disease-specific protection, and whether their elimination from the diet results in adverse long term health outcomes.

Quantitative ethnobotanical and food frequency surveys conducted in Koki, Kalo and Wanigela identified plants with differential patterns of use in each community. Betel quid, guava bud tea and to some degree, noni, were consumed mostly in Kalo. Koki residents used noni products regularly and ate mangrove bean once or twice per week, while Wanigela villagers subsisted on the "beans" as their primary source of energy. Recognizing the potential diabetogenicity of betel quid, we postulated that certain plants accelerate or delay insulin resistance when interfaced with other environmental diabetogenic factors. As a preliminary test, we used a cell culture system to examine the glucose transport-mediating effect of plants. Manuscript 4 thus bridges the field and laboratory components of the thesis and attempts to extrapolate experimental findings to community usage patterns.

CHAPTER 6

MANUSCRIPT 4

CONSUMPTION OF GUAVA (*PSIDIUM GUAJAVA* L.) AND NONI (*MORINDA CITRIFOLIA* L.) MAY COUNTER BETEL QUID DIABETOGENICITY IN COASTAL PAPUA NEW GUINEA

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ABSTRACT

Despite compelling evidence supporting the diabetogenicity of betel quid (BQ), BQ-chewing Kalo residents of coastal Papua New Guinea have a low incidence of type 2 diabetes (DM2). In contrast, neighbouring Wanigela, who abstain from BQ, have an unusually high incidence of the disease. A comparative dietary and ethnomedical analysis between the two communities was undertaken to identify traditional plants that may counteract the diabetogenicity of BQ or mediate DM2 risk. In Kalo, guava bud (*Psidium guajava* L.) and noni (*Morinda citrifolia* L.) were consumed more frequently than in Wanigela, whereas the inverse was observed for mangrove bean (*Bruguiera gymnorrhiza* (L.) Lam.). These plants, along with BQ and its component ingredients areca nut (*Areca catechu* L.) and *Piper betle* L. inflorescence, were assessed for their ability to mediate insulin-dependent and -independent glucose transport in cultured 3T3-L1 adipocytes. Results indicate a dose-dependent inhibition of insulin action from methanolic extracts of BQ, areca nut (AN) and *P. betle* inflorescence (PBI), supporting the plant's status as a diabetogenic agent. Conversely, guava bud (GB) extract displayed significant insulin-mimetic and potentiating activity. Noni fruit (NF), leaf (NL), and commercial juice (NJ), all displayed insulin-like activity but had little or no effect on insulin action. Habitual intake of these plants is proposed to offer some protection against DM2 development and/or betel quid diabetogenicity, whereas the modest insulin-mimetic effect displayed by cooked mangrove bean (MBC) may not.

INTRODUCTION

Compelling epidemiological and animal studies suggest an association between betel quid (BQ) chewing and type 2 diabetes mellitus (DM2) (Boucher *et al.*, 1994; Mannan *et al.*, 2000; Tung *et al.*, 2004). In Papua New Guinea, chewing BQ was the predominant independent risk factor for DM2 (odds ratio 3.4; 95% CI. 2.0-5.9) (Benjamin, 2001) . However, DM2 is rare in Kalo, a coastal community where residents habitually and avidly chew BQ (Dowse, 1994). In contrast, the ethnically similar and geographically adjacent Wanigela people have been recorded as having an unusually high incidence of DM2 in both urban and rural settings (Dowse *et al.*, 1994), yet as Seventh-Day Adventists they abstain from chewing BQ.

Beyond their macronutrient composition and glycemic index, several food and medicinal plants have antidiabetic effects attributed to phytochemicals that are able to stimulate pancreatic insulin secretion, reduce peripheral insulin resistance or decrease carbohydrate absorption (Marles & Farnsworth, 1995). Conversely, plants such as areca nut can be diabetogenic (Tung *et al.*, 2004). Differential consumption patterns of pro- and antidiabetic traditional plants may mediate DM2 risk over and above the risk imposed by dietary and lifestyle changes associated with acculturation.

The aim of this study was to identify traditional plants that are utilized or absent from the food and medical systems of Kalo and Wanigela that may impart some protection or risk towards DM2 and BQ diabetogenicity. An ethnomedical survey was conducted in each community and plants that were quantitatively and/or qualitatively significant were selected for further analysis. Insulin-mimetic and -potentiating effects of methanolic plant extracts were assessed using cultured 3T3-L1 adipocytes, a cell line that contains both the GLUT 1 and GLUT 4 glucose transporters and is used extensively to study insulin resistance (Thomson *et al.*, 1997).

MATERIALS AND METHODS

Study area

A quantitative ethnobotanical survey of medicinal and food plants was conducted in Kalo (Central Province, 10.050° S, 148.200° E), Koki (National Capital District, 9.483° S, 147.167° E), and Wanigela (Central Province, 10.050° S, 147.783° E) from January to August, 2004. Wanigela is a lagoon stilt village located approximately 400 km east from the capitol. Some residents engaged in subsistence fishing and farming, while others relied on city relatives to provide trade store goods. Koki, a permanent Wanigela settlement located in a relatively affluent suburb of Port Moresby, had direct access to grocery stores and open air markets. Both Wanigela and Koki practice the Seventh-Day Adventist religion which emphasizes health and diet by advocating vegetarianism and abstinence from stimulants such as betel quid, alcohol, caffeine and tobacco. Residents of Kalo, a road-connected village located roughly equidistant between Koki and Wanigela, are ethnically similar and are predominantly subsistence farmers and fishermen.

Participants were selected by stratified random sampling according to gender and age and administered a quantitative questionnaire modeled after the Expanded Diabetes Diagnostic Criteria (EDDC) developed by Carlson (1995). The questionnaire invited participants to list all plants indicated for DM2 symptoms. All individuals above 16 years were eligible to participate. The survey covered a final sample of 365 participants roughly divided between the three villages. Approval from the head of each local level government was obtained, followed by a public information session and individual prior informed consent before initiation of the study. Permission and ethics approval was obtained from the McGill University Ethics Committee, the Papua New Guinea Medical Advisory Board and the Papua New Guinea Department of Environment and Conservation.

Anthropometric measurements and blood glucose determination

Methods used to measure weight, height, waist and hip circumference, tricep skinfold thickness (TSF), body fat composition, systolic (SBP) and diastolic (DBP) blood

pressure and fasting blood glucose (FBG) levels are described elsewhere (Owen *et al.*, 2007a).

Plant selection and extract preparation

Plants included in the present study were selected from a combination of quantitative and qualitative measurements determined by the frequency of their usage, the EDDC questionnaire and their local sociocultural importance. The protocol used for methanolic extraction is described elsewhere (Owen *et al.*, 2007b). Voucher specimens were deposited at the University of Papua New Guinea and McGill University herbarium. Betel quid was prepared by combining approximately 66.6% AN, 26.6% PBI and 6.6% calcium hydroxide before extraction. Cooked mangrove bean was prepared according to traditional methods: thin slices were soaked for 1 h and boiled in two changes of sea water. Noni juice was purchased from a local health food store (Flora Manufacturing & Distributing Ltd, Burnaby, BC).

Cell Culture

3T3-L1 murine pre-adipocytes were purchased from American Type Cell Collection (ATCC; Chicago, IL) and cultured in a humidified 37 °C 5% CO_2 : 95% air atmosphere in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1.0% penicillin-streptomycin antibiotics (Invitrogen Life Technologies, Burlington, ON). Upon 80% confluence, differentiation was initiated by adding 250 µmol/L 3-isobutylmethylxanthine (IBMX), 1 µmol/L dexamethasone (DMX), and 670 nmol/L insulin for approximately 2 d and further continued with FBS and insulin until at least 90% of the adipocytes developed lipid droplets.

Determination of plant extract concentration for cell culture

Plant extracts were solubilized in dimethyl sulfoxide (DMSO) such that final cell medium concentration was 0.1%. Aliquots of plant extract were stored at -20 °C. A trial to determine maximal non-toxic concentrations by observing cellular morphological changes showed that all plants except PBI were well tolerated at concentrations up to 0.20 mg/mL. All extracts were tested at 50, 100 and 200 µg/mL with additional smaller concentrations of 25 and 12.5 µg/mL for PBI.

Glucose Uptake Assay

Following the methods of Martineau et al. (2006), differentiated and confluent 3T3-L1 adipocytes grown in 12-well plates were incubated with either vehicle (DMSO), plant extract or positive control for 18 hours. After this period, cells were incubated for an additional 3 h in serum-free medium. Thereafter, cells were rinsed twice with Krebs Ringer phosphate buffer solution at 37 °C and then treated with 0, 1, or 100 nM insulin in this buffer for 30 minutes in the presence or absence of plant extract. Cells were then washed twice with glucose-free Krebs and treated with 0.5 μCi/mL 2-deoxy-D-[1-^{3}H]-glucose (TRK-383, Amersham Biosciences, Baie d'Urfé, QC) for 10 minutes at 37 °C without extracts. After incubation, cells were placed on ice and immediately rinsed three times with ice-cold Krebs-phosphate buffer, lysed with 0.1 mol/L NaOH for 30 minutes and scraped. The lysate was added to 1 mL of liquid scintillation gel (Ready-Gel 586601; Beckman Coulter Inc., Fullerton, CA) along with 1.0 mL distilled water and incorporated radioactivity was measured in a scintillation counter. A well-recognized hypoglycemic plant extract, fenugreek seed (*Trigonella foenum-graecum* Linn.) methanolic extract (Vats *et al.*, 2002) was used as a positive control at a non-toxic dose of 75 μg/mL (data not shown). Results are derived from the average of three plates from three independent experiments and expressed as the change in glucose uptake activity relative to basal levels obtained from incubation with the vehicle.

Statistical Analysis

Differences in consumption patterns, anthropometric and clinical measures between genders and between villages were assessed using independent t-tests. Spearman's correlation for non-normally distributed data was used to find associations between consumption patterns and health parameters. To explore the associations between plant intake and FBG, FBG was first normalized by inverse transformation (100-1/FBG) and multivariate linear regression performed with age, waist circumference and weight as covariates. The insulin-like and insulin potentiating effect of plant extracts in cell culture experiments were compared to insulin in the absence of extract using independent t-test. All data are presented as mean ± SEM from at least 3 independent

experiments performed in duplicate with significance set at $p \leq 0.05$. Analysis was performed with SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Plant and human population characteristics

Collection details of the plants used in this study, their ethnopharmacological indications and extract yield are presented in **Table 6.1**. Although Papua New Guineans consider the classification of foods and medicines as a continuum, most recognize mangrove bean as principally a food, noni and guava bud as principally medicines, and betel quid as a habit akin to chewing gum, but with recognized medical properties.

Consumption patterns of these plants along with the health characteristics of males and females of each community are presented in **Table 6.2**. Both genders living in rural Wanigela were significantly less heavy, had a lower BMI, a lower WHR indicating less prevalence of abdominal obesity, and a lower total body fat percentage compared to age and gender-matched residents of urban Koki and semi-rural Kalo. Despite this, Wanigelans had a prevalence of DM2 (males: 13.3%; females: 2.8%) and hypertension (males: 18.3%; females: 23.1%) equal to that of their urban counterparts. One dietary factor that is shared between the two communities is the consumption of mangrove bean. Almost all of the Wanigelans interviewed relied on mangrove bean as their primary source of energy, and roughly half of the males and 87% of the females in Koki had consumed it at least once during the week. Considered more of a famine food in Kalo, none of the villagers had consumed mangrove bean during the survey period. However, virtually all Kalo villagers regularly chewed BQ, and roughly 15% of the population had consumed an infusion of guava buds and young leaves at least once during the week, and about a fifth had used noni (part not specified). Urban and rural gender differences in noni use differed in that slightly more women in Koki had used noni (8.2 %), while in rural Wanigela, males were more frequent users (11.7 %).

Insulin-like activity of plant extracts in 3T3-L1 adipocytes

The ability of plant extracts to stimulate glucose uptake in 3T3-L1 adipocytes in the absence of insulin is depicted by the left-most data points in Figures 1-4. Of the BQ

ingredients, AN elicited a 40-60% increase in glucose uptake (**Figure 6.1.A**) while PBI had no effect at 12.5 μg/mL and at higher concentrations inhibited glucose transport (**Figure 6.1.B**). Betel quid extract exhibited a combination of these effects, where its AN content produced a 50% increase in glucose uptake at 50 μg/mL, but at higher concentrations, produced an effect more akin to its PBI component as it became progressively antagonistic with higher doses (**Figure 6.1.C**). Of the extracts tested, GB had the strongest insulin-like activity, increasing glucose uptake in a dose-dependent manner up to more than 150% at a concentration of 200 μg/mL (**Figure 6.2.**). Except for the root, all noni extracts at all tested concentrations had significant insulin-like activity, the strongest being the commercially prepared fruit juice which increased glucose uptake by 92% from baseline at 100 μg/mL (**Figure 6.3.**). In contrast, NR significantly inhibited glucose influx (**Figure 6.3.D**). Comparable to GB, MBR exhibited strong activity at all tested concentrations (**Figure 6.4.A**). However, when tannins were removed after traditional processing, mangrove bean's insulin mimetic activity was reduced by ~65%, suggesting that tannins were the primary responsible agent (**Figure 6.4.B**).

Insulin potentiating activity of plant extracts in 3T3-L1 adipocytes

The ability of plant extracts to mediate insulin's action on glucose uptake was assessed at insulin concentrations of 1 and 100 nmol/L, depicted in Figures 1-4 as the middle and right-most data points, respectively. Areca nut extract reduced insulin's action on glucose uptake in 3T3-L1 adipocytes in a dose-dependent manner (**Figure 6.1.A**) while all tested concentrations of PBI inhibited insulin action altogether (**Figure 6.1.B**). When considered together in the BQ admixture, glucose uptake was progressively inhibited in the presence of insulin with increased dose (**Figure 6.1.C**). In contrast, GB displayed significant insulin potentiating activity at higher doses (200 μg/mL), increasing glucose uptake by 2.5 times greater than insulin alone at 1 nmol/L, and 1.6 times greater than at 100 nmol/L (**Figure 6.2.**). No insulin potentiating effect was observed for any of the noni extracts (**Figure 6.3.A-D**), and in the case of NL and NR, 200 μg/mL decreased insulin action. A potent insulin-potentiating effect was observed for the tannin-rich extract MBR, where 50 μg/mL produced a 140% increase in glucose transport at an insulin concentration of 1 nmol/L and a 45% increase at 100 nmol/L (**Figure 6.4.A**). The methanolic extract of the processed form of mangrove bean (MBC) had no effect on

insulin's action (**Figure 6.4.B**), supporting the assumption that tannins were the active agent.

Association between plant intake and population health parameters

Associations between the frequency and amount of plant ingested and selected health parameters were assessed using Spearman's correlation for non-normally distributed data (**Table 6.3.**). Since medicinal plants were not ingested as frequently as food plants, but rather used to relieve symptoms as they arose, correlations could not be reliably calculated for GB and noni. As an alternative, guava bud tea intake data were replaced with frequency data of guava fruit, and this was found to have an inverse correlation with FBG (r=-0.21, p<0.0001) and prevalence of DM2 (r=-0.12, p<0.05). This relationship remained significant when FBG was inverse-transformed and controlled for age, central adiposity and weight (β=-0.117, p=0.024). A simple inverse correlation between BQ and FBG and DM2 was also observed (r=-0.15, p<0.005; r=-0.11, p<0.05, respectively), but this disappeared when covariates were included (β=-0.-014, p=0.787). In contrast, a significant causal relationship between MBC and FBG was observed (r=0.14, p<0.05) and remained significant when controlled for age, weight and central adiposity (β=0.139, p=0.010). There was no correlation however between MBC consumption and DM2 prevalence.

Mangrove bean consumption was associated with lighter body weight, shorter stature, smaller BMI, a thinner waistline and less subcutaneous fat as assessed by TSF thickness. In contrast, all these parameters increased significantly with BQ consumption. Those who consumed more guava fruit tended to have less % body fat (r=-0.11, p<0.05) and lower DBP (r=-0.13, p<0.05), however the latter was more strongly correlated with BQ (r=-0.18, p<0.0001) and MBC (r=-0.19, p<0.0001) intake.

DISCUSSION

The low incidence of DM2 despite relatively high rates of obesity and adiposity, compounded by BQ indulgence in Kalo, suggests exposure to protective environmental factors, likely via diet and ingestion of functional botanicals. Although GB and noni were

quantitatively insignificant in the diet compared to other foods, our findings cannot exclude the possibility of a modest reduction of DM2 associated with long-term use. Phytochemicals are able to exert an antidiabetic effect by interacting with an array of metabolic targets, and insulin-mimetic or insulin-sensitizing activity represents only a fraction of their functionality. These may also affect DM2 risk indirectly through their antioxidant, anti-obesity and anti-hypertensive properties and may also negate the harmful effects of diabetogenic agents.

Diabetogenicity of betel quid and its components

In PNG multivariate analysis had identified BQ chewing as an independent predictor of high fasting capillary blood glucose (p=0.005), surpassing the effect of age (p=0.028), BMI (p=0.061) and region of origin (p=0.056) (Benjamin, 2001). The unstable free-radical generating nitrosated derivates of AN alkaloids are thought to be diabetogenic because of their glucose-like ring-shaped moiety that is able to bind to islet β-cells (Boucher & Mannan, 2002). When young CD1 mice were fed AN in standard feed for 2-6 days, permanent diabetes developed in 8.3% of the animals. The offspring of the areca-fed mice, especially males, developed diabetes in 10.6-30.0% of the various test litters, suggesting that AN consumption induced heritable abnormalities resulting in increased DM2 risk (Boucher *et al.*, 1994). Our cell culture experiments support the hypothesis that BQ is diabetogenic since the extract inhibited insulin-mediated glucose intake in 3T3-L1 adipocytes, and at 200 μg/mL reduced glucose uptake to below baseline levels (**Figure 6.1.C**).

Analysis of BQ's individual plant components showed that AN promoted glucose transport in the absence of insulin, but was a dose-dependent inhibitor when insulin was introduced. This property provides an additional possible explanation for the reported ability of arecoline, the major AN alkaloid, to cause short-term hypoglycemia (Chempakan, 1993). This mechanism is believed to be due to arecoline's ability to inhibit GABA neurotransmitter receptors in islet cells leading to increased secretion of glucagon and somatotropin with compensatory insulin release resulting in hypoglycemia (Boucher & Mannan, 2002). If the insulin-mimetic effect of AN observed in this study operated *in vivo*, ingestion would cause an immediate increase in blood glucose clearance via

incorporation into peripheral tissues, contributing to the hypoglycemic response. Further elucidation of mechanisms is required to evaluate the effect of AN alkaloids on insulin receptors and GLUT4 translocation in insulin-sensitive tissues.

The second BQ ingredient, PBI, had the more negative influence on deoxyglucose transport. PBI extract was the most cytotoxic to 3T3-L1 adipocytes, requiring no more than 12.5 μg/mL to avoid an inhibitory effect and maintain a glucose intake rate equal to that of baseline levels. The cytotoxicity of PBI extract was also observed previously with cultured bovine arterial endothelial cells (Owen *et al.*, 2007b), oral mucosal fibroblasts (Jeng *et al.*, 1994) and oral keratinocytes (Jeng *et al.*, 1999), probably because of the extract's content of safrole, a known human carcinogen (IARC, 2004). Epidemiological findings suggest that chewers who include piper inflorescence rather than the leaf in their masticant have increased risk of developing oral cancer (Ko *et al.*, 1995). In an animal study, the presence of PBI in BQ exacerbated the occurrence of hyperkeratosis to AN alone despite an increase in survival rate (Chiang *et al.*, 2004). From these studies and our own observations, we can surmise that the glucose transport inhibitory activity of PBI was a result of impaired cellular membrane integrity rather than through direct inhibition of the GLUT4 signaling pathway.

Despite epidemiological and laboratory evidence supporting the diabetogenicity of BQ, there is little support for an ecological relationship. The practice of BQ chewing dates from before 500 BC, and DM1 or DM2 have been historically uncommon in populations who chew. Nevertheless, a relationship between BQ chewing and DM2 may be justified if the myriad of confounders are considered. Betel quid may indirectly increase susceptibility to DM2 by promoting weight gain and increased waist size. Among a Bangladeshi population residing in east London, waist size and weight increased with increasing use of *paan* BQ, independent of other established risk factors such as central obesity, age, smoking and parity (Mannan *et al.*, 2000). This agrees with the present study, where a significant relationship between BQ chewing and weight (r=0.27, p<0.0001), BMI (r=0.10, p<0.05) and waist circumference (r=0.11, p<0.05) was observed, despite an inverse correlation with DM2 (r=-0.111, p<0.05). However, the latter was rendered non-significant when age, weight and central obesity were accounted

for in the analysis (β=0.014, p=0.787). Additionally, it may also be possible that susceptibility to BQ diabetogenicity could itself be genetically determined in humans, as is the case for the diabetogenic nitroso-compound streptozotocin (STZ) in the mouse where susceptibility depended on the H2 region of the major histocompatability complex (Tanaka *et al.*, 1990).

Insulin-mimetic and potentiating activity of guava

Guava bud and leaves are rich in phenolic compounds, particularly quercetin and gallic acid, which are considered the responsible agents for the plant's therapeutic effects. An antidiabetic effect of guava leaf has been reported in genetically (Oh *et al.*, 2005) and chemically-induced (Ojewole, 2005) animal models of DM2. However, quercetin has been shown to interfere with glucose uptake in adipocytes by possibly interacting with GLUT4 directly rather than by a mechanism related to protein-tyrosine kinase and insulin signaling inhibition (Strobel *et al.*, 2005). Gallic acid on the other hand, reportedly stimulates glucose uptake and enhances insulin sensitivity by activating peroxisome proliferator-activated receptor (PPAR)-γ, a ligand-dependent transcription factor that regulates key metabolic pathways that controls fatty acid oxidation, adipocyte differentiation and insulin sensitivity (Huang *et al.*, 2005b). Gallic acid, along with other guava leaf components including flavonoids, terpenoids, alkaloids and tannins, would explain the particularly strong dose-dependent insulin-mimetic and potentiating effects of the extract on deoxyglucose transport in 3T3-L1 adipocytes observed in this study.

Unfortunately, guava bud and leaf intake was too infrequent to calculate a reliable relationship with population health parameters. However, when guava fruit was considered, we found a significant inverse relationship with DM2 (r=-0.12, p<0.05) and FBG (r=-0.21, p<0.0001), which remained significant after controlling for age, weight and central obesity (β=-0.117, p=0.024). Guava fruit consumption was also favourably associated with body fat composition (r=-0.11, p<0.05) and DBP (r=-0.13, p<0.05). This is not surprising given the soluble fiber and phenolic antioxidant content of the fruit. Guava juice was reported to be hypoglycemic in healthy and alloxan diabetic mice as well as in healthy and diabetic humans (Cheng & Yang, 1983), and in a randomized, single-blind controlled trial, hypertensive patients fed 100g/day guava fruit had lower blood

pressure, significant decreases in serum total cholesterol, triglycerides and an increase in HDL after 4 weeks compared to a control group (Singh *et al.*, 1993).

Insulin mimetic activity of noni

With the exception of the root, all noni tissue extracts stimulated glucose uptake in the absence of insulin, the strongest from the commercial fruit juice (100μg/mL caused a 92.0 ± 22.3 % increase compared to basal glucose intake levels). The responsible bioactive compounds of noni are believed to be anthraquinones, a group of phenolic compounds that have various biological properties depending on the number and position of functional hydroxyl groups and glycosides. There is some evidence supporting the insulin-mimetic action of some anthraquinones and anthraquinone derivatives. Sennidin from *Rheum palmatum* stimulated glucose incorporation into rat adipocytes via the IR, phosphatidylinositol 3-kinase (PI3K)- and Akt-dependent pathway (Abe *et al.*, 2006). The molecule, however, had no potentiating effect when 100 nM insulin was added to the medium, a finding that is similar to our study where all noni extracts did not significantly affect insulin action. Furthermore, NL and NR at 200 μg/mL acted as insulin agonists. This pattern, in which noni displayed insulin-mimetic and dose-dependent insulin inhibitory activity could help explain a previous report for a related species, *Morinda officinale*, where the ethanolic root extract displayed both hypo- and hyperglycemic effects in STZ-diabetic mice depending on the dose (Soon & Tan, 2002). It is possible that a relatively high dose of anthraquinones in noni could alter the structure of the protein factors IR and Akt such that it would no longer relay the glucose uptake signal initiated by insulin. This would explain why noni root extract was a stronger insulin agonist, since the anthraquinones content is higher relative to the rest of the plant. Such a mechanism was reported for the traditional Filipino antidiabetic plant *Lagerstroemia speciosa* L. (30) where the bioactive components were tannins (Liu *et al.*, 2005).

Insulin mimetic activity of mangrove bean

Tannins are polyphenolic compounds that rarely possess biomedical properties, in part because of their astringency and poor bioavailability. Nevertheless, tannins from red wine were reported to have antidiabetic activity in DM2 patients (Gin *et al.*, 1999) which suggests that other tannin-rich food or medicinal plants would have a similar effect. In

Wanigela, unpalatable tannins from mangrove beans were removed by soaking and boiling sliced hypocotyls in two changes of water, resulting in an 80% loss of total phenol content (Owen *et al.*, 2007b). Consequently, cooked mangrove bean extract lost roughly 65% of the insulin-like activity observed in the extract of the raw plant, strongly suggesting that tannins were the responsible agent. Nevertheless, a modest yet significant insulin-mimetic effect suggests that cooked mangrove bean could be of some benefit in preventing DM2. This, however, was not observed in our survey. A positive correlation between MBC frequency intake and FBG (r=0.14, p<0.05) remained significant when controlled for age, weight and central adiposity (β=0.139, p=0.010), despite an otherwise favourable association with weight (r=-0.32, p<0.0001), BMI (r=-0.15, p<0.005), waist circumference (r=-0.19, p<0.0001), TSF (r=-0.15, p<0.005), and DBP (r=-0.19, p<0.0001). It is possible that the starches in MBC are rapidly digested or transformed into glucose, causing a large increase in post-prandial blood glucose and insulin. As of yet, the glycemic index of MBC is unknown. If MBC was a contributor to high FBG concentrations, this would have been detected in the earliest diabetes surveys conducted in Wanigela because of the importance of the plant as a staple food. However, DM2 had only been detected in the village in the late 80s, concurrent with the introduction of non-staple items such as polished rice, refined flour, sugar and edible oils (Patel *et al.*, 1986). The association between MBC and FBG is therefore likely due to other factors that have statistical collinearity with residence in Wanigela, thereby limiting our ability to distinguish the effect of a single food item on chronic disease development.

CONCLUSION

The present study was designed to provide the first step in determining whether consumption of a particular plant could affect DM2 risk or its comorbidities in light of the purported risk incurred by chewing BQ. Of the plants that form Kalo's traditional food and medical systems, guava bud and noni stood out as being distinctive from those of rural Wanigela. Using cultured 3T3-L1 adipocytes as a model of insulin resistance, we demonstrated that guava bud and noni extract possess potent insulin-mimetic activity, the former also having effective insulin-potentiating activity. It is possible that long-term

ingestion of these may protect against BQ diabetogenicity or DM2 associated with socioeconomic transition. A large prospective study that controls for confounders is required to reliably discern any dietary association with lifestyle-related diseases, especially for infrequently consumed plants such as GB and noni. Likewise, different experimental models, particularly animal studies and clinical studies are needed to more accurately explore their antidiabetic potential.

Table 6.1. Characteristics of plants, the area of collection, ethnomedical indications and methanolic extract yield.

Family, species	Plant part	English, local name	Ethnomedical indications[a]	Area collected	Extract yield (%)
Arecaceae					
Areca catechu L.	Nut	Betelnut, Buai	Stimulant / sedative, appetite suppressor / stimulant, anti-malarial	Koki, Kalo	24.35
Myrtaceae					
Psidium guajava L.	Bud	Guava, Tuava	Antidiabetic, antibacterial, anti-malarial, anti-inflammatory, antidiarrheal, gastrointestinal tonic	Kalo	18.35
Piperaceae					
Piper betle L.	Inflorescence	Pepper, Daka	Antibacterial	Koki, Kalo	12.50
Rhizophoraceae					
Bruguiera gymnorrhiza (L.) Lam.	Cooked hypocotyls	Mangrove bean, Kavela	Antimicrobial, insecticidal	Wanigela	2.1
	Raw hypocotyls			Wanigela	23.5
Rubiaceae					
Morinda citrifolia L.	Fruit	Noni, Nono	Analgesic, anti-inflammatory, hypotensive, antibacterial, tonic	Kalo	29.35
	Juice			Commercial	
	Leaf			Kalo	23.30
	Root			Koki	17.25
Betel quid					13.25

[a] Ethnomedical information was obtained from interviews with participants and traditional healers.

Table 6.2. Population characteristics of adult men and women of Kalo, and the Wanigelan urban and rural communities Koki and Wanigela, Papua New Guinean. Data expressed as mean ± SD. The proportion of people who have consumed the plant at least once during the survey week is also included.

	Kalo		**Wanigela**			
	(semi-rural)		Koki (urban)		Wanigela (rural)	
	Male	Female	Male	Female	Male	Female
n	60	61	60	60	60	65
Age (y)	43.2 ± 17.9	42.6 ± 15.3	35.8 ±17.8	42.0 ± 16.5	40.7 ± 16.2	40.7 ± 19.4
Fasting blood glucose (mmol/L)	3.5 ± 1.3	3.6 ± 1.3	4.2 ± 3.5	3.8 ± 1.3	4.8 ± 3.5	3.8 ± 1.3
Diabetes (%)[1]	3.3^{ab*}	1.6	10.2*	5.0	13.3^{*}	4.6
Weight (kg)	$68.3 \pm 15.3^{c*}$	59.1 ± 13.2^{bc}	67.0 ± 17.9^{c}	61.7 ± 17.4^{c}	$59.1 \pm 10.2^{*}$	48.7 ± 8.3
Stature (m)	$1.7 \pm 0.6^{ab*}$	1.6 ± 0.1^{ab}	$1.6 \pm 0.7^{*}$	1.5 ± 0.1	$1.6 \pm 0.1^{*}$	1.5 ± 0.1
Body mass index (kg/m^2)	23.6 ± 4.8	23.1 ± 4.4^{a}	25.3 ± 5.4^{c}	26.0 ± 6.2^{c}	$22.3 \pm 3.1^{*}$	21.0 ± 3.0
Overweight (%)[2]	26.6^{ab}	26.2^{ab}	48.3^{c}	46.6^{c}	6.7	8.5
Waist circumference (cm)	85.6 ± 13.6	85.6 ± 14.7	87.4 ± 14.43	85.4 ± 13.2	80.4 ± 8.85	77.14 ± 8.3
Abdominal Obesity (%)[3]	15.0^{ab*}	32.8^{ab}	13.6^{c*}	35.0^{c}	1.7^{*}	10.8
Waist: hip ratio (cm)	0.93 ± 0.11	0.90 ± 0.13^{ab}	0.94 ± 0.09^{c}	0.87 ± 0.10^{c}	0.92 ± 0.07	0.89 ± 0.06
% Body fat	$21.6 \pm 5.5^{b*}$	32.5 ± 6.7^{ab}	$23.6 \pm 7.7^{c*}$	36.3 ± 6.9^{c}	$18.4 \pm 4.7^{*}$	29.4 ± 5.4
Tricep skinfold thickness (mm)	19.0 ± 6.7	19.0 ± 6.7	13.9 ± 8.2	22.5 ± 9.3	9.4 ± 4.0	13.5 ± 4.9
Excess body fat (%)[4]	49.2^{ab*}	49.2^{ab}	67.2^{c*}	75.0^{c}	33.3^{*}	27.7
Systolic blood pressure (mm Hg)	130.5 ± 18.5	127.5 ± 14.6	129.1 ± 15.9	129.9 ± 18.1	128.4 ± 16.5	132.8 ± 16.7

	Kalo		Wanigela			
	(semi-rural)		Koki (urban)		Wanigela (rural)	
	Male	Female	Male	Female	Male	Female
Diastolic blood pressure (mm Hg)	75.9 ± 10.2[a]	75.5 ± 7.8[ab]	81.4 ± 7	81.1 ± 12.3	79.9 ± 11.3	80.6 ± 7.5
Hypertension (%)[5]	15.0[*]	13.1[ab]	22.0[*]	28.3	16.7	23.1
Proportion who consumed the plant (%)						
Betel quid	93.3[ab]	93.4[ab]	33.3[c]	1.7	6.7[*]	0.00
Guava bud	15.0[ab]	14.8[ab]	1.7	2.0	3.3[*]	0.00
Noni	21.7[ab]	18.0[ab]	2.0[*]	8.2	11.7[*]	0.00
Mangrove bean	0.0[ab]	0.0[ab]	48.3[c*]	86.7	93.3[*]	100.00

[1] Defined as hyperglycemia, fasting blood glucose >0.7 mmol/L; [2] BMI>25.0; [3] Waist circumference >0.102 cm for men; >0.80 cm for women; [4] % body fat >20% for men; >32% for women (American Council on Exercise); [5] Systolic/diastolic blood pressure >140/90 mm Hg; [a]$p<0.05$ between same gender of Kalo vs. Koki; [b]$p<0.05$ between same gender of Kalo vs. Wanigela; [c]$p<0.05$ between same gender of Koki vs. Wanigela; [*]$p<0.05$ between genders of same village

Table 6.3. Spearman's correlation coefficients for the relationship between intake of betel quid, guava and mangrove bean, and fasting blood glucose, diabetes status and selected anthropometric and vascular antecedents.

	Betel quid (g/wk)	**Guava fruit (g/wk)[1]**	**Mangrove bean (g/wk)**
Fasting blood glucose (mmol/L)	-0.154**	-0.213***	0.137*
Type 2 diabetes[2]	-0.111*	-0.116*	0.047 [ns]
Standardized β[3]	-0.01 [ns]	-0.12*	0.14*
Weight (kg)	0.273***	0.092[ns]	-0.315***
Stature (m)	0.409***	0.233***	-0.397***
Body Mass Index (kg/m^2)	0.101*	-0.040 [ns]	-0.149**
Waist circumference (cm)	0.109*	-0.032 [ns]	-0.190***
% Body fat	-0.08 [ns]	-0.109*	-0.023 [ns]
Tricep skinfold thickness (cm)	0.176**	-0.008 [ns]	-0.154**
Systolic blood pressure (mm Hg)	-0.081 [ns]	-0.091 [ns]	0.071 [ns]
Diastolic blood pressure (mm Hg)	-0.184***	-0.132*	-0.190***

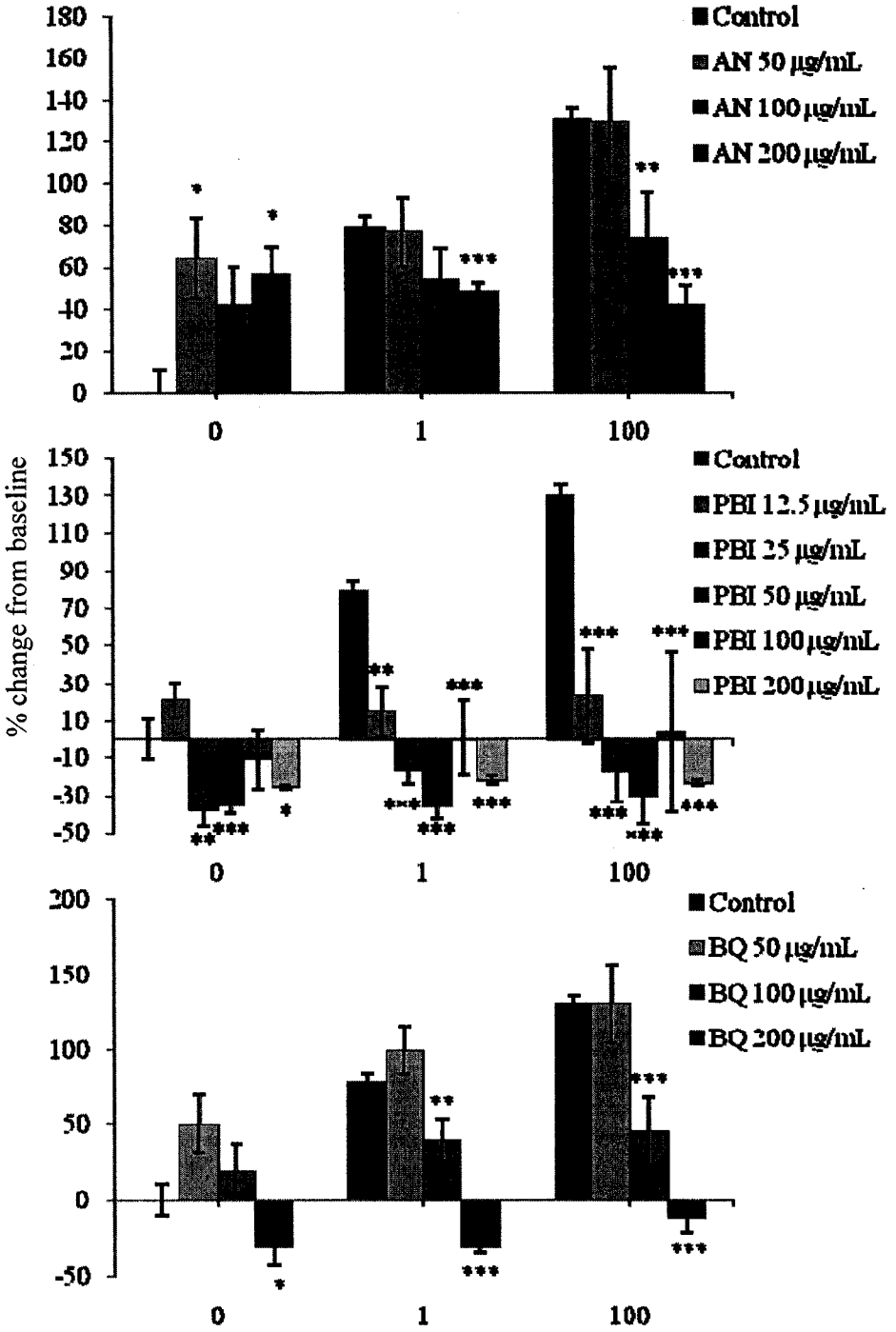
[1]Guava bud tea intake data was supplemented with guava fruit because of infrequent intake.

[2]Nondiabetic=0; diabetic=1.

[3]Continuous dependent variable fasting blood glucose was normalized by inverse transformation (100-1/p) and food intake was entered in multivariable analysis with age, weight and waist circumference as covariables.

*p<0.05; **p<0.005; ***p<0.0001; [ns] =non-significant

Figure 6.1. Percent change from vehicle (DMSO) in glucose uptake in 3T3-L1 adipocytes with various concentrations of A: areca nut (AN), B: Piper betle inflorescence (PBI) and C: betel quid (BQ) MeOH extracts in the absence (0 nmol/L) and presence of 1 and 100 nmol/L insulin. Bars represent the mean ± SEM of n=9 samples from 3 independent experiments. $*p<0.05$; $**p<0.01$; $***p<0.0001$ vs. insulin (control). Bars higher than the control indicate insulin-potentiating activity; lower bars indicate insulin-negating activity.


Control
AN 50 µg/mL
AN 100 µg/mL
AN 200 µg/mL
180
160
140
120
100
80
60
40
20
0
0
1
100
Control
PBI 12.5 µg/mL
PBI 25 µg/mL
PBI 50 µg/mL
PBI 100 µg/mL
PBI 200 µg/mL
% change from baseline
150
130
110
90
70
50
30
10
-10
-30
-50
0
1
100
Control
BQ 50 µg/mL
BQ 100 µg/mL
BQ 200 µg/mL
200
150
100
50
0
-50
0
1
100
Insulin nmol/L

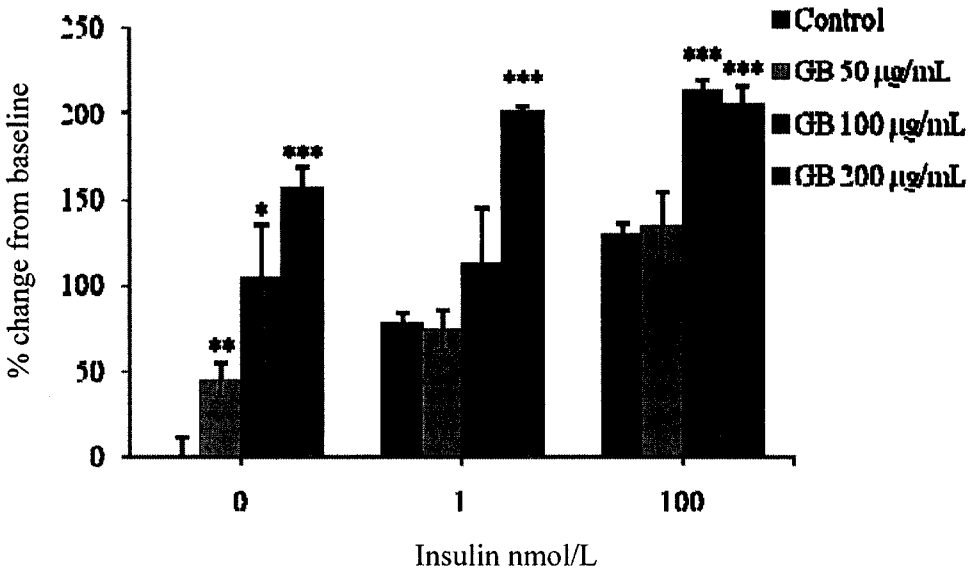
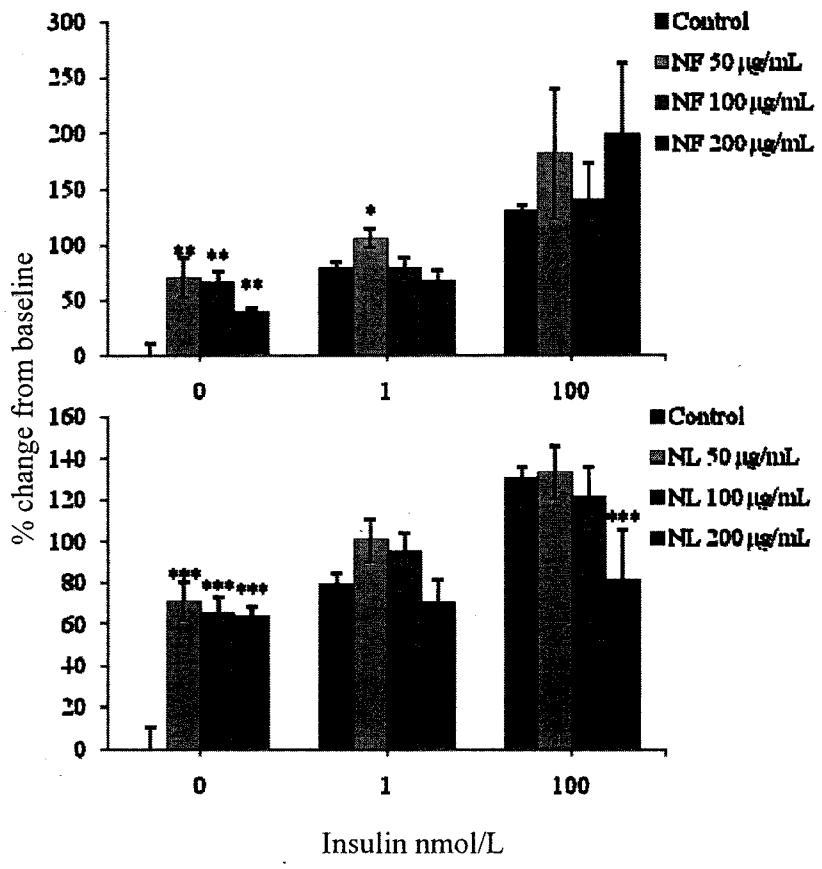
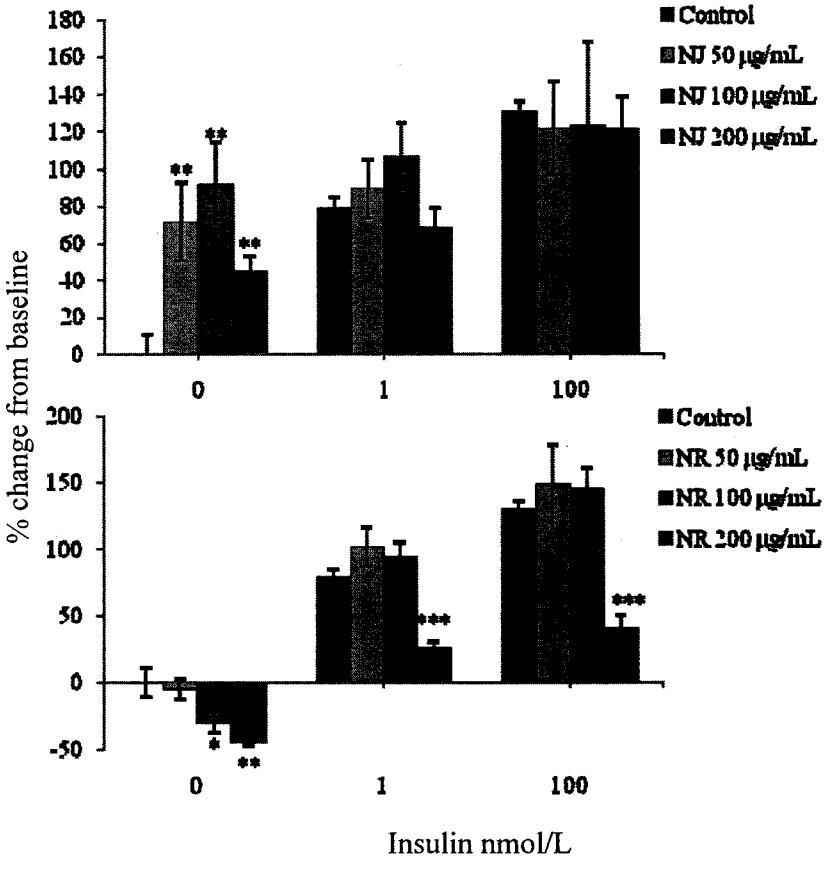


Figure 6.2. Percent change from vehicle (DMSO) in glucose uptake in 3T3-L1 adipocytes with various concentrations of guava bud (GB) MeOH extracts in the absence (0 nmol/L) and presence of 1 and 100 nmol/L insulin. Bars represent the mean ± SEM of n=9 samples from 3 independent experiments. *p<0.05; **p<0.01; ***p<0.0001 vs. insulin (control).

Figure 6.3. Percent change from vehicle (DMSO) in glucose uptake in 3T3-L1 adipocytes with various concentrations of A: noni fruit (NF), B: fruit juice (NJ), C: leaf (NL), and D: root (NR) MeOH extracts in the absence (0 nmol/L) and presence of 1 and 100 nmol/L insulin. Bars represent the mean ± SEM of n=9 samples from 3 independent experiments. *p<0.05; **p<0.01; ***p<0.0001 vs. insulin (control). Bars higher than the control indicate insulin-potentiating activity; lower bars indicate insulin-negating activity.


% change from baseline
Control
NF 50 µg/mL
NF 100 µg/mL
NF 200 µg/mL
Control
NL 50 µg/mL
NL 100 µg/mL
NL 200 µg/mL
Insulin nmol/L


% change from baseline
Control
NJ 50 µg/mL
NJ 100 µg/mL
NJ 200 µg/mL
Control
NR 50 µg/mL
NR 100 µg/mL
NR 200 µg/mL
Insulin nmol/L

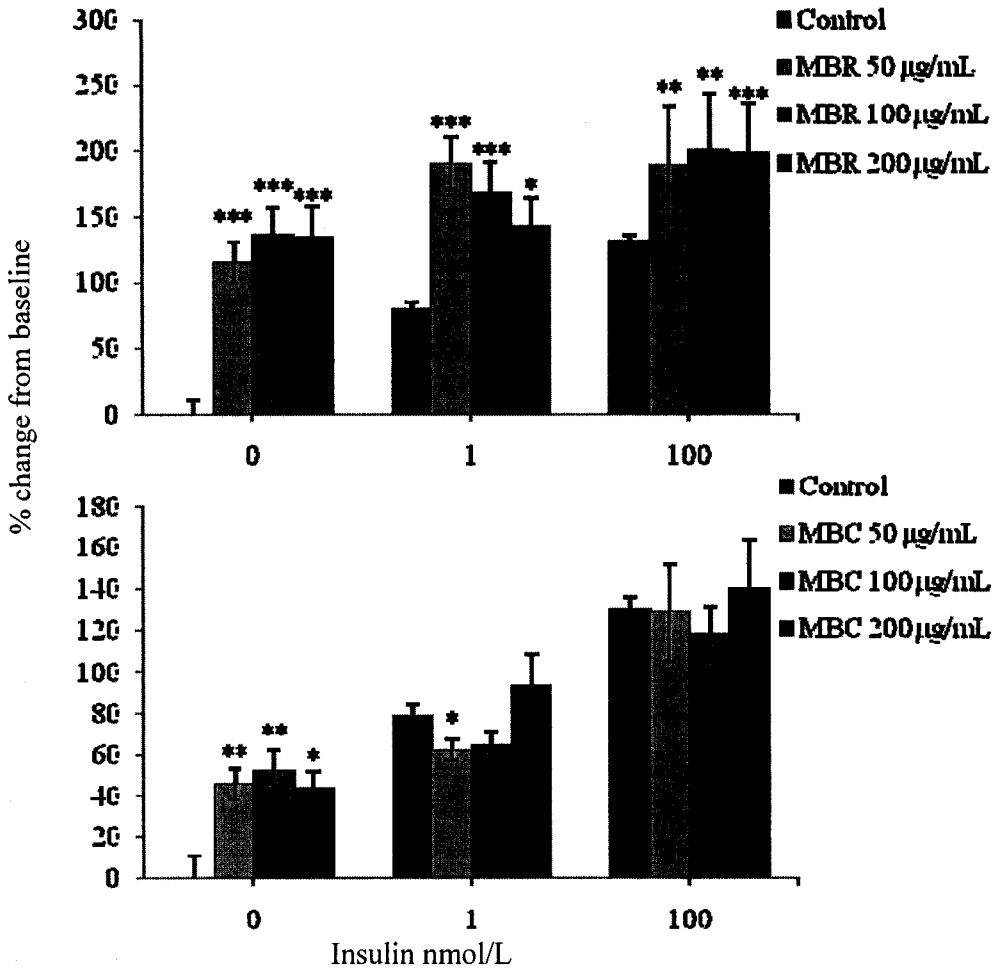


Figure 6.4. Percent change from vehicle (DMSO) in glucose uptake in 3T3-L1 adipocytes with various concentrations of A: raw (MBR) and B: cooked (MBC) mangrove bean MeOH extracts in the absence (0 nmol/L) and presence of 1 and 100 nmol/L insulin. Bars represent the mean ± SEM of n=9 samples from 3 independent experiments. *p<0.05; **p<0.01; ***p<0.0001 vs. insulin (control).

BRIDGE 4

The preceding chapter explored an activity-specific property of plants that suggest potential applicability towards reducing insulin resistance. The focus in the proceeding manuscript shifts to a generic property: that of antioxidant activity. Research acknowledges the important contribution of plant polyphenols as sources of dietary antioxidants towards reducing total plasma oxidative stress and ameliorating cardiovascular health (Kim *et al.*, 2003a). Oxidative stress is an interconnecting condition that links IR to cardiovascular disease, both in their etiologies and as an outcome (Bakker *et al.*, 2000). A key initial step in the genesis of macrovascular disease involves alterations in endothelial function caused by oxLDL cytotoxicity (Tesfamariam, 1994). Several vitamin and phytochemical antioxidants have demonstrated an *in vitro* ability to protect endothelial cells from oxLDL-induced apoptosis via non-antioxidative mechanisms (Mabile *et al.*, 1995; Furman *et al.*, 2001; Martin-Nizard *et al.*, 2002; Tsai *et al.*, 2003b; Steffen *et al.*, 2005). Few studies, however, have used this model to test multi-component preparations or crude extracts.

In Manuscript 5, associations are made between an extract's phenolic content, its antioxidant activity towards LDL peroxidation, and its ability to protect cultured endothelial cells from oxLDL. The model also enabled the determination of an extract's toxicity in the absence of oxLDL. Possible responsible constituents and extracellular interactions are discussed.

CHAPTER 7

MANUSCRIPT 5

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ENDOTHELIAL CYTOPROTECTION FROM OXIDIZED LDL BY SOME CRUDE MELANESIAN PLANT EXTRACTS IS NOT RELATED TO THEIR ANTIOXIDANT CAPACITY

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ABSTRACT

Habitual consumption of some Melanesian medicinal and food plants may influence atherosclerosis development via their antioxidant capacity at the endothelial level. Areca nut (AN; *Areca catechu*), piper inflorescence (PBI; *Piper betle*), betel quid (BQ), guava buds (GB; *Psidium guajava*), the leaves (NL), juice (NJ), fruit (NF) and root (NR) of noni (*Morinda citrifolia*) and the propagules of raw (MBR) and cooked (MBC) mangrove (*Bruguiera gymnorrhiza*) were evaluated for their ability to scavenge the 1,1-diphenyl-2-picryl-hydrazyle (DPPH) radical, to protect human LDL from Cu^{2+}-catalyzed oxidation and to protect cultured bovine aortal endothelial cells (BAEC) from oxidized LDL (oxLDL)-induced cytotoxicity. Polyphenol-rich extracts AN, PBI and BQ were potent DPPH scavengers, having similar activity to quercetin and able protect LDL from oxidation in a dose-dependent manner at concentrations higher than 10 μg/mL, but were pro-oxidants at lower concentrations. These extracts were cytotoxic to BAEC at concentrations above 10 μg/mL and were unable to prevent oxLDL endotheliopathy. GB and NR at 10 μg/mL displayed both the ability to delay LDL oxidation and prevent oxLDL cytotoxicity, although the latter lacked the ability to scavenge the DPPH radical. The remaining noni extracts NF, NJ, NL, and both mangrove extracts MBC and MBR were unable to protect LDL from oxidation at all tested concentrations, but were effective cytoprotective agents at 50 μg/mL. All extracts were able to prevent an oxLDL-mediated increase in intracellular aldehyde generation but had little effect on extracellular peroxidation as measured by TBARS. Based on this model system, we conclude that the antioxidant benefits of AN, PBI and BQ may be offset by their ability to enhance the cytotoxic effects of oxLDL towards BAEC, while GB and low concentrations of noni and mangrove may be considered antiatherogenic. The discrepancies between our *in vitro* and cellular culture experiments emphasize the importance of experimental conditions in evaluating the antioxidant potential of crude plant extracts.

Keywords: Antioxidants; LDL; bovine aortic endothelial cells; LDH; TBARS; Melanesian plant extracts

INTRODUCTION

Differential rates of metabolic disease in Melanesia are underlined by contrasts in dietary and medicinal plant use. In an epidemiological investigation of traditional dietary and medicinal plant use patterns and cardiovascular health in coastal Papua New Guinea, plants that were routinely used in one setting and absent in others were collected for laboratory testing. Betel quid (BQ), a socioculturally important masticatory comprised of areca nut (AN; *Areca catechu*), the inflorescence of *Piper betle* (PBI) and slaked lime is chewed by the vast majority of the population as a psychoactive stimulant. An infusion of guava buds (GB; *Psidium guajava*) was consumed habitually in a few coastal villages as a gastrointestinal and circulatory tonic. Noni (*Morinda citrifolia*), perhaps the most popular medicinal in the Pacific region, was used by most coastal communities as a tonic. Traditionally, the fruit and its juice were eaten for the treatment of asthma, tuberculosis and diarrhea. Leaves were most often used externally to treat wounds and sores but occasionally consumed for mouth sores and stomach aches. The root tea was ingested as a treatment for urinary disorders. The hypocotyls of wild mangrove (*Bruguiera gymnorrhiza*) form the primary energy staple of a small number of remote rural villages.

Growing evidence supports an association between the consumption of antioxidant-rich foods and reduced risk of atherosclerosis (Dragland *et al.*, 2003). A common hypothesis concerns the inhibition of low-density lipoprotein (LDL) oxidation, a key step that would otherwise initiate a pathological response resulting in chemotaxic migration of monocytes to the subintima of blood vessels and formation of characteristic macrophage-foam cells that are inherent in atherosclerotic lesions (Aviram & Fuhrman, 1998). Other biological effects of oxidized LDL (oxLDL) include up-regulation of endothelial adhesion molecules, expression and release of growth factors and cytokines, and proliferation of smooth muscle cells (Holvoet & Collen, 1998). Oxidized LDL is further atherogenic in promoting vascular dysfunction by directly exerting cytotoxicity. Concomitant intracellular increases in TBARS and Ca^{2+} are also associated with oxLDL cytotoxicity towards cultured endothelial cells, resulting in cell death (Cominacini *et al.*, 2001).

Several dietary factors such as α-tocopherol (Mabile *et al.*, 1995) and plant phenolics (Vieira *et al.*, 1998) are able to protect endothelial cells from oxLDL cytotoxicity through a non-antioxidant mechanism involving inhibition of intracellular signaling events. However, other phenolic compounds, such as quercetin, display conditional pro-oxidant activity and cytotoxicity, highlighting the importance of a phenol's molecular structure and experimental conditions (Furman *et al.*, 2001).

Concomitant with a loss of traditional dietary and medicinal practices in developing nations is a dramatic increase in the incidence of cardiovascular disease. The need to identify protective elements in indigenous systems as a culturally sensitive health-promotion strategy prompted us to examine the antioxidant properties of selected plants in relation to cardiovascular health, irrespective of their traditional use. In this study, crude MeOH plant extracts were screened for general free-radical scavenging activity using the stable radical DPPH. Various extract concentrations were then tested *in vitro* for their ability to affect lag time before the appearance of conjugated dienes and production of TBARS resulting from Cu^{2+}-induced LDL oxidation. Lastly, extracts were incubated for 24 h with bovine aortic endothelial cells (BAEC) to determine maximal nontoxic concentrations by measuring extracellular LDH release, after which oxLDL was added and cytotoxicity monitored up to 6 h. Extra- and intracellular generation of aldehydes resulting from lipid peroxidation was measured as TBARS in the medium and lysate, respectively, to assess an extract's ability to confer cellular resistance to oxLDL-induced oxidative stress. Although biomechanisms were not elucidated, possible bioactive components that explain association patterns between the antioxidative and cytoprotective properties of plant extracts are discussed.

MATERIALS AND METHODS

Plant collection and extract preparation

Fresh plant material was collected in Papua New Guinea (**Table 1.**), oven-dried at 100° C for 1 h and brought back to Canada for methanolic extraction as described in Owen and Johns (2002). Voucher specimens were deposited at the University of Papua

New Guinea and McGill University herbarium. Betel quid (BQ) was prepared by combining approximately 66.6 % *A. catechu*, 26.6 % *P. betel* and 6.6 % calcium hydroxide before extraction. Cooked mangrove bean (MBC) was prepared according to traditional methods: thin slices were soaked for 1 h and boiled in two changes of sea water. Noni juice (NJ) was purchased in a local health food store (Flora Manufacturing & Distributing Ltd, Burnaby, BC).

Total and water-soluble phenol concentration

Total and water-soluble phenol concentrations were determined using the Folin-Ciocalteau method as described in Owen and Johns (1999). Phenol concentration was obtained from a standard curve of tannin and expressed as mg tannic acid equivalents (TAE) / g extract.

Free radical scavenging activity

Methanol crude extracts were screened for non-specific free radical scavenging activity using the 1,1-diphenyl-2-picryl-hydrazyle (DPPH) radical as described in Owen and Johns (2002). The efficiency concentration at 50% (EC_{50}) was calculated by determining the extract concentration required to quench all DPPH radicals in solution, determined by observing no further change in absorbance with increased concentration, and dividing it in half. Results were standardized to account for interference from pigments. Ascorbic acid, quercetin and epicatechin (Sigma Chem. Co.) were used as standards.

Low-density lipoprotein preparation

Human LDL in solution containing 0.15 NaCl and 0.01% EDTA (Intracel, Frederick, MD) was diluted in 4 mL PBS and passed through a Sephadex PD-10 column (Pharmacia Biotech, Uppsala) to remove most of the NaCl and EDTA. Lipoprotein concentration was estimated with Sigma Diagnostics protein assay kit (Sigma Chem. Co.) using bovine serum albumin as a standard. For cell culture, extensive modification of LDL (MDA-rich with high lipid peroxide content) was obtained by overnight (8 h) incubation with 15 μM $CuSO_4$ at 37°C, sterilized by filtration (0.2 μm Millipore membrane).

Conjugated dienes formation

Lag time was measured using the methods of Esterbauer et al. (1992). In a UV-transparent microtiter plate, 100 μg/mL LDL protein, 5 μM $CuSO_4$ dissolved in PBS, and 1, 5, 10 and 25 μg/mL plant extract dissolved in 50% MeOH were mixed to a final volume of 200 μL and absorbance continuously monitored at 234 nm using a uQuant™ universal microplate spectrophotometer (Bio-Tek Instruments, Inc.). Corresponding concentrations for ascorbic acid (5.67, 28.39, 56.78 and 141.95 μM) and Trolox® (4, 19.98, 39.95 and 99.89 μM) were used as positive controls (data not shown). Results are expressed as percent (%) increase or decrease in lag time relative to the lag time of Cu^{2+}-oxidized LDL (oxLDL) incubated without extract or standard.

Measurement of thiobarbituric acid reactive substances (TBARS)

Following the methods of Sobal et al. (2000), 100 μg/mL LDL protein, 5 μM $CuSO_4$ dissolved in PBS, and 1, 5, 10 and 25 μg/mL plant extract dissolved in 50% MeOH were made up to 1 mL with PBS and left to incubate at room temperature. At 0, 90, 180 and 360 minutes, 120 μL was removed in duplicate, placed on ice for 5 min and 10 μL each of 10 μM BHT and 400 μM EDTA added to halt oxidation. Thereafter, 50 μL 50% (w/v) trichloroacetic acid (Sigma Chem. Co.) and 75 μL 1.3% (w/v) thiobarbituric acid was added and the reaction mixture incubated at 60°C for 40 min. Tubes were then cooled on ice for 5 min, followed by centrifugation at 2000 g for 10 min, after which 200 μL of the supernatant was transferred to a microtiter plate and fluorescence (excitation wavelength 510 nm / emission wavelength 553 nm) recorded using a Wallac Victor^2 multilabel counter (PerkinElmer Inc.). Concentration of TBARS was obtained using a 1, 1, 3, 3-tetraethoxypropane (malondialdehyde, MDA) standard curve and results expressed as percent (%) increase or decrease relative to the TBARS levels generated from Cu^{2+}-oxidized LDL (oxLDL) incubated without extract or standard. Ascorbic acid and Trolox® were used as positive controls (data not shown).

Cell Preparation

Bovine aortic endothelial cells (BAEC) were maintained in 12-well plates containing Dulbelco's modified Eagle's medium (DMEM; Life Tech. Inc., Burlington,

ON) with 0.3 g/L glutamine, supplemented with 10 % (v/v) fetal bovine serum (FBS), 20,000 U/L penicillin and 20,000 μg/L streptomycin in a humidified 37°C atmosphere containing 5% CO_2 and 95% air. Cells had fewer than 5 passages and all experiments performed 2-d post-confluence.

Cytotoxicity of plant extracts and oxLDL

Cytotoxicity expressed as percent (%) lactate dehydrogenase (LDH) release ([extracellular LDH] / [intracellular LDH]*100) was measured using a commercially available kit (Sigma Chem. Co.). Plant extracts and the positive control BHT were dissolved in DMSO and added to wells at 0.01% final volume. DMSO was tested alone to ensure that any activity was not due to the vehicle. Prior to the addition of oxLDL, plant extracts, 10 μM BHT and DMSO were incubated for 24 h in order to allow any possible incorporation into BAEC. The highest concentration of extract tolerated by BAEC without incurring any significant increases in LDH release over 24 hours was found to be 10 μg/mL for AN, PBI, BQ, GB and NR, and 50 μg/mL for the remaining extracts. Following 24 h incubation, 100 μg oxLDL was added to the medium containing plant extract, BHT or DMSO and LDH release measured in the supernatant and lysate at 0, 180 and 360 min. Native LDL was measured in the absence of test samples. Results are expressed as percent (%) LDH increase relative to normal baseline levels of cells grown in the absence of oxLDL and plant extracts.

Measurement of cellular TBARS

Extra- and intracellular aldehyde generation were determined as TBARS in the medium and lysate respectively. At 0, 180 and 360 min, plates were placed on ice for 5 min and the medium immediately collected and separated into aliquots of 120 μL to which 10 μL of 10 μM BHT and 400 μM EDTA were added to halt oxidation. For lysates, cold PBS was added and cells were scraped, sonicated for 10 min, centrifuged at 250x g for 5 min and supernatant collected and treated as per the protocol for the medium. Determination of TBARS was as above. Results are expressed as percent (%) increase or decrease in TBARS concentration relative to normal baseline levels of cells incubated in the absence of oxLDL and plant extracts.

Statistics

Results are expressed as mean ± SEM from at least three independent experiments performed in duplicate. A one-tailed ANOVA with post-hoc Tukey's was used to test significant differences between samples for DPPH. Differences in lag time, in vitro and cellular TBARS and cellular LDH were assessed using Student's t-test. Spearman's correlation was used to assess associations between assays. Significance was established at $p<0.05$.

RESULTS

DPPH scavenging assay

Several phenolic compounds are known antioxidants able to scavenge free radicals. In this assay, plant extracts that had a higher content of total and water-soluble phenols (**Table 7.1.**) were better scavengers of DPPH than those with little or no phenolic content. Thus BQ and its constituents AN and PBI, which had a phenol content of 9.97 ± 0.70, 17.83 ± 1.78 and 11.48 ± 0.37 mg TAE / g extract, respectively, were relatively strong scavengers of DPPH, with activity comparable to that of the antioxidant flavonoid quercetin (**Figure 7.1.**). Guava bud (GB) extract (11.52 g TAE / g extract) demonstrated the highest scavenging capacity of the extracts tested, with an EC_{50} = 8.05 ± 0.49 μg/mL. Raw mangrove bean (MBR) extract also exhibited significant DPPH scavenging activity (EC_{50} = 12.22 ± 0.50) but removal of tannins and phenols via traditional processing (approximately 80% of total phenols in MBR were lost when preparing MBC) caused a complete loss of activity. Similarly, none of the noni extracts were able to scavenge DPPH, likely due to their low phenol content. Spearman's coefficient (r = -0.79, p = 0.0062) indicated a significant association between the total phenol content of an extract and its DPPH scavenging activity.

In vitro *inhibition of Cu^{2+}-mediated LDL oxidation*

To determine whether plant extracts could prolong the lag time before the appearance of conjugated dienes, a product of lipid peroxidation, different concentrations

of extract were incubated with LDL and $CuSO_4$, and continuously monitored at 234 nm. In the absence of antioxidants, progressive oxidation and deterioration of the LDL particle occurred after approximately 180 min after addition of $CuSO_4$. Lag time was substantially prolonged by extracts with relatively high phenol content, but only at concentrations higher than 10 μg/mL (**Figure 7.2.**). Lower concentrations were found to be pro-oxidant. Potent antioxidant activity was demonstrated by BQ because of its PBI constituent, which was able to prolong lag time by 164 % at 9 μg/mL relative to LDL oxidized in the absence of extract or standard. The AN component of BQ also displayed an ability to prolong lag time, but only at concentrations higher than 8 μg/mL. The root of noni was the only extract from the plant able to extend lag time, although its effect was inferior in comparison to PBI, AN, BQ and GB. In contrast, the remaining noni extracts and both mangrove bean extracts were only able to extend lag time for an additional 20 minutes at concentrations below 5 μg/mL, and at higher concentrations, actually enhanced LDL oxidation (data not shown). An exception to this was NJ, which prolonged lag time by 20 minutes for all concentrations tested (1-25 μg/mL). The same pattern was observed for the positive control, Trolox (data not shown).

Another byproduct of lipid peroxidation of the LDL membrane is the generation of aldehydes, measured as TBARS. Analysis of TBARS formation confirmed that the propagation phase of Cu^{2+}-mediated LDL oxidation occurred after 180 min. As expected, native LDL in the absence of Cu^{2+} did not produce significant TBARS (data not shown). Consistent with our conjugated dienes data, phenolic-rich plant extracts, as well as NR, significantly decreased TBARS levels after 3 hours incubation at extract concentrations above 5 μg/mL, except for AN which required 10 μg/mL. Below this concentration, BQ, PBI, AN and GB exacerbated lipid peroxidation ($p < 0.001$) and increased TBARS levels compared to LDL oxidized in the absence of extracts (**Figure 7.3.**). The remaining noni extracts, along with both mangrove bean extracts, either promoted TBARS formation or provided no additional protection against LDL oxidation (data not shown). Because these had similar oxidation patterns, the observed activity for MBR was included in Figure 3 as a representative extract.

Effects of plant extracts on cytotoxicity induced by oxLDL

Histomorphological observations of BAEC 2-day post-confluence revealed the characteristic "cobblestone" monolayer growth pattern. Incubation with 0.1 mg/mL native LDL did not induce any alterations, whereas 6 h incubation with Cu^{2+}-oxLDL resulted in major morphological cellular changes. Cellular contraction and elongation were first observed along with cytoplasmic vacuolization, followed by detachment from the bottom of the well. Substantial cell death occurred after 6 h post-incubation with oxLDL in the presence of phenolic-rich extracts BQ, AN, PBI, GB, as well as NR, indicating enhanced cytotoxic effects of the extracts. Consequently, this required a maximal non-toxic concentration of 10 μg/mL, whereas the remaining extracts NF, NJ, NL, MBC and MBR elicited no change in BAEC morphology at concentrations up to 50 μg/mL. These were therefore chosen as the effective concentrations for our cell culture experiments.

Loss of cell membrane integrity is an indication of toxicity and ensuing cell death. As a result of increased membrane permeability, the intracellular enzyme lactate dehydrogenase (LDH) is released into the extracellular space and its intra- / extracellular ratio used as a marker of cytotoxicity. Incubation of BAEC with oxLDL resulted in a 13 % (p<0.0001) increase in extracellular LDH levels after 6 hours relative to BAEC incubated without oxLDL (**Figure 7.4.**). In comparison, native LDL (nLDL) only elicited a 4% increase. Our positive control, BHT 10 μM, displayed only a modest effect in reducing oxLDL-induced cytotoxicity. Interestingly, BQ (7.72 ± 3.63 % increase in LDH) reduced oxLDL cytotoxicity to a degree lower than both its component ingredients AN (11.64 ± 2.63 %) and PBI (20.12 ± 4.29 %), although the high variance in our data resulted in no statistical difference between BQ and oxLDL after 6 h incubation (p = 0.109). Considering that PBI was the only extract found to promote cytotoxicity after 3 h (p = 0.03) suggests a possible negating effect resulting from the BQ admixture.

The remaining extracts all displayed the ability to decrease (NF, NL, MBR) or altogether inhibit (GB, NL, NR, MBC) LDH release after 6 h incubation. Our findings establish that processing mangrove bean (MBC) enhances BAEC cytoprotection against oxLDL compared to its raw form (MBR).

Extracellular and intracellular production of TBARS in BAEC

Cytotoxicity of cultured BAEC is also characterized by an increase in the lipid peroxidation of the plasma membrane and organelles, resulting in increased generation of extracellular and intracellular aldehydes, respectively, measured as TBARS. Addition of nLDL did not produce significant extracellular or intracellular TBARS for the duration of the incubation time. After 6 h of having added oxLDL to the medium however, extracellular TBARS levels rose to 120 % ($p<0.001$) and intracellular levels to 83 % ($p<0.01$) relative to BAEC cultured without oxLDL, indicating enhanced oxidative stress. None of the extracts were able to inhibit extracellular TBARS generation except for BQ which had a modest yet statistically significant ($p=0.03$) protective effect (**Figure 7.5.**). Every extract tested however, was able to inhibit intracellular oxidation, indicated by TBARS levels that were lower or no different than BAEC incubated without oxLDL.

DISCUSSION

This study demonstrates the ability of crude plant extracts to protect endothelial cells from oxLDL-induced cytotoxicity is independent of their ability to prevent Cu^{2+}-mediated LDL oxidation. This has potentially important implications when considering the benefits of dietary components vis-à-vis atherosclerosis. The pivotal first step in atherogenesis is considered the oxidative modification of LDL and the supplementation of exogenous antioxidants has been shown to prevent or delay this process (Jialal & Fuller, 1995). An important contributor of dietary antioxidants include the polyphenolic phytochemicals that are found abundantly in teas, fruits and vegetables, the consumption of which is almost always positively associated with reduced risk of chronic disease (Duffy & Vita, 2003). When assessed for antioxidant activity in *in vitro* conditions however, several dietary phenolic compounds display both antioxidant and pro-oxidant activity, depending on the structure of the phenol, the functional groups it contains and its consequential redox potential (Cao *et al.*, 1997; Fijisawa *et al.*, 2002). Extract concentration and the presence of transition metals also determine activity outcome. Likewise, these factors have a heavy influence in cell culture systems, where, depending

on the cell line, a compound or extract can exert a cytoprotective or cytotoxic effect (Vieira *et al.*, 1998; Fujisawa *et al.*, 2004).

Based on the DPPH free-radical scavenging assay, the Cu^{2+}-oxidized LDL assay and assessment of oxLDL-induced BAEC cytotoxicity, three different patterns of activity could be described: 1) Phenol-rich extracts that inhibited Cu^{2+}-catalyzed LDL oxidation, but were cytotoxic in themselves or exacerbated the cytotoxic properties of oxLDL; 2) Extracts that inhibited Cu^{2+}-catalyzed LDL oxidation and reduced the toxic effects of oxLDL; and 3) Phenol-poor extracts that exacerbated Cu^{2+}-catalyzed LDL oxidation yet were cytoprotective against oxLDL.

Pattern 1: Antioxidant phenol-rich extracts with cytotoxic tendencies

Pattern 1 includes the extracts BQ and its two ingredients AN and PBI. These were potent DPPH scavengers and able to inhibit or prolong the onset of Cu^{2+}-mediated LDL oxidation at concentrations above 10 μg/mL but were pro-oxidants at 1 μg/mL (Figures 2 & 3). Both AN and PBI contain the phenol hydroxychavicol, a known antioxidant able to effectively scavenge a variety of reactive oxygen species (ROS) (Chang *et al.*, 2002). The molecule was also found however, to induce oxidative stress and cytotoxicity towards cultured Chinese hamster ovary cells and $HepG_2$ cells (Lee-Chen *et al.*, 1996; Chen *et al.*, 2000). Similarly, the flavonoids quercetin, eugenol, and isoeugenol, all of which occur in PBI, have been reported to be potent inhibitors of Cu^{2+}-mediated LDL oxidation, but at lower concentrations (10 μM), exacerbated lipid peroxidation (Naidu & Thippeswamy, 2002). In a previous study, an aqueous extract of PBI exhibited strong ROS scavenging activity (2003) but was also found to promote oxidative stress and cytotoxicity towards cultured oral mucosal fibroblasts and oral keratinocytes, causing DNA breaks (1994; 1999). The dual anti- / pro-oxidant activity demonstrated by several phenols is due to the compound's redox potential at a given concentration that favours the formation of the stable phenoxyl radical, which is able to further react with lipid peroxides (Fijisawa *et al.*, 2002).

Transition metals are critical to *in vitro* LDL oxidation mediated by cultured cells, but their role under physiologically plausible conditions is not clear (Esterbauer *et al.*, 1992). Since Cu^{2+} was added simultaneously with the extracts in our *in vitro* assays, it is possible that some plant phenols were able to chelate Cu^{2+}, thus acting as indirect antioxidants by inhibiting binding of the metal to LDL. This is particularly relevant for tannins, phenolic macromolecules able to bind and sequester a number of compounds, including many free radicals and transitional metals (Chung *et al.*, 1998). Tannins are especially abundant in AN and may account for its antioxidant activity. However, AN also contains a relatively high concentration of the transitional metals copper (3-188 μg/g dry weight) and iron (~75 μg/g) (Trivedy *et al.*, 1997) which can exacerbate the oxidation of phenols and lipids. This would explain why a higher concentration of AN (8 μg/mL), relative to the other phenol-rich extracts, was needed to convert the extract from a pro-oxidant to an antioxidant in our LDL oxidation assays (**Figures 7.2. & 7.3.**). The AN phenol / transition metal ratio at concentrations above 8 μg/mL shifted the extract's redox potential towards antioxidation.

In our cell culture experiment conditions, cells were preincubated for 24 hours with the extract, the medium removed and the extract reintroduced along with extensively oxidized LDL. It is important to note that Cu^{2+} added singly to BAEC did not induce significant LDH release or TBARS generation after 6 h incubation (data not shown). Therefore, the source of toxic compounds came from the oxLDL particle (such as oxidized polyunsaturated fatty acids (PUFA), oxysterols, and aldehydes), extract phytochemicals or the product of their biochemical interaction. In the case of AN, PBI and BQ, any concentration above 10 μg/mL was found to be cytotoxic after 24 h incubation with BAEC. At this maximal non-toxic concentration, none were able to offer any additional benefit in protecting BAEC from oxLDL-induced cytotoxicity, and in the case of PBI, exacerbated the condition after only 3 h (Figure 4).

Interestingly, the *in vitro* pro-oxidative tendencies of AN and PBI at 1 μg/mL were substantially reduced when combined to form BQ. No effect on lag time was observed for BQ at 1 μg/mL (**Figure 7.2.**) and TBARS production after 6h was lower than either AN or PBI (**Figure 7.3.**), suggesting a sort of synergistic action. This effect

was also observed in our cell culture experiment where BQ was less cytotoxic than AN and PBI and was the only extract able to reduce extracellular TBARS formation 6 h after addition of oxLDL. Given the content of metal ions in AN and the polyphenol content in PBI, the resulting redox potential of BQ essentially depended on the balance between the rate of free radical generation from AN metals and the rate at which they could be quenched by AN and PBI polyphenols. The presence of slaked lime potentially affected the redox potential of phenols by raising the pH. In alkaline conditions ($pH \geq 9.5$), areca polyphenols rapidly undergo autoxidation to form $O_2^{-\bullet}$ and H_2O_2, a reaction that is aggravated by the presence of metal ions (Nair *et al.*, 1992). Our data demonstrated that BQ had an antioxidant capacity between that of PBI and AN, but much closer to that of the former, indicating that PBI polyphenols could easily contend with the enhanced presence of metal ions.

Pattern 2: Antioxidant extracts with cytoprotective effects

Pattern 2 includes the phenol-rich extract GB and the phenol-poor root extract of the noni tree, NR. Both significantly prolonged the antioxidative capacity of LDL against Cu^{2+}oxidation (Figures 2 & 3) and inhibited oxLDL cytotoxicity towards BAEC (Figure 4). GB shares a common property with AN and PBI, in that it displayed pro-oxidant activity at low concentrations (1 μg/mL), indicated by a decrease in lag time (Figure 2) and a rise in TBARS (Figure 3) from Cu^{2+}-oxidation of LDL. One of the more abundant phenolic compounds in GB is quercetin (Liang *et al.*, 2005), which would explain the pro-oxidant / antioxidant duality of the extract, as well as its potent DPPH scavenging capacity. In a previous report, an extract of guava leaf was found to possess equipotent free radical scavenging activity to quercetin (2002). Unlike AN and PBI however, GB exhibited a cytoprotective tendency and inhibited oxLDL cytotoxicity towards BAEC after 6 hours (Figure 4), a property that is likely due to its content of the phenol (+)-gallocatechin. This compound was previously identified as a strong antimutagen, able to protect *Escherichia coli* from UV-induced mutations (Matsuo *et al.*, 1994). This was attributed to the molecular structure of (+)-gallocatechin which contains three adjacent hydroxyl groups; an apparent requirement for phenols to possess this activity. It is plausible that (+)-gallocatechin could equally have protected BAEC from oxLDL-induced cytotoxicity, although further studies are necessary to confirm this.

Noni root extract stands apart from the remaining noni extracts obtained from the leaf (NL), fruit (NF), and fruit juice (NJ) in that it contains a greater content of anthraquinones, a group of compounds that possess antioxidant or pro-oxidant activity depending on the number and position of hydroxyl groups and glycosides on the phenolic structure (Cai *et al.*, 2004). Our results mirror that of Zin et al. (2002; 2006) who observed antioxidant activity in the MeOH extract of NR, but not NF or NL using the ferric thiocyanate method (FYC) and TBARS assay. In their study, further fractionation and retesting of the extracts found no correlation between phenol content and antioxidant activity, suggesting the presence of non-phenolic antioxidants. Although clearly an antioxidant according to our LDL oxidation studies, NR was inferior compared to the phenol-rich extracts AN, PBI, BQ and GB, and possessed virtually no DPPH scavenging ability. In an earlier study, a lack of free radical scavenging activity was also observed for the anthraquinone-rich roots of *Rumex patienta* (Demirezer *et al.*, 2001). Anthraquinones have been reported to be cytotoxic or cytoprotective, depending on such familiar factors as concentration, chemical structure and the cell line used. At relatively high concentrations, anthraquinones are thought to induce oxidative stress in hepatocytes, which might account for the few case reports of hepatoxicity in those who overly consumed noni (Millonig *et al.*, 2005). On the other hand, when an anthraquinone-rich plant extract was incubated in isolated perfused rat hearts, a dose-dependent protective effect against ischemia-reperfusion injury was observed as evidenced by a significant decrease in LDH leakage. Protection in this case was found to be associated with enhanced myocardial glutathione status (Yim *et al.*, 1998). In the present study, NR extract concentrations greater than 10 μg/mL was found to be cytotoxic towards BAEC but at lower concentrations, was able to protect the cells from oxLDL-induced injury. In light of what is known about the biological effects of anthraquinones, they could account for the properties exhibited by NR in this study.

Pattern 3: Pro-oxidant extracts with cytoprotective effects

Pattern 3 includes the remaining noni extracts NF, NJ and NL, as well as the raw (MBR) and cooked (MBC) extracts of the mangrove bean. These were relatively poor in phenol content (Table 1) and lacked the ability to scavenge the DPPH radical (with the exception of tannin-rich MBR, which had an EC_{50} similar to quercetin and the other

phenol-rich extracts AN, PBI, BQ and GB). None were able to protect LDL from Cu^{2+}-oxidation (Figures 2 & 3), and with the exception of NJ, actually shortened lag time and exacerbated TBARS generation with increasing extract concentration. Yet at a concentration of 50 μg/mL, all were able to inhibit oxLDL-induced cytotoxicity towards BAEC for up to 6 hours (Figure 5).

In an earlier study, a 50 μg/mL MeOH and EtOAc fraction of noni juice was reportedly able to inhibit 88 and 96 %, respectively, *in vitro* TBARS production from Cu^{2+}-catalyzed LDL oxidation. The isolated active compounds were found to be a series of lignans whose potency depended on the number of phenolic hydroxyl groups (Salleh *et al.*, 2002; Kamiya *et al.*, 2004). Similarly, a lignan isolated from the n-BuOH soluble portion of noni fruit was found to be a potent DPPH and $ONOO^-$ scavenger (Su *et al.*, 2005). These findings are contradictory to ours, in which noni juice demonstrated very poor DPPH scavenging activity. This discrepancy may be due to differences in sample preparation since we used the juice in its commercial form rather than as an extraction, which may have diluted any antioxidant lignans. The juice did have a modest antioxidant effect in prolonging lag time (16 % longer at 25 μg/mL) before the appearance of conjugated dienes. However, TBARS production suggested a pro-oxidative effect with increasing concentration (data not shown). Likewise our NF extract, which may more closely relate to the juice extract used by Kamiya et al. (2004), also displayed poor DPPH scavenging activity. A lack of antioxidant activity in a MeOH extract of NF was also reported by Zin et al. (2002). In the present study, antioxidant effects were noted at low concentrations (<5 μg/mL) where 1 μg/mL prolonged lag time by 26 % and reduced TBARS by 20 %, but at higher concentrations displayed pro-oxidant activity, shortening lag time by 29% at 25 μg/mL, a concentration half of that used by Kamiya et al.

Our results for NL extract concur with the findings of Salleh et al. (2002), who reported no LDL protective effects against Cu^{2+}-catalysis and only 5 % TBARS inhibition at a concentration of 12.5 μg/mL. Similarly, a NL MeOH extract failed to exhibit antioxidant activity using the ferric thiocyanate method (FYC) and TBARS assay, although like NF, the ethyl acetate fraction was more active (Zin *et al.*, 2002). In this study, a similar NL concentration as that used by Salleh et al. (10 μg/mL) shortened lag

time by a modest 14 % ($p < 0.01$), but had no effect in reducing TBARS after 6 h. Like the roots (NR), noni fruit (NF), leaves (NL) and juice (NJ) contain antioxidant anthraquinones, albeit in much smaller concentrations, which is why their maximal non-toxic concentration was 50 μg/mL rather than the 10 μg/mL required for NR. Differences in anthraquinone concentration may account for the observed pro-oxidant activity in our LDL oxidation experiments, but this did not affect the extracts' ability to inhibit oxLDL-induced cytotoxicity. All were able to prevent LDH leakage after 6 h incubation with oxLDL.

In regards to mangrove bean, our study demonstrates that cooking of the bean via traditional methods removes approximately 80 % of the total phenol content occurring in the raw plant, causing a complete loss of DPPH scavenging activity, but without otherwise affecting its activity towards LDL oxidation and BAEC cytoprotection. Nutritional composition analysis of mangrove propagule shows that it contains 20-50 g starch / 100 g dry weight (Hanashiro *et al.*, 2004). Considering that mangrove starches are more highly branched and viscous compared to other common food sources, it is possible that it prevented the binding of oxLDL to BAEC by either sequestering oxLDL or creating a mechanical barrier via cross-linking with the endothelial surface glycocalyx. Tannins, likewise, would have this same ability and may have been the responsible agent for the cytoprotection displayed by MBR. In the same way, mangrove polysaccharides and tannins could bind to endothelial surfaces, causing alterations in membrane permeability and permselectivity and preventing LDH leakage into the culture medium.

Internalization of oxLDL is mediated by the lectin-like LDL receptor-1 (LOX-1), and is immediately associated with an increase in intracellular oxidative stress and TBARS (Cominacini *et al.*, 2001). Intense and sustained rises in cytoplasmic Ca^{2+} and mitochondrial generation of ROS (Zmijewski *et al.*, 2005) following oxLDL uptake triggers the activation of degradative enzymes resulting in irreversible damage of cellular components and eventual cell necrosis (Furman *et al.*, 1999). In the present study, all extracts were able to block intracellular signaling pathways leading to oxidative stress. In light of the findings of Vieira et al. (1998), who noted that certain phenols were unable to integrate into endothelial cells, it is plausible that the extracts blocked an early event in

the signaling pathway, perhaps at the ligand-binding site. Although all extracts were able to prevent an intracellular rise in TBARS, none were able to contend with the pro-oxidant effects of oxLDL on BAEC lipid membranes after 6 h incubation, represented as extracellular TBARS (**Figure 7.5.**). We know that TBARS in the culture medium did not arise from intracellular sources and subsequently leaked into the medium since there was no correlation between LDH leakage and extracellular TBARS 6 h postincubation. Also, TBARS could not have come from the oxLDL particle or they would have been detected in earlier (3 h) measurements (data not shown). The lack of association between antioxidant activity and cytoprotection in this study is supported by the lack of activity seen with our positive antioxidant control, BHT. This however may be a question of dosage, since a previous study using fibroblasts found that 10 μM BHT had no effect in inhibiting oxLDL-induced toxicity (Morel *et al.*, 1983), whereas a higher dose (50 μM) proved effective (Thomas *et al.*, 1993).

In light of these findings, the association between antioxidant-rich plants and health may not be as strong as purported. Indeed, some antioxidant compounds like those in AN and PBI may inhibit peroxidation but the resulting phenoxyl radicals can exert cytotoxic or mutagenic effects. Likewise, some plant extracts like NF, NJ, NL, and MBC may be able to protect endothelial tissues despite being poor radical scavengers. Clearly antiatherogenic factors need not be antioxidant in nature as long as vascular tissues can be protected from the cytotoxic presence of oxLDL.

CONCLUSION

In the present study, a preliminary examination of the effects of various plant extracts on LDL oxidation and cytoprotection of cultured BAEC in the absence and presence of oxLDL was undertaken without further elucidation of biomechanisms. Methanolic extracts of areca nut (AN), *P. betle* inflorescence (PBI) and betel quid (BQ) contained high concentrations of antioxidant polyphenols able to protect LDL from Ca^{2+}-catalyzed oxidation, but had no effect on oxLDL-induced endotheliopathy. Guava bud (GB) and noni root (NR) extract were also able to effectively inhibit LDL oxidation in a

dose-dependent manner, but also prevented oxLDL cytotoxicity. The same may be said for small concentrations of NF, NJ, NL, MBC and MBR, although greater concentrations exhibited pro-oxidant activity. From these observations, we can conclude that the antioxidant ability of a plant extract is independent of its ability to protect BAEC from the toxic byproducts of extensively oxidized LDL. Further research into functional dietary compounds able to mediate atherogenesis is required in order to develop culturally-specific strategies to maintain cardiovascular health.

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Table 7.1. Characteristics of plants, extract yield, and content of total and water-soluble phenols. TAE: tannic acid equivalents.

Family, species	Plant part	English, local name	Extract yield (%)[a]	Voucher No.	Total Phenols (mg TAE / g extract)	Water soluble phenols (mg TAE / g extract)
Arecaceae						
Areca catechu L.	Nut	Betelnut, Buai	24.35	KAL23	17.83 ± 1.78	10.72 ± 0.71
Myrtaceae						
Psidium guajava L.	Bud	Guava, Tuava	18.35	KAL01	11.52 ± 0.77	9.40 ± 0.18
Piperaceae						
Piper betle L.	Inflorescence	Pepper, Daka	12.50	KAL24	11.48 ± 0.37	7.98 ± 1.32
Rhizophoraceae						
Bruguiera gymnorrhiza (L.) Lam.	Cooked hypocotyls	Mangrove bean, Kavela	2.1	WAN01	1.35 ± 0.94	0.68 ± 0.08
	Raw hypocotyls		23.5		6.38 ± 1.12	4.23 ± 0.12
Rubiaceae						
Morinda citrifolia L.	Fruit	Noni, Nono	29.35	KAL03	1.36 ± 0.36	0.45 ± 0.00
	Commercial juice				0.64 ± 0.26	-0.26 ± 0.01
	Leaf		23.30		2.06 ± 0.99	1.33 ± 0.05
	Root		17.25		1.82 ± 0.07	0.30 ± 0.06
Betel quid			13.25		9.97 ± 0.70	6.15 ± 0.20

[a] Extract yield = (g extract / g dried plant material) x 100

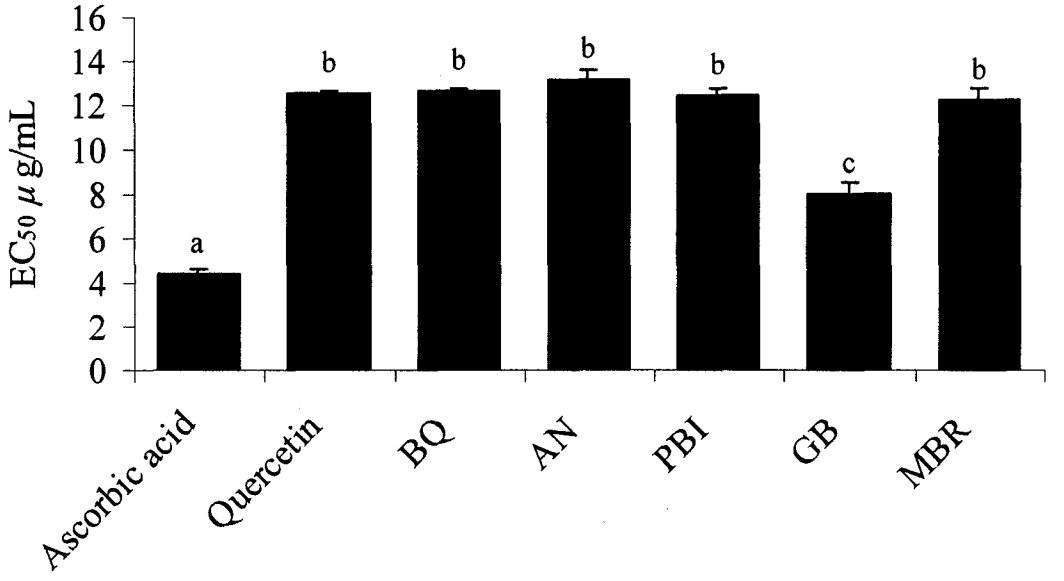

Figure 7.1. Free radical scavenging activity of selected crude plant extracts using the stable radical 1,1-diphenyl-2-picryl-hydrazyle (DPPH). Results are presented as the efficacy concentration at 50 % (EC_{50}) which is the concentration required to quench half of the DPPH radicals in the solution. The antioxidant vitamin ascorbic acid and flavonoid quercetin are included as references. BQ: betel quid; AN: areca nut; PBI: *P. betle* inflorescence; GB: guava bud; MBR: mangrove bean, raw. Different letters represent significant differences at $p<0.05$.

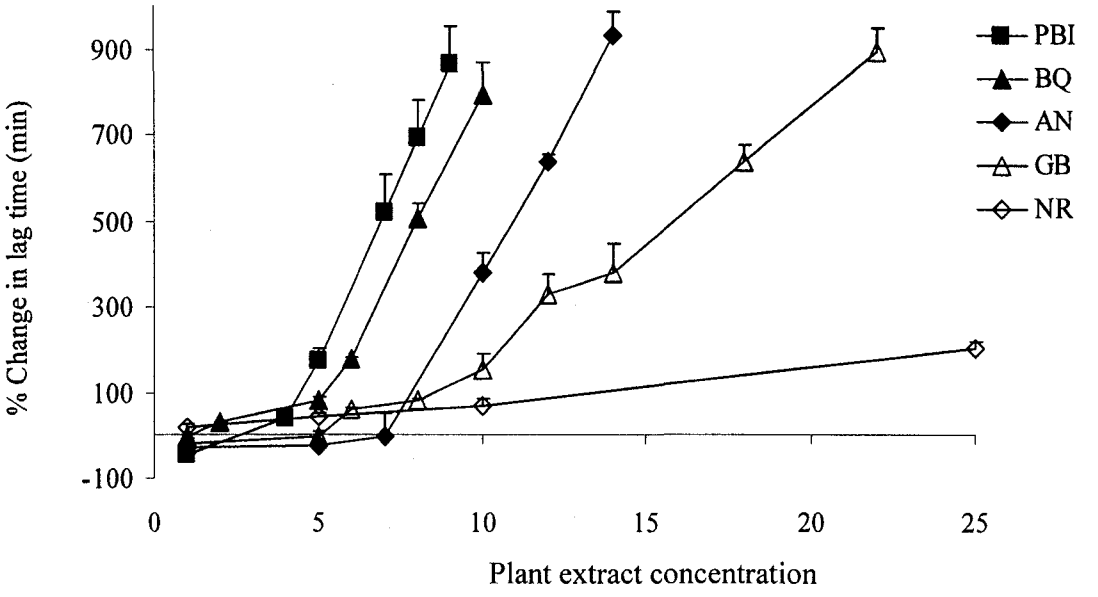

Figure 7.2. *In vitro* antioxidant activity of various concentrations of selected plant extracts on Cu^{2+}-mediated LDL oxidation measured as lag time (min) before the appearance of conjugated dienes. Results are expressed as % change in lag time relative to LDL oxidized in the absence of plant extracts (oxLDL). Points above the x-axis represent a prolongation of lag time and an increased ability of a plant extract to protect LDL from oxidation. Points below the x-axis represent pro-oxidant activity and a shortening of lag time. Results are the average of at least three independent experiments performed in duplicate. BQ: betel quid; AN: areca nut; PBI: *P. betle* inflorescence; GB: guava bud; NR: noni root. All points for plant extract concentrations of 5 μg/mL and higher are significant at $p<0.05$ vs. oxLDL, except for GB at 5 μg/mL. AN, PBI and GB at 1 μg/mL are significant pro-oxidants at $p<0.05$ vs. oxLDL.

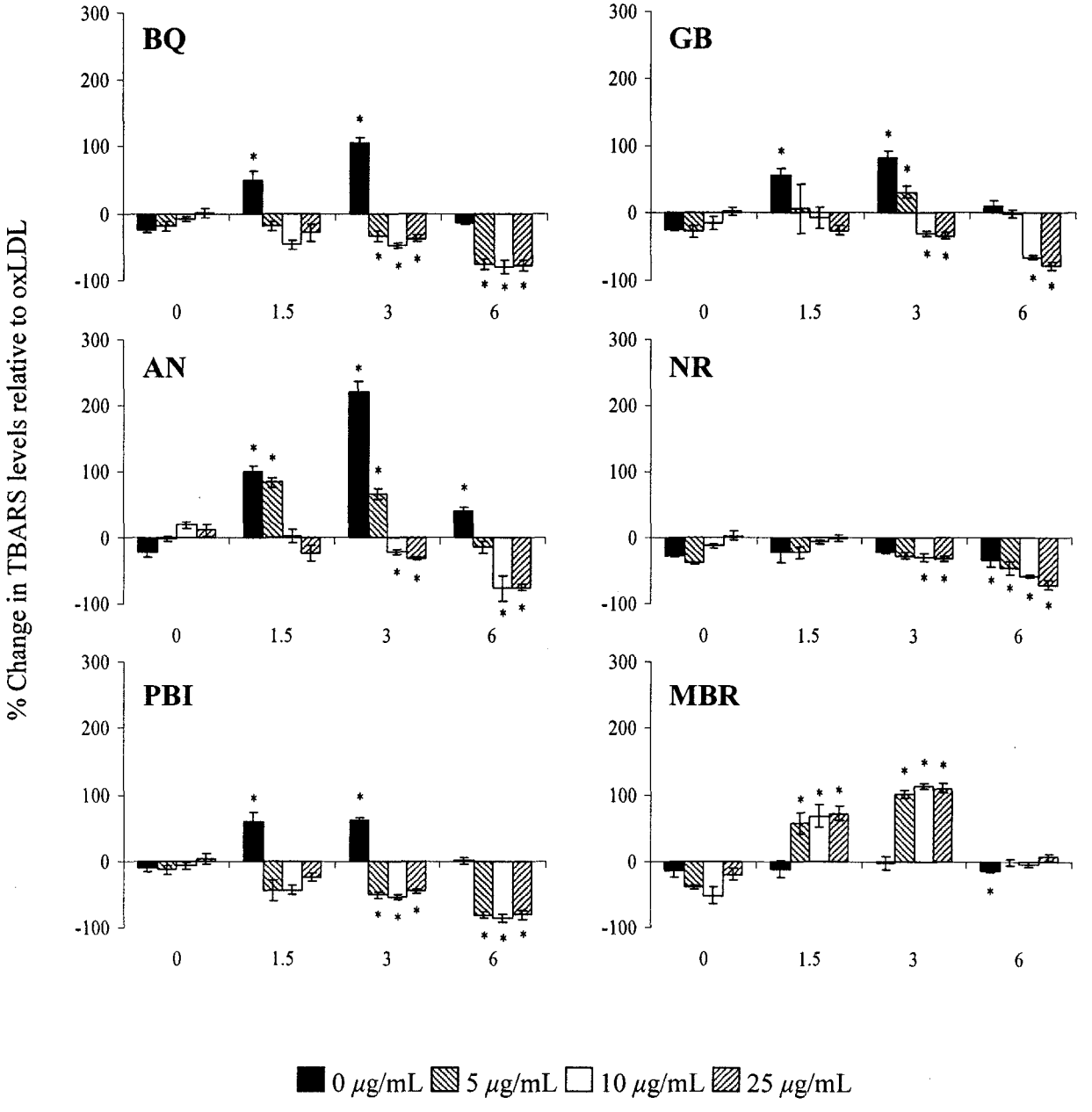
% Change in TBARS levels relative to oxLDL
BQ
GB
AN
NR
PBI
MBR
300
200
100
0
-100
0
1.5
3
6
0 μg/mL
5 μg/mL
10 μg/mL
25 μg/mL

Figure 7.3. *In vitro* anti- and pro-oxidant activity of various concentrations of selected plant extracts on Cu^{2+}-mediated LDL oxidation measured as TBARS generation over an incubation period of 6 h. Results are expressed as % change in TBARS generation relative to LDL oxidized in the absence of plant extract (oxLDL). Bars above the x-axis represent an increase in TBARS and exacerbation of oxidation compared to normal oxLDL. Bars below the x-axis represent antioxidant activity and a reduction in TBARS generation. Results are the average of at least three independent experiments performed in duplicate. BQ: betel quid; AN: areca nut; PBI: *P. betle* inflorescence; GB: guava bud; NR: noni root; MBR: mangrove bean, raw. *$p<0.05$ vs. oxLDL.

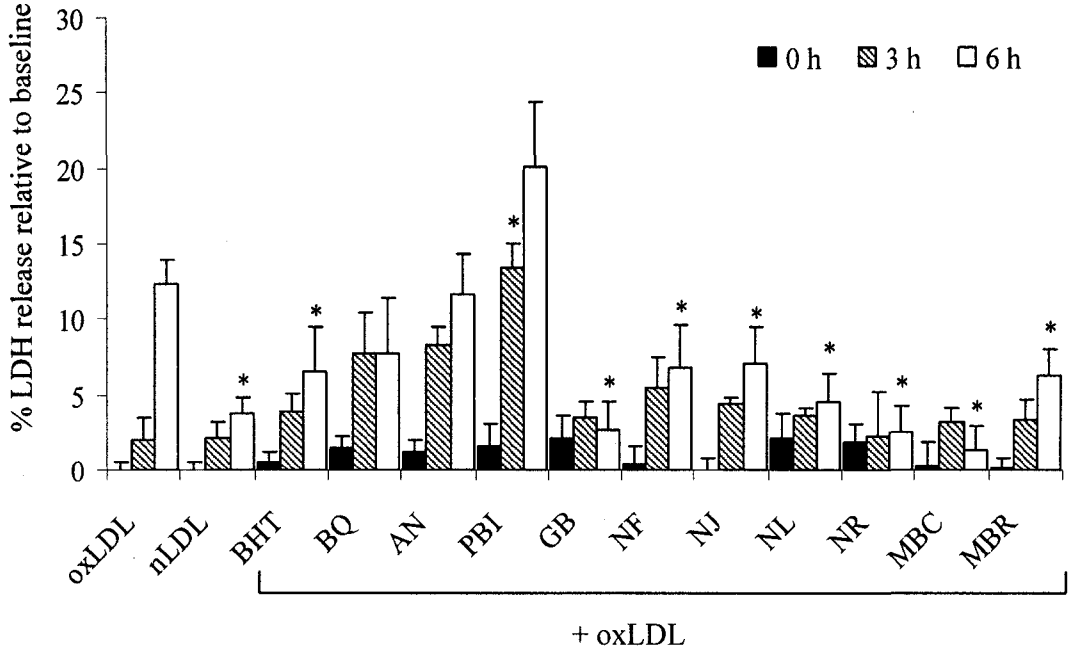

Figure 7.4. Cytotoxicity of maximal non-toxic concentrations of plant extracts in the absence (time 0 h) and presence of 0.10 mg/mL oxLDL in cultured BAEC. Results are presented as % lactate dehydrogenase (LDH) release relative to normal baseline levels of cells grown in the absence of oxLDL and plant extracts. Bars represent the average ± SEM of at least three independent experiments performed in triplicate. oxLDL: oxidized LDL; nLDL: native LDL; BQ: betel quid; AN: areca nut; PBI: *P. betle* inflorescence; GB: guava bud; NF: noni fruit; NJ: noni juice; NL: noni leaf; NR: noni root; MBC: mangrove bean, cooked; MBR: mangrove bean, raw. The maximal 24 h non-toxic concentration for AN, PBI, BQ, GB and NR was 10 μg/mL; for NF, NJ, NL, MBC, MBR, it was 50 μg/mL. *$p<0.05$ vs. oxLDL.

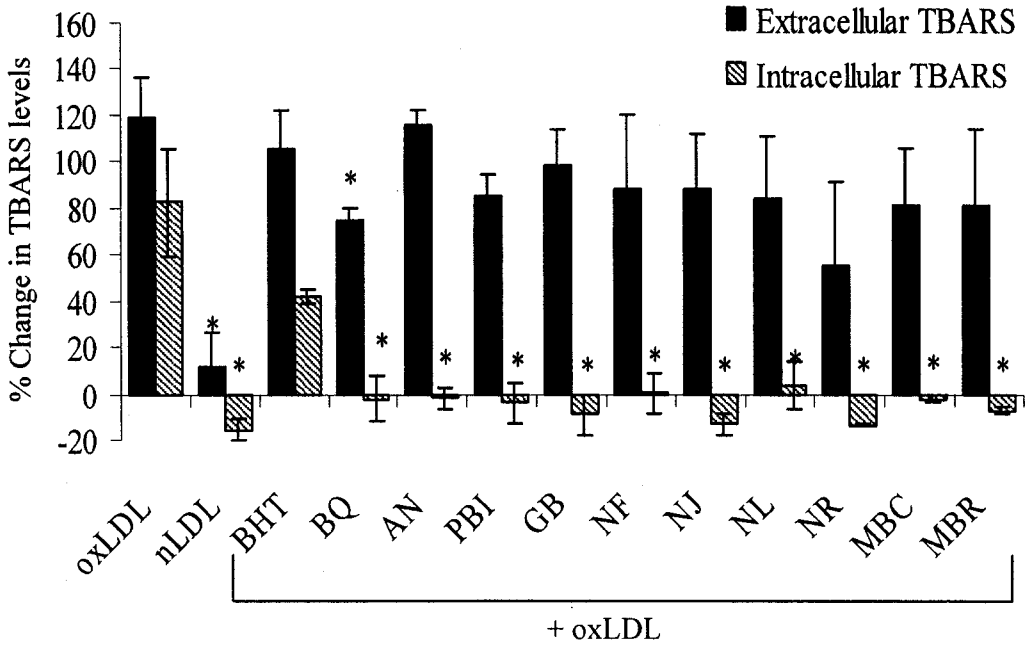

Figure 7.5. Extracellular and intracellular generation of TBARS as a measure of oxidative stress in cultured BAEC incubated for 6 h with 0.10 mg/mL oxLDL and maximal non-toxic concentrations of plant extracts. Results are presented as % change from baseline TBARS concentrations of cells incubated without oxLDL or plant extracts, and calculated as the mean of at least three independent experiments performed in triplicate. Bars above the x-axis represent an increase in TBARS and exacerbation of oxidation compared to normal baseline TBARS levels. Bars below the x-axis represent antioxidant activity and a reduction in TBARS. oxLDL: oxidized LDL; nLDL, native LDL; BQ: betel quid; AN: areca nut; PBI: *P. betle* inflorescence; GB: guava bud; NF: noni fruit; NJ: noni juice; NL: noni leaf; NR: noni root; MBC: mangrove bean, cooked; MBR: mangrove bean, raw. The maximal 24 h non-toxic concentration for AN, PBI, BQ, GB and NR was 10 μg/mL; for NF, NJ, NL, MBC, MBR, it was 50 μg/mL. *p<0.05 vs. oxLDL.

CHAPTER 8
GENERAL DISCUSSION AND CONCLUSIONS

Economic development and acculturation in developing countries need not entail a concomitant rise in NCD. Successful food systems in transition effectively retain elements of local food culture that are often associated with cultural identity and social well-being, much like the composite traditional/Westernized food systems of some Asian and Mediterranean diets (Trichopoulou & Vaasilopoulou, 2000). Implementation of this process, however, requires a paradigm shift in individual behaviours, market economies, public health policy, agricultural practice and ecosystem management (Johns & Sthapit, 2004). Policy platforms based on empirical documentation facilitate microeconomic and social marketing strategies that reinforce indigenous food production and consumption. Scientific evaluation of the functional properties of local cultivated and wild species is thus a research priority.

Increased recognition of the multidimensional nature of diets has elevated dietary patterns as an alternative or adjunct to the traditional approach of using single nutrients as exposures for examining diet-health associations. Central to this is the concept of diversity, which was thought to ensure adequate nutrient intake of essential nutrients and to promote health. In developing countries, a simple count of foods or food groups has been associated with nutrient intake, nutrient adequacy, child nutrition status and growth, food security, various household economic factors and various biomarkers of chronic diseases. This thesis focuses on incorporating the concept of functionality as an additional virtue of dietary diversity. Intuitively, a dietary pattern diverse in foods delivers bioactive elements that mediate metabolism and reduce chronic disease risk.

Testing of this hypothesis required an ecosystems approach that integrated dietary pattern assessment, nutrient composition analysis, phytochemistry and pharmacology, contextualized within cultural-specific socioeconomic and ecological spheres. Firstly, a description of dietary patterns and medicinal plant use in three adjacent communities with

disparate economic conditions, socio-religious ethos and ecological / agricultural patterns provided an overview of the links between dietary diversity and metabolic health. This association was further explored in manuscript 2, where nutrient composition data allowed comparisons of the commonly employed FVS and DDS with a recently developed index, the QUANTIDD. Although more complex in its calculations, the QUANTIDD takes into account portion sizes (expressed as energy or weight) and corrects for inter-food group variability. Consequently, we have demonstrated that it provided new information on dietary patterns not detected by FVS or DDS, and proved to be a superior predictor of nutrient adequacy.

The concept of dietary pattern functionality was introduced in manuscript 3 with the creation and application of the DFI. For the first time, we have demonstrated that a quantitative measure of total dietary functionality is associated with improved metabolic indicators of NCD. Additionally, our findings suggest that the long-term health benefits of consuming a variety of fruits and vegetables are due to their non-nutrient content. From there, the thesis focused on the pharmacological properties of selected plants as a means to provide support for their functionality towards NCD risk and prevalence *vis-à-vis* their consumption patterns. The research design thus allowed the extrapolation of experimental findings to epidemiological conditions, linking biochemical processes to population disease patterns.

This multidisciplinary ecosystems approach enabled a more holistic overview of the complex processes involved in NCD development. Recognition that human populations are not merely influenced by the biotic, abiotic and social elements that surround them, but are rather embedded within them, required a different theoretical approach to incorporate potential cross-stage interactions. Current disciplinary-bound approaches to health, which focus on biomedical and personal behavioural issues, often view disease as a linear process in which outcomes are first defined and studies are designed to identify determinants of those outcomes. This model follows the systems theory, in which connections between various elements are delineated by a certain boundary. While facilitating deduction of disease causality and risk, such systems rarely

consider interactions with other systems embodying agricultural and economic policies, social expectations created by media advertising, cultural habits and evolutionary ecology. The control of many tropical infectious diseases, for example, had as much to do with advances in biomedical science than with ameliorations in housing, nutrition and water management (Waltner-Toews, 2001).

The ecosystems approach to human health is thus firmly rooted in complex systems theory, characterized by patterns of interactions occurring in nested hierarchies, termed holarchies, in which a system is conceptualized as being both a whole thing, and part of something larger. For example, cells make up organs, which are parts of individuals, who are parts of families, and are in turn parts of villages and larger communities. This means that initiatives made at any scale will have implications for systems of which it is part as well as the systems of which it is comprised. Well-intended interventions aimed at improving the health status of a population may thus have negative feedback consequences on another system (Ole Nielsen, 2001; Waltner-Toews, 2001). Policies and practices resulting in increased availability of cheap cereals in developing countries served primarily to prevent starvation, but had the drawback of undercutting the economic and ecological sustainability of farmers, while creating a new array of nutritional disorders culminating in unprecedented rates of NCDs. The green revolution that had occurred since the 1960s contributed to the systemic changes that have resulted in more than two billion people with serious micronutrient deficiencies. Subsidized higher-yield species translated into higher productivity and profits for local farmers, but displaced a variety of other crops that consequently restricted the diet of billions of people. Moreover, the increased use of fertilizers and pesticides meant increased dependence on fossil fuels, contributing to the mining of non-renewable resources, global warming and the emergence of new diseases (Welch *et al.*, 1997). Thus, the solution for a caloric shortage problem resulted in the reorganization of the system in such a way that it was rendered unsustainable.

The emergence of new diseases can be viewed as being the result of disruptions in socio-ecological systems and inherently reflects our failure to understand the connections within these systems. Diabetes in Wanigela, for example, likely originated from the

communal decision to relocate the village to the mudflats of Marshall Lagoon as a solution to escape inter-tribal conflicts (see manuscript 1, page 80). The physical and ecological constraints of the mudflats ecosystem required new adaptive strategies that were ultimately not conducive towards sustaining human health. Restricted access to arable land meant that sustenance had to be obtained from comparatively less diverse edible wild plant populations and marine resources. With access to vast expanses of mangrove forests, uncultivated mangrove beans formed the staple of Wanigela, and fish became an item that was traded with neighbouring villages for vegetables, tubers and other commodities. Greater reliance on trade rather than agriculture as a food source compelled the Wanigelans to sell their catch in the urban waterfront markets of Port Moresby, where large-scale post-war urban migration was occurring. Vendors initially lived on their canoes, which they gradually converted into permanent residents on the shoreline. Though some Koki residents are self-employed as fishermen, the majority now seek full-time employment in the city. Globalization and international trade policies of the 1960s introduced rice, flour, salt, vegetable oil, and sugar into urban markets, which swiftly entered the food supply of adjacent coastal villages. The construction of the Magi highway in the 1960s and its extension in the 1980s to eastern regions of Central Province further increased the accessibility and availability of Western foods to rural regions. Today, some families in Wanigela rely almost exclusively on food brought back from their urban relatives. Having had no previous exposure to low glycemic index carbohydrates, the evolutionary response of the Wanigelans was enhanced insulin production, leading to peripheral tissue insulin resistance and ultimately, overt diabetes.

Public health policies aimed at reducing DM2 rates in Wanigela would justifiably espouse a strategy that combined increased physical activity and greater attention to diet. However, an ecosystems approach may find that relocation back to the mainland may adequately correct the human-ecology imbalance, indirectly reducing DM2 susceptibility. Firstly, infectious diseases and parasitic infections related to mudflat habitation would disappear, resulting in greater overall health and immune function. Second, more families would participate in agriculture, which would enhance dietary diversity and increase exposure to functional phytochemicals. Greater participation in farming would also result in increased physical exertion and amelioration of muscular insulin sensitivity.

Increased consumption of a variety of fruits and vegetables alone may be sufficient to curtail DM2 risk, regardless of the presence of non-traditional foods in diet systems. Consider the residents of Kalo, who consume greater amounts of rice, flour, sugar, biscuits and soda relative to Wanigela, yet despite higher rates of obesity, have lower rates of DM2 and hypertension. The comparatively richer dietary diversity of Kalo may explain, in part, this observation. Here, we have identified guava and noni, examples of two species that were consumed more frequently in Kalo than in Wanigela, as possible antidiabetogenic and antiatherogenic agents that may confer some protection against DM2 development. If Wanigelans retained cultural elements of their indigenous food systems during the nutrition transition, there is a strong likelihood that a relocation of a single kilometer to the east onto the mainland would ameliorate long-term FBG levels.

One of the more noteworthy findings from this study is the statistical significance observed between guava intake and glycemic control. This kind of reductive research, in that a single food or nutrient is attributed to improved health effects, is a common practice in nutrition science. For chronic diseases however, single foods usually alter risk by amounts too small to measure except through large, costly population studies. Yet, the fact that Papua New Guinean diets are comparatively less diverse and more consistent in their patterns relative to that of most developed countries could potentially amplify the health effects of individual food items so that it is quantifiable. Continued research on Melanesian plant phytochemistry and functionality is thus a priority that is needed to support agricultural and economic policies aimed at encouraging local food production and allowing consumers to make healther food choices. The simple message of incorporating more locally produced and traditional foods, particularly fruits and vegetables, into the diet, is a sensible practice that can coexist with the Westernization of diets without incurring costs on metabolic health. Including more tubers and consuming less rice in the diet, for example, would be a feasible, inexpensive dietary modification that would translate into improved long-term weight control. Papua New Guineans have long depended on tubers as their primary energy staple since topographical and climatic restrictions have historically precluded grain cultivation, and thus an evolutionary adaptation to metabolizing tuber starch more efficiently could be presumed. Considering the large variation in cultivars and varieties, tubers can provide more fiber, nutrients and

phytochemicals than polished white rice. Rice, which provides 27% of the dietary energy supply of the world (Fresco, 2005), has a relatively high glycemic index (Donduran *et al.*, 1999) and may promote hyperinsulinemia in sensitive individuals, leading to increased DM2 risk (Ayuo & Ettyang, 1996).

The complex theoretical basis of the ecosystem approach inherently involves drawbacks in its conceptualization and operationalization. As Puccia & Levins (1985) stated, "no model can be general, precise and realistic". Incorporating socio-ecological components into a model limits the ability to predict outcomes, in particular because of the kinds of phenomena associated with life in a continuously changing world. Without a clear causal model for health problems, any scientifically sound intervention would have to have some degree of flexibility in its options. Scientific research designs that employ an ecosystems approach necessarily compromise precision for generalization, and these seldom produce unambiguous results. Since ambiguous results require interpretation, the implications of the research are left to the influence of the researcher's point of view which can become thoroughly intertwined with the science. The results obtained in the present thesis are not sufficiently specific to provide direct evidence of causality concerning the benefits of dietary diversity and functionality towards NCD development, or whether the consumption of guava, betel quid, noni and mangrove bean truly affect insulin sensitivity and endothelial integrity. However, our generalist approach provided valuable information towards a better understanding of the complex multidimensional interconnections between health, non-communcable disease and environment. As with any study, certain limitations in design, methodology and analysis influence the ability to interpret results. Below is a discussion of the major limitations of this study and recommendations as to how to circumnavigate these in future research.

LIMITATIONS AND FUTURE WORK

Dietary assessment

Food frequency questionnaires have become the dominant tool for assessing food consumption patterns in epidemiological studies. As a result, several studies have focused on identifying errors in dietary FFQ collection and addressed their validity (accuracy) and reliability (consistency). Most inaccuracies result from cognitive errors in estimating portion size and frequency of consumption, which are influenced by age, gender, education and culture (Vuckovic *et al.*, 2000). Although FFQs can be validated to some degree by comparison with an additional reference method such as indirect observation, diet history and food record data, validation against a purely objective reference that eliminates reporting bias remains a major obstacle. Direct observation, food weighing and chemical analysis of duplicate food samples provide the best reference validation tools, but are expensive and impractical for free-living subjects. Studies have shown that FFQs are more useful for comparing intakes of groups rather than individuals, as not all nutrients are properly validated to represent absolute intake (Mullen *et al.*, 1984; Zulkifli & Yu, 1992). Validity is further improved if non-food items such as supplements and condiments are considered, and if visual aids such as food models, cups and measuring spoons are used (Hanson *et al.*, 2000).

The FFQ used in this thesis was modified from the one developed by Hodge et al. (1996b), who had pilot-tested their questionnaire in Hanuabada, a neighbouring village of Koki with similar diets. Their FFQ included 87 food and beverage items and was designed to assess food and macronutrient intake over the preceding 12 months in order to account for seasonality and pay period. We included an additional 48 items, such as alcohol, masticants, spices, wild vegetables and medicinal plants, and shortened the recall period to seven days in order to reduce cognitive error. Measuring cups and spoons were used as visual aids and special attention given to hidden foods such as coconut cream which was used almost ubiquitously in meals. The questionnaire was pre-tested on a small number of individuals in Kalo in order to standardize interview techniques and

identify additional foods to be included. The Golberg cut-off (Black, 2000) was used to identify under-reporters although none were detected in our population. Moreover, nutrient intake values in our study were comparable to those reported in other areas of PNG (Ulijaszek & Pumuye, 1985; Hodge *et al.*, 1996b) and other developing countries (Hatløy *et al.*, 1998; Ogle *et al.*, 2001; Torheim *et al.*, 2004; Savy *et al.*, 2005). However, no proper validation technique was used to test our FFQ, so food consumption estimates and absolute nutrient intake should not be construed as being accurate. Nutrient composition data in this study was used primarily as a comparative tool between Koki, Kalo and Wanigela. For nutritional interventions where absolute nutrient intake information is crucial, a more extensive food use questionnaire such as a food record covering seven to 14 non-consecutive days should accompany the FFQ (Zulkifli & Yu, 1992).

Dietary diversity measurement issues

Despite the recognized importance of eating a wide variety of foods, there remains a lack of consensus on how dietary diversity should be defined and measured. A simple count of food items or food groups oversimplifies the concept of diversity and its association to diet quality. Originally conceived with the idea that increased diversity buffers the risk of nutrient deficiency, growing concerns in developing countries regarding overnutrition and nutrient excess have led to global shifts in redefining dietary quality to include proportionality and moderation (Ruel, 2002). The FVS and DDS, often employed in developing countries for its simplicity, does not consider current dietary recommendations concerning saturated fat, cholesterol, sodium and refined sugar intake. Indeed, there is evidence that increased dietary diversity enhances energy and nutrient intake, leading to obesity and no improvement in nutritional quality (McCrory *et al.*, 1999; Brown *et al.*, 2002; Ponce *et al.*, 2006). Clearly, diversity within food groups is a better indicator of diet quality, especially in the fruits and vegetables groups, and less so in the cereal category.

A lack of uniformity in food or food group classification systems, as well as different reference periods, scoring systems and cutoff points to indicate low and high

diversity, are important issues that need to be addressed to enable cross-cultural comparisons. In this study, a few of these issues were addressed by incorporating the QUANTIDD and comparing its usefulness as an indicator of nutrient adequacy to the more popular FVS and DDS. The scores reported here can be compared to all future dietary diversity studies that calculate the QUANTIDD, irrespective of the number of food items per food group. The index is, however, influenced by food group categorization. Issues such as whether dairy products and eggs should be lumped in the meat category, or whether processed cereals should be separated from whole meal as it was done in this study, can affect the final QUANTIDD score. Recalculating the score with alternative food group classifications and retesting its association with nutrient adequacy ratios and NCD risk parameters would not be a difficult task to perform. The same could be done for FVS and DDS. Future work on dietary diversity indices should use such different methodological perspectives in order to ameliorate their validity.

Experimental models

The *in vitro* antioxidant activity of botanical extracts is strongly dependent on a multitude of factors, including the phase solubility of the antioxidant, the colloidal properties of the substrates and the conditions and stages of oxidation. In a review by Frankel and Meyer (2000), the authors warn against using one-dimensional assays to evaluate multifunctional food and biological antioxidants. In order to capture the multifaceted nature of antioxidants, the authors suggested that testing protocols should properly 1) choose biologically relevant substrates; 2) test various oxidation conditions; 3) analyze both initial and secondary oxidation products; 4) compare antioxidants at comparable molar concentrations of active components; and 5) quantify on the basis of induction period, percent inhibition, rate of hydroperoxidation or decomposition, or IC_{50} (concentration to achieve 50% inhibition). Based on the chemical reactions involved, Huang et al. (2005a) divided antioxidant assays into two broad categories: Those based on hydrogen atom transfer, and those based on electron transfer. Hydrogen atom transfer-based assays apply a competitive reaction scheme (hydrogen atom donating capacity), whereas electron transfer-based assays measure the capacity of an antioxidant in the

reduction of an oxidant, which usually changes colour when reduced. To comprehensively study different aspects of antioxidant action, the authors suggest that at least one assay from each category be used.

The choice of antioxidant assays used in the present study complies with most of these recommendations:

- Polyunsaturated lipids on the surface of the human LDL particle and the plasma membrane of cultured endothelial cells were used as oxidation substrates to represent sequential processes in atherosclerosis and endothelial dysfunction.

- Various oxidation conditions were tested by using different concentrations of extract and reference antioxidants. During protocol development, various dilutions of copper and LDL were tested before settling on a concentration that maximized time and energy efficiency, although these were not reported.

- Initial oxidation products of lipid peroxidation were measured as conjugated dienes, while TBARS represented secondary (decomposition) oxidation products.

- The concentration ratios of catalytic inducers/antioxidants and antioxidants/substrate used here are comparable to other studies that have evaluated activity in botanical extracts. In this study, we compared extracts and reference antioxidants at identical concentrations expressed as μg/mL. To enable molar comparisons, extracts should have been tested based on their total phenol content. Since total and water-soluble phenol content were measured, it would not be difficult to extrapolate our results and express them according to phenol concentration. Results would be more valid, however, if our assays were repeated with the correct corresponding extract concentration.

- Oxidation was quantified as percent inhibition for the DPPH assay; conjugated dienes measured the rate of hydroperoxidation; and TBARS measured the rate of decomposition.

- The DPPH assay is an electron-transfer-based assay, whereas the autoxidation of LDL and BAEC membranes is a hydrogen atom transfer-based reaction.

Future work would include compositional data of the extracts and bioassay-guided fractionation to isolate active antioxidant compounds. Better characterization of extract/phytochemical antioxidant activity would be achieved using different test models. Some of the more common free-radical trapping protocols include the total radical-trapping parameter (TRAP) assay, superoxide anion scavenging, the Trolox equivalent antioxidant activity (TEAC) assay, the ferric-reducing antioxidant power (FRAP) assay, and the oxygen radical absorbance capacity (ORAC) assay. Each uses a different free radical-generating system and method for oxidation end-point observations. However, as non-specific one-dimensional assays, they do not allow investigations of the mechanism of antioxidant protection in complex biological systems.

The use of cell culture systems to evaluate antioxidant activity of botanical extracts is not commonly encountered in the literature primarily due to the complexities in investigating multicomponent reactions. Without genomic and/or proteomic data, it is uncertain as to whether an observed inhibition of ROS generation is due to enzyme inhibition, antioxidant enzyme recycling, or radical scavenging activity. Future research would thus focus on the antioxidant and cytotoxic effects of isolated plant compounds, accompanied with work on cellular gene (mRNA) and protein expression. To more accurately reflect *in vivo* conditions, test compounds should be in a concentration and metabolite form as they would occur in plasma.

Antidiabetic activity

Since NCDs affect all systems of an organism, animal models would be ideal for evaluating the antiatherosclerotic and antidiabetic properties of botanical extracts. Analyses of antidiabetic plants have been primarily compelled by the search for novel oral hypoglycemic agents suitable for pharmaceutical development. As such, research

protocols were designed to test plant samples in animal models displaying mid- to advanced stages of DM2 characterized by hypoinsulinemia. The most common method employed is the chemical induction of DM2 using streptozotocin (STZ) or alloxan which possess specific pancreatic β-cell cytotoxicity (e.g. Abdel-Hassan *et al.*, 2000; Cetto *et al.*, 2000). The glucose moiety of STZ confers specificity for β-cells and depresses local concentrations of NAD and NADP levels so that glutathione (GSH) activity is reduced. In addition to the resultant oxidative stress, the alkylating action of STZ can cause DNA cross-linkage and strand scission that cannot be blocked by superoxide dismutase (SOD) or catalase (CAT) (Bailey & Flatt, 1990). Alloxan functions by producing toxic peroxide free radicals and thus destroys β-cell function. Diabetes is therefore brought about by destruction of β-cells and decreased insulin secretion, resulting in systemic hyperglycemia.

The chemically-induced diabetes model is appropriate to examine the hypoglycemic activity of a test sample but offers little insight into physiological and etiological mechanisms. A biological environment characteristic of early stages of DM2 includes insulin resistance, hyperinsulinemia and impaired glycemic control. In this regard, animals with genetic predispositions to obesity offer a more accurate model of human DM2 and are thus better predictors of the potential success of effective treatments. The three most extensively studied models of insulin resistance are the obese animal syndromes *fa/fa* rat, *db/db* mouse and *ob/ob* mouse. The latter displays particularly severe insulin resistance, requiring over 100 times the insulin dosage to achieve the same hypoglycemic effect as their lean, non-diabetic littermates (Flatt & Bailey, 1981). These three syndromes display hyperinsulinemia before there is a measurable decline in insulin action, which closely resembles conditions in humans. In the context of "nutrition as preventative medicine", genetic animal models offer the greatest relevancy in assessing the physiological roles of dietary and non-nutrient factors in DM2 etiology.

CONCLUSION

This dissertation contributes valuable empirical support for the role of non-nutrients as metabolic mediators of chronic disease development. We have demonstrated that dietary diversity, dietary functionality, consumption patterns and the absence or presence of certain foods in transitional diets are associated with NCD risk parameters. Our laboratory findings strongly imply a beneficial health effect associated with increased intake of guava fruit or leaf bud infusion, and noni (especially the root), while betel quid use may exacerbate CVD and DM2 progression. Mangrove bean on the other hand, does not seem to offer any significant protection from NCD development.

Having achieved the specific objectives addressed by the individual manuscripts, it is possible to formulate a more informed conclusion as to whether dietary functionality influences susceptibility to NCD risk. The pharmacological activities of our selected species provide important insights into their possible biological functions in human individuals. Considering that these plants form only a small fraction of the community's food supply, the fact that our statistics detected a significant association between certain NCD risk parameters and their consumption frequency suggests that the elimination or introduction of certain foods can significantly influence health outcome. A dietary pattern this is diverse and includes the right balance of introduced and traditional foods would conceivably supply adequate nutrients and functional compounds to maintain metabolic homeostasis and provide protection from chronic disease. This was effectively demonstrated using our compound quantitative index, the Dietary Functionality Index. With time, new pharmacognostic information will strengthen the predictive power of the DFI, validating its use as a tool to identify dietary patterns with low functionality.

Additional research is clearly needed to further investigate the toxicity/functionality of betel quid, guava, noni, and mangrove bean. More importantly, the nutritional and medical properties of traditional Papua New Guinean foods and medicines as a whole need to be documented, characterized and scientifically evaluated to assess their impact on long-term health. This information would improve the validity of the DFI score and reinforce the perception of traditional foods as preventative medicine. Confronted with rapid rates of acculturation and urbanization, economic and public health

policy platforms would greatly benefit from such empirical evidence as a means of promoting consumption of locally grown foods while retaining elements of indigenous food systems that reflect the rich biodiversity with which Papua New Guinea is blessed.

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APPENDIX I: ETHICS REVIEW FORM

ETHICS REVIEW FORM

CONSENT BY THE PARTICIPANT OF RESEARCH PROTOCOL

Code #_____

Name_____

Date_____

Project Title: Phytochemical Diversity and Human Health: Type 2 Diabetes Mellitus in Transitional Communities of Papua New Guinea

Institutions involved: School of Dietetics and Human Nutrition, McGill University, Canada
School of Medicine and Health Sciences, University of Papua New Guinea
Researchers: Patrick Owen, Ph.D. candidate, Dr. Timothy Johns, Dr. Lohi Matainaho, Dr. Todd Capson.
Contact information: In Canada: Dr. T. Johns, CINE, Macdonald Campus of McGill University, 21,111 Lakeshore road, Sainte-Anne-de-Bellevue, PQ, H9X 3V9.
In PNG: Dr. L. Matainaho, School of Medicine and Health Sciences, UPNG, Boroko, Port Moresby, PNG.

I,_____, the undersigned, hereby consent to participate as a subject in the above-named research project conducted by McGill University and the University of Papua New Guinea. The nature of the procedure or treatment, its risks and/or benefits, and possible alternatives, follow:

1. Public Health Objective

Dietary factors are associated with 5 of the 10 leading causes of death in the world. As communities become more modernized, dietary patterns and lifestyles change, often resulting in increased prevalence of obesity and diabetes. The objective of this study is to quantify all dietary and non-dietary ingested material in three communities undergoing different stages of modernization in Papua New Guinea. Since type 2 diabetes is a common metabolic disease associated with modernization, we will analyze some of these plants for their ability to lower blood glucose and lower insulin resistance in diabetic animals.

2. Protocol

- An in-person interview methodology will be used for all dietary interviews. The dietary interviewer will record the information reported by the respondent on a 20-page questionnaire consisting of two (2) sections: 1- a food frequency questionnaire and; 2 - an ethnomedical questionnaire.
- Measurement aids and visuals including charts and photographs will be used by the respondent to quantify the foods and beverages that are reported.

Time Allotment:
Depending on the number of items reported in the questionnaire, the interview length ranges from 60 to 90 minutes.

Health Measures:

- The interviewer will measure body weights and heights of the respondent. The respondent will be asked to remove shoes and wear light clothing. A scale will be provided.
- Hip and waist circumference will be measured with a standard measuring tape.
- Systematic and diastolic blood pressure will be measured using a sphygmomanometer and stethoscope while the respondent is relaxed and seated.
- Physical activity levels will be assessed according to factors such as occupation and sports participation.
- Fasting glucose will be determined using a portable glucometer. The participant is asked to not consume any food or drink other than water the morning of the test. A small prick on the side of the index finger will be made to draw a single drop of blood for analysis. All procedures will involve sterilized equipment and the wound treated with a disinfectant.

Eligibility
All survey participants above 16 years old are eligible for the interview. Translators may assist respondents when needed, and proxy reporting is permitted.

Exclusion Criteria
None, except for circumstances where communication or cognitive difficulties make it impossible for the participant to provide the necessary information and a proxy reporter is not available to complete the interview.

Risks:
There are no risks associated with the interview or health measures. Slight pain may be experience during the blood draw for glucose analysis.

Report of findings:
Findings will be presented in an information pamphlet that display the results of this study to communities involved. The information will be used to increase awareness of the health benefits of fruits and vegetables against chronic disease development, and relate possible beneficial properties of some medicinal plants.

3. Intellectual Property Rights

In all instances, all research, collection, databases and publications inherent in this study will respect what is regarded as sacred, secret or confidential by tradition, and no information will be divulged without prior informed consent of the respondent. This study adheres to the ethical guidelines outlined by the Convention on Biodiversity, the International Society of Ethnobiology and the Papua New Guinea Institute of Biodiversity. This study has no commercial interests. In the unlikely event of commercial development of a medicinal plant, no action will be taken without consultation and discussion with PNG BioNet.

APPENDIX II: MODERNITY SCORE SYSTEM

No.	Date:
	Score:

1. Area of Origin: **Score**

a. Length of contact period

80-100 years	5
60-79 years	4
40-59 years	3
20-39 years	2
10-19 years	1
0-9 years	0

b. Access to urban center

Periurban – within 1 hr walk	5
1 hr travel by car or boat	4
Return trip possible in one day	3
Access by road or sea	2
Outstation visitable in one day	1
"The big bush"	0

2. Education: **Score**

Standard 10+	5
Standards 7-9	4
Standard 6	3
Standard 1-5	2
Mission education only	1
No education	0

3. Employment: **Score**

Large business, management, professional, service officers	5
Skilled work, service ranks	4
Semi-skilled work, small business	3
Unskilled work	2
Some cash income (family, marketing, smallholding)	1
Subsistence	0

4. Length of employment: **Score**

45+ years	10
40-44 years	9
35-39 years	8
30-34 years	7
25-29 years	6
20-24 years	5
15-19 years	4
10-14 years	3
5-9 years	2
1-4 years	1
Less than one year	0

5. Number of years spent in urban centre: **Score**

30+ years	5
20-29 years	4
10-19 years	3
5-9 years	2
1-4 years	1
Less than a year	0

6. **Father's employment:** Scores as for own employment (see item 3)

0 1 2 3 4 5

7. Type of housing: **Score**

Constructed house with modern facilities	5
Constructed house without modern facilites	4
Framed house	3
High grade house in traditional materials	2
'Improved' traditional house (partitions, floor)	1
Simple traditional dwelling	0

8. Spouse increment: **ADD**

Spouse's score

30+	5
25-29	4
20-24	3
15-19	2
10-14	1
<10	0

APPENDIX III: ANTHROPOMETRY AND PERSONAL INFORMATION

No.		Date: d/m/y

Age:	Weight (kg)	Height (m)	BMI(kg/m^2)
Gender: M F	1.	1.	
Religion:	2.	2.	
Place of birth:	Avg:	Avg:	

Years spent in village:	Waist circ. (cm)	Hip circ. (cm)	WHR: (w/h)
	1.	1.	
Marital Status:	2.	2.	
	Avg:	Avg:	

Smoker: Y N If so, # per day?	Systolic BP:	Diastolic BP:	Blood Pressure:
	1.	1.	
	2.	2.	
	Avg.	Avg.	

Skinfold Thickness

Tricep (TSF)(mm)	Biceps (mm)	Subscapular (mm)	Suprailiac (mm)	Sum of 4 (mm):
1.	1.	1.	1.	
2.	2.	2.	2.	% Body Fat:
3.	3.	3.	3.	
Avg:	Avg:	Avg:	Avg:	

	Mid-upper arm circumference (cm)	Mid-arm muscle circumference (cm) = MUAC (cm) – [0.314XTSF (mm)].
	1.	
	2.	
	Avg.	

Physical Activity Factor (circle one)		Resting Energy Expenditure
1.3. Sedentary	e.g. house-bound and office workers	
1.6. Light	e.g. sales, housework	Energy Expenditure (REE x Phys. Act.)
1.7. Moderate	e.g. trades workers, flat gardening	
2.4. Heavy	e.g. hill gardening, labourers, regular aerobic sports	BMR:

Fasting Glucose Conc. (mmol/L):	Time:

Have you been diagnosed with diabetes by a doctor? Y N When?	Have you modified your diet in the past year (give reasons)? Y N
Date of last hospital visit?	

Notes

APPENDIX IV: FOOD FREQUENCY QUESTIONNAIRE

No.			Date:	
Food	Standard Serving Size	How often	Comments	Season
Cereal Foods				
Rice - white polished	1 cup (cooked)			
- brown	1 cup (cooked)			
Bread – white	1 slice			
- brown	1 slice			
Breakfast cereal	1 cup			
Noodles (Maggi)	¼ cup (boiled)			
Spaghetti (tinned)	1 tin			
Biscuits - Pacific Island	1 pack (4 biscuits)			
- plain/savoury/small	1 pack (4 biscuits)			
-plain/sweet/small	1 pack (4 biscuits)			
Scones	1 cup			
Fried flour	½ cup			

Starchy Foods				
Sweet potato (*KauKau*)	1 cup			
Taro Singapore Chinese (*Kongkong*)	1 cup			
Taro (*Taro tru*)	1 cup			
Yam -Greater (*Yam tru*)	1 cup			
-Lesser (*Mami*)	1 cup			
-Potato	1 cup			
Breadfruit (*Kapiak*)	1 cup			
Cassava (*Tapiok*)	1 cup			
Cooking banana/Plantain	1 long			

No.			Date:	
Food	Standard Serving Size	How often	Comments	Season
European potato (*Pateta*)	1 cup			
Jackfruit	1 cup			
Mangrove bean	1 cup			
Sago (*Saksak*)	1 cup			

Legumes				
Peanuts (*Pinat*) -raw	1 cup			
-cooked	1 cup			
Winged bean (*Asbin*)	1 cup			
Common bean pods	1 cup			
Pea	1 cup			
Snake bean	1 cup			

Vegetables				
Leafy vegetables (cooked)				
-Aibika	1 cup			
-Pumpkin leaves	1 cup			
-Choko leaves	1 cup			
Cabbage -Chinese	1 cup			
-English	1 cup			
Broccoli	1 cup			
Cauliflour	1 cup			
Corn (maize)	1 medium cob			
Pumpkin/Squash	1 cup			
Carrots	1 medium			
Lettuce	1 cup			
Cucumber	1 medium			

No.			Date:	
Food	Standard Serving Size	How often	Comments	Season
Pitpit	1 segment (internode)			
Onion	1 medium			
Celery	1 cup			
Eggplant	1 cup			
Tomato	1 medium			

Fruit				
Apple	1 small			
Banana	1 large			
Guava	1 fruit			
Mango	1 medium			
Watermelon (*Melon*)	1 large slice (1/2 cup)			
Pear	1 medium			
Passionfruit	1 medium			
Grapes	10 grapes			
Paw Paw (*Popo*)	1 medium slice			
Pineapple (*Pinap*)	1 cup			
Orange (*Swit muli*)	1 medium			
Mandarin (*Swit muli*)	1 small			
Lime (*Muli*)	1 fruit			
Starfruit (*Faiv kona*)	1 fruit			
Kiwifruit	1 small			
Sugarcane	1 segment (internode)			
Malay apple (*Laulau*)				

No.			Date:	
Food	Standard Serving Size	How often	Comments	Season
Nuts				
Pandanus	1 cup			
Canarium almonds (*Galip*)	1 cup			
Java almonds (*Talus*)	1 cup			
Okari nuts (*Okari*)	1 cup			
Poa nuts (*Poa*)	1 cup			
Pecan	1 cup			
Tahitian chestnut	1 cup			
Beach almond	1 cup			

Coconut Products				
Coconut, water	From 1 coconut			
Coconut, flesh (young)	From 1 coconut			
Coconut, flesh (old)	From 1 coconut			
Coconut, cream	1 tbs			
Coconut, grated	1 cup			
Number of coconuts used every meal:				
Number of adults and children in family:				

SPICES, HERBS AND CONDIMENTS

No.				Date:	
Item	Standard Serving Size	Food associated	How Often	Comments	Season
Chili pepper	1 small				
Curry powder	1 tsp				
Garlic	1 clove				
Ginger, market	1 tbs minced				
Ginger, wild (*Golgol*)	1 tbs minced				
Lemon grass	¼ cup				
Mint	¼ cup				
Pepper, black	1 tsp				
Salt	1 tsp				
Soy sauce	1 tbs				
Turmeric	1 tsp				

MASTICANTS

Item	Standard serving size	How often	Comments
Betel nut (*Buai*)	1 medium		
Mustard stick (*Daka*)	1 medium		
Lime	1 tsp		
Big boy bubble gum			

BEVERAGES

Item	Standard serving size	How often	Comments (sugar, milk cream added? Amounts?)
Coffee	1 cup		
Tea -black	1 cup		
Herbal teas (specify)	1 cup		
Soft drinks	250 ml (1 can)		
Beer (SP)	1 stubbie		
Cordial	1 cup		

Item	Standard Serving Size	How Often	Comments
Meats			
Fish	½ cup		
Tin fish	½ cup		
Chicken	½ cup		
Lamb flaps	½ cup		
Beef	½ cup		
Tin beef (corned beef)	½ cup		
Pork	½ cup		
Crab	½ cup		
Shrimp, crayfish	½ cup		

Appendix IV con't

MISCELLANEOUS

Item	Size	How often	Comments

Traditional Medicinal Plants

No.			Date
Plants that are used	Frequency of use	Indications	Method of preperation

Plant that are known			

APPENDIX V: ETHNOMEDICAL QUESTIONNAIRE

Instructions:

Read the symptom to the individual and provide further explanation if required. List as many plants as they can. If more than one plant is mentioned, inquire as to the importance of each and what would be the treatment of choice by the individual. Verify that the vernacular plant name is spelled correctly. For each plant listed, inquire as to how these are prepared, mode and method of administration, whether there are any adjunct plants or therapies, whether it is collected in the wild or purchased at a market, the plant part harvested, and any other indication for which the plant is traditionally used. If the individual does not know a remedy, note it down. Do not urge or influence an answer in any way.

No.			Date		
Indication	Plants	Mode & method of administration	Comments	Rank	Vouch. No.
Tonic					
Weakness /fatigue or to increase strength or vitality					
Pain-numbness of feet/legs and/or hands/arms					

No.			Date		
Indication	Plants	Mode & method of administration	Comments	Rank	Vouch. No.
Pain, headache, backpain, general body pain					
Impotence / aphrodisiac for men					
Fungal infection (e.g. Candidal infection of vagina, feet or hands)					
Thirst					
Muscle weakness					
Muscle pain					

No.			Date		
Indication	Plants	Mode & method of administration	Comments	Rank	Vouch. No.
Weak legs					
Intermittent limping					
Obesity					
Appetite suppressant					
Chronic foot sores that do not heal					
Gangrene of toes or feet					
Fainting spells or dizziness					
Excessive sweating					

No.			Date		
Indication	Plants	Mode & method of administration	Comments	Rank	Vouch. No.
Chest pain or heart disease					
Kidney problems or urination difficulties (non-infectious)					
Diarrhea at night					
Intermittent blurred vision or blindness					
Round, brown slightly raised painless lesions on shins or back of hand					
Yellowish lipid deposits (xanthomas) on skin					

Antidiabetic Plants

Indication	Plants	Mode & method of administration	Comments	Rank	Vou. No.

Why do you think people get diabetes; how is it caused?
What do you think people should do to treat diabetes?

Case Presentations

A middle aged woman reports feeling fatigued and weak with increased thirst and increased amount of urination. Her urine tastes sweet and insects got to the urine on the ground.

A middle-aged woman is tired and weak and has recurrent/persistent vaginal/vulvar itching with irritated red vulvar skin with thick milky-coloured of cottage cheese-like vaginal discharge. The woman may also have irritated red skin under her breasts. She may also have increased thirst and increased amounts of urination
A middle-aged or old person is tired and weak, has numb painful legs and a foot sore that will not heal. Person also has intermittent limping and may also have increased thirst and increased amounts of urination.
A middle-aged or old man is tired and weak, and has difficulty achieving an erection (impotence). His feet are sometimes numb and painful. He may also have increased thirst and increased amounts of urination.
A middle aged or old person is tired, weak and intermittently dizzy, sometimes fainting, especially when standing up quickly. Person also has abnormal increased sweating. Person may also have increased thirst and increased amounts of urination.

APPENDIX VI: ETHNOBOTANICAL QUESTIONNAIRE


McGill

Voucher No: ☐☐☐☐☐☐☐☐ Date of collection: d☐☐ m☐☐ y☐☐

VERNACULAR NAME_____

Other names _____Binomial:_____

THE INTERVIEWEE History, age and gender	Collection site (area, soil, neighboring spp. etc)

1. PARTS COLLECTED AND USE

1. Part collected_____Use:_____

2. Part collected_____Use:_____

3. Part collected_____Use:_____

2. PREPARATION AND STORAGE

Part(s) used. Other ingredients. Amounts used. Processing methods. Is the preparation used fresh, or can it be stored? If stored, where, how and for how long?

3. Ethnomedical and Ethnopharmacological Aspects

Local disease name or term. Local disease etymology. Symptoms treated. Response to therapy. Supposed pharmacological action(s).

4. Route of Administration or Application

5. Adjunct Therapies

Is treatment preceded, accompanied, or followed by any other treatment, therapy or ritual? If so, for how long, and why?

6. Toxicity

7. Storage

Is or can the collected plant or plant part be stored before use? How is it stored?, and for how long?

8. Context of Collection

Who collects? When? Do planting, collecting and harvesting activities occur at certain times of the day, lunar cycle or season?

9. PLANT RESOURCE STATUS

Source of use knowledge. Percentage of community involved in plant product collection and use. Cost of use (labor, time, expense). Frequency of collection. Economic role and importance.

10. MANAGEMENT STATUS

Is the plant managed? Native or introduced? What amounts are harvested? Is the resource perceived to be more or less abundant than in the past? Has harvesting or planting increased/decreased with time?

11. NOTES

APPENDIX VII: KALO WOMEN'S FELLOWSHIP FOCUS GROUP

Nutrition, Food and Diabetes **March 3, 2004**

1. Arigi gau ganigani pita ganira, gani veganai, mageria veveakava ita taunipararai?
   What constitutes a balanced meal?

2. Ita raka ganigani vetaira pita ganira taunipara pia tsiliga e pia kwalimu?
   What constitutes a healthful diet?

3. Gemi numa taurana raka ganigani vetaira geririwara namarana?
   What foods are preferred by family members?

   1.
   2.
   3.
   4.
   5.

4. Kivani arigia rauna gevegarururana?
   How long are babies breast-fed?

5. Kivani pia tawa ularana arigi gau ganiganina gegupurana?
   What are the first foods introduced to weaning babies?

6. Ita raka tsiwa ganigani geletamo taganirana?
What factors affect food choice? Discuss which foods are selected according to:

A	Arigi gau ganigani lagarai ge miatagona gaurai taganirana *Availability*
B	Arigi gau ganigani voira kei pa nama lagi gaurai taganirana *Affordability*
C	Arigi gau ganigani mamira nama pakurai taganirana *Taste*
D	Arigi gau ganigani ita tauniparara geveakavarana gaurai taganirana *Nutritious quality*

E	Arigi gau ganigani rupuna e ririwarana gaurai taganirana *Religious beliefs*
F	Arigi gau ganigani gapigapira atsi vekwalavira gaurai taganirana *Convenience*

7. Gomi gemi tugamagiai, walagani guluna e etoma guluna pe geinaguluna e gekalavo-kalavona gelegele?
   In your opinion, do people get more, less or the same amount of exercise as they did a generation ago?

8. Gulu kunerana pene vogomai ewagumona aonai, ita gera ganigani kolemara raka gema irau tsiwana? Gomi gemi tugamagiai, gulu kapira geria ganigani kolemara maki pia irau pa aikina? Vo raka tsiwa?

   In the same period (generation), how has diet changed? How do you predict diet will be affected for the next generation?

APPENDIX VIII. Food Functionality Index (FFI) categories of plant pharmacological activity queried in the NAPRALERT and MEDLINE database that were used to build the Dietary Functionality Index (DFI).

Glycemic control

Antidiabetogenic
α-glucosidase inhibition
Antihyperglycemic activity
Antinephrotoxic
β-glucosidase inhibition
Carbohydrate absorption inhibition
Diabetic angiopathy improvement
Diabetic cataract inhibition
Diabetic nephropathy improvement
Diabetic neuropathy improvement
Diabetic retinopathy improvement
Diabetic vasculopathy improvement
Glucose metabolism stimulation
Glucose transport stimulation
Glucose-6-phosphatase stimulation
Hypoglycemic activity
Insulin activity
Insulin binding activity
Insulin biosynthesis stimulation
Insulin degradation inhibition
Insulin induction
Insulin level increase
Insulin potentiating effect
Insulin receptor binding effect
Insulin receptor increased
Insulin release stimulation
Insulin resistance inhibition

Inflammation / Stress

Antianxiety effect
Antiinflammatory activity
Antistress activity
Corticosterone level decrease
Cortisol decrease
Cyclooxygenase inhibition
Immunomodulator activity
Interleukin-1 inhibition
Interleukin-6 inhibition
Cytokine inhibition
Myocardial antiinflammatory activity
Nuclear factor κ-β inhibition
Prostaglandin E2 inhibition
Prostaglandin release inhibition
Prostaglandin synthesis inhibition
Tumor necrosing factor-α gene expression inhibition
Tumor necrosing factor-α synthesis inhibition
Tumor necrosing factor-α secretion inhibition

Lipid profile amelioration

Antihypercholesterolemic activity
Cholesterol absorption inhibition
Cholesterol inhibition
Cholesterol synthesis inhibition
Fatty acid release stimulation
Fatty acid synthesis inhibition
HDL elevating effect
Hypocholesterolemic activity
Hypotriglyceridemia activity
LDL receptor binding stimulation
LDL receptor upregulation
LDL uptake stimulation
Lipid absorption inhibition
Lipid metabolism effects
Lipid profile alteration
Lipid synthesis inhibition
Lipogenesis inhibition
Lipolysis stimulation
Lipolytic effect
Lipoprotein lipase stimulation

Coronary / Peripheral cardiovascular health

Antiarrhythmic activity
Antiatherogenic activity
Antiatherosclerotic activity
Angioprotective activity
Antianginal activity
Anticoagulant activity
Antifibrillatory activity
Antiischemic effect
Antiplatelet activity
Anti-stroke activity
Antithrombotic effect
Cardioprotective activity
Cardiotonic activity
Coronary blood flow decreased
Coronary resistance decreased
Coronary vasodilator activity
Fibrinogen conversion inhibition
Fibrinogen level decreased
Fibrinolytic activity
Myocardial circulation enhancement
Myocardial infarction treatment
Myocardial ischemic improvement
Plaque-forming cell supression
Platelet activitation inhibition
Platelet adhesion inhibition
Platelet aggregation inhibition

Obesity

Adipocyte conversion inhibition
Adipokinetic activity
Antiobesity activity
Appetite suppressant
Fatty acid content decrease
Metabolism stimulation
Thermogenic activity
Weight gain inhibition
Weight loss

Oxidative stress

α-tocopherol depletion prevention
α -tocopherol level increase
Antiaging activity
Ascorbic acid level increase
Ascorbic acid transport stimulation
B-carotene depletion prevention
Fatty acid oxidation inhibition
Free radical formation inhibition
Glycation inhibition
LDL oxidation inhibition
Lipid peroxidase inhibition
Lipid peroxide formation inhibition
Oxygen radical inhibition
Radical scavenger effect
Reactive oxygen species generation inhibition
Superoxide dismutase stimulation
Superoxide inhibition
Superoxide production inhibition
Superoxide radical scavenging activity
Superoxide scavenging activity increase
Thiobarbiturate reacting substance inhibition

Vascular Tension

Angiotensin-converting enzyme inhibition
Antihypertensive activity
Blood flow decrease
Blood viscosity
Hypotensive activity
Renin inhibition
Vasoconstriction inhibition activity
Vasodilator activity
Vasorelaxation activity
Diuretic activity

APPENDIX IX. Bibliography for the Food Functionality Index (FFI) pharmacological categories used to build the Dietary Functionality Index (DFI). Each food that had been consumed during the survey period was queried using the NAPRALERT[SM] database (www.napralert.org) accessed 16 / 05 / 2006, and MEDLINE (www.pubmed.gov), accessed 09 / 06 / 2007. In order for a reference to be included, research must have employed an in vivo model and the plant or its extract administered orally. For fish, only review articles are listed.

	Food Functionality Index (FFI) pharmacological categories						
Food group Common name Scientific nomenclature	Glycemic control	Inflammation	Lipid profile amelioration	Coronary/ peripheral vascular health	Obesity	Oxidative stress	Vascular tension.
Grains / cereals							
Rice (brown) *Oryza sativa* L.	(1-3)		(4-9)				
Wheat (whole) *Triticum aestivum* L.	(2, 3, 10, 11)		(11-13)				
Starchy foods / tubers							
Banana *Musa* spp.	(14-21)		(14, 22)	(18, 23, 24)			(25, 26)
Breadfruit *Artocarpus altilis* (Park.) Fosberg		(27)					
Cassava *Manihot esculenta* Crantz			(28)				
Sago *Metroxylon sagu* Rottb.			(29)	(29)		(30)	
Sweet Potato *Ipomoea batatas* (L.) Lam.	(31-33)		(33)			(34-36)	(37)

Food group Common name Scientific nomenclature	Food Functionality Index (FFI) pharmacological categories						
	Glycemic control	Inflammation	Lipid profile amelioration	Coronary/ peripheral vascular health	Obesity	Oxidative stress	Vascular tension.
Yam *Dioscorea alata* L.	(38, 39)		(40, 41)	(42)		(40)	
Legumes							
Bean *Vicia faba* L.	(43)		(44)				
Broad Bean *Phaseolus vulgaris* L.	(3, 45, 46)		(47-50)		(51, 52)		
Pea *Pisum sativum* L.	(53, 54)		(49, 55)			(56)	
Peanut *Arachis hypogaea* L.	(57, 58)		(59)	(58-61)		(62)	
Snakebean *Vigna unguiculata subsp. Sesquipedalis* (L.) Verdc.			(50, 63)			(56)	
Wingbean *Psophocarpus tetragonolobus* (L.) D.C.						(56)	
Vegetables							
Aibika *Hibiscus manihot* (L.) Medik	(64)	(64)					

Food group Common name Scientific nomenclature	Food Functionality Index (FFI) pharmacological categories						
	Glycemic control	Inflammation	Lipid profile amelioration	Coronary/ peripheral vascular health	Obesity	Oxidative stress	Vascular tension.
Amaranth *Amaranthus caudatus* L.			(65)				
Brassica *Brassica oleracea* var *capitata* L.; *Brassica oleracea* var *botrytis* L.; *Brassica oleracea* var *italica* Plenck	(18, 45)		(66, 67)			(68-72)	
Carrot *Daucus carota* L.			(73) (74)			(75-77)	
Chinese cabbage *Brassica chinensis* L.						(78)	
Choko *Sechium edule* (Jacq.)							(79)
Corn *Zea mays* L.	(57, 72, 80-82)		(72, 81)	(83, 84)	(85)		(86)
Celery *Apium graveolens var. dulce* (Mill.) D.C.		(87)	(88)	(89)		(75, 77)	(89)
Cucumber *Curcumis sativus* L.	(45)						
Eggplant *Solanum melongena* L.			(90)	(91)	(90)	(75, 90-95)	(90)

Food group Common name Scientific nomenclature	Food Functionality Index (FFI) pharmacological categories						
	Glycemic control	Inflammation	Lipid profile amelioration	Coronary/ peripheral vascular health	Obesity	Oxidative stress	Vascular tension.
Lettuce *Lactuca sativa* L.	(45, 96)	(84)					
Onion *Allium cepa* L.	(45, 97-107)		(97, 106, 108-116)	(104, 117-123)		(105, 106, 124-128)	(129-131)
Pumpkin *Cucurbita pepo* L.			(86)			(92)	
Tomato *Lycopersicon esculentum* Mill.			(132, 133)	(134, 135)		(92, 136, 137)	
Fruit							
Apple *Malus domestica* Borkh.						(34, 138, 139)	
Custard Apple *Annona squamosa* L.						(140)	
Guava *Psidium guajava L.*	(141-144)		(145)			(146)	(145)
Lemon *Citrus* x *limon* (L.) Burm.f.			(147)				(79)
Lime *Citrus aurantiifolia* (Christm.) Swing.	(148)	(148)			(149, 150)		
Mandarin *Citrus reticulata* Blanco.						(92)	

Food group Common name Scientific nomenclature	Food Functionality Index (FFI) pharmacological categories						
	Glycemic control	Inflammation	Lipid profile amelioration	Coronary/ peripheral vascular health	Obesity	Oxidative stress	Vascular tension.
Mango *Mangifera indica* L.	(16, 151, 152)	(153)	(154)			(153, 155)	
Orange *Citrus sinensis* (L.) Osbeck.			(156, 157)	(158)			
Papaya *Carica papaya* L.	(159)					(160-162)	(37)
Passionfruit *Passiflora edulis* Sims.			(163)	(164)			
Pineapple *Ananas comosus*(L.) Merr.						(34, 92)	
Soursop *Annona muricata* L.	(165, 166)	(167)					
Starfruit *Averrhoa carambola* L.	(148)	(148)					
Sugarcane *Saccharum officinarum* L.	(168, 169)	(170)			(171)		
Watermelon *Citrullus lanatus* (Thunb.) Matsum. & Nakai						(172-174)	
Nuts							
Coconut *Cocos nucifera* L.	(148)	(148)				(62)	

Food group Common name Scientific nomenclature	Food Functionality Index (FFI) pharmacological categories						
	Glycemic control	Inflammation	Lipid profile amelioration	Coronary/ peripheral vascular health	Obesity	Oxidative stress	Vascular tension.
Pandanus *Pandanus jiulianettii* Martelli	(148, 175)	(148)	(175)				
Sea Almond *Terminalia catappa* L.	(176)		(176)				
Spices							
Black Pepper *Piper nigrum* L.	(177, 178)	(179)	(177)			(178, 180)	(181)
Chili *Capsicum frutescens* L.	(182, 183)		(184)		(185-189)	(190)	
Curry *Murraya koenigii* (L.) Sprengel	(191, 192)						(37, 193)
Garlic *Allium sativum* L.	(194-199)		(195, 200-220)	(119, 195, 203, 204, 221-235)		(225, 226, 229, 233, 236-239)	(119, 191, 217, 223, 240-249)
Ginger *Zingiber officinale* Rosc.	(250, 251)	(250)	(251-253)	(252, 253)			(254)
Masticants							
Betelnut *Areca catechu* L.	(255)		(256-258)			(259)	

Food group Common name Scientific nomenclature	Food Functionality Index (FFI) pharmacological categories						
	Glycemic control	Inflammation	Lipid profile amelioration	Coronary/ peripheral vascular health	Obesity	Oxidative stress	Vascular tension.
Piper *Piper betle* L.			(260)				(37)
Beverages							
Coffee *Coffea Arabica* L.			(261)		(262)		
Tea *Camellia sinensis* (L.) Kuntze			(263)	(264)	(265)	(266-271)	
Medicinal Plants							
Comfrey *Symphytum officinale* L.	(183)	(272)					
Fig *Ficus septic* Burm.f.						(273)	
Hibiscus *Hibiscus rosa-sinensis* L.	(274-277)						(278-280)
Lemongrass *Cymbopogon citratus* Stapf.		(281)	(282)				(37, 281)
Mint *Ocimum americanum* L.	(283-285)	(286)	(283, 284, 287)				
Neem *Azadirachta indica* A. Juss.	(103, 288-296)	(297, 298)	(296, 299)	(300)		(298, 301-305)	

Food group Common name Scientific nomenclature	**Food Functionality Index (FFI) pharmacological categories**						
	Glycemic control	Inflammation	Lipid profile amelioration	Coronary/ peripheral vascular health	Obesity	Oxidative stress	Vascular tension.
Noni *Morinda citrifolia* L.	(300, 306)					(306)	
Peppermint *Mentha x piperita* L.		(84)					
Tridax *Tridax procumbens* L.		(307)					
Fish							
Reef fish (composite)		(308-312)	(313-317)	(318-327)			(314, 319, 324, 328-332)

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APPENDIX X: ETHNOBOTANY OF KALO AND WANIGELA

The majority of species included here were identified as having medicinal properties by local herbalists. In some cases, plants in flower or fruit were collected randomly and their importance dicussed with herbalists.
Voucher specimens have been deposited at the University of Papua New Guinea, the National Agricultural Research Institute (Lae, PNG) and McGill University. Information has been collected with full consent from the herbalists, all of whom belong to the recently formed Papua New Guinea Traditional Medicine Practioners Association. The ethnobotanical information presented here has been entered in the PNG Traditional Medicine Database managed by the National Department of Health.
Species are arranged according to Voucher number.

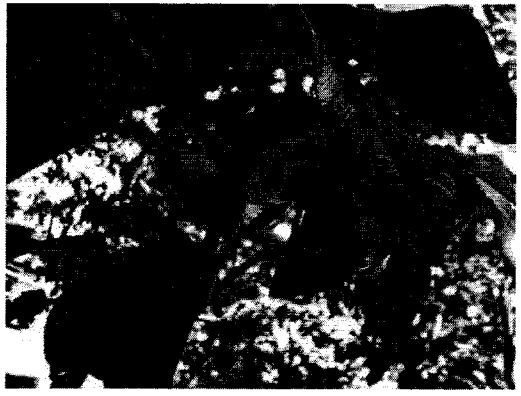
Voucher #	Species
KAL-01	*Psidium guajava* L.
KAL-02	*Dracaena angustifolia* Roxburgh
KAL-03	*Morinda citrifolia* L.
KAL-04	*Premna dallachyana* Benth.
KAL-05	*Tridax procumbens* L.
KAL-06	*Eugenia javanica* L.
KAL-07	*Dolichandrone spathacea* (L.f.) Schum.
KAL-08	*Ocimum tenuflorum* L.
KAL-09	*Desmodium brachypodium* A. Gray.
KAL-10	*Hibiscus tiliaceous* L.
KAL-11	*Crotalaria pallida* Ait.
KAL-12	*Murdannia nudiflora* (L.) Brennan
KAL-13	*Sida acuta* Burm. f.
KAL-14	*Indigofera linifolia* (L.f.) Retz.
KAL-15	*Pterocarpus indicus* Willd.
KAL-16	*Erigeron sumatrensis* Retz.
KAL-17	*Averrhoa carambola* L.
KAL-18	*Codiaeum variegatum* (L.) A. H. L. Jussieu
KAL-19	*Euodia anisodora* Laut. & K. Sch.
KAL-20	*Ficus septica* Burm. f.
KAL-21	*Ficus botryocarpa* Miq.
KAL-22	*Caesalpinia bolduc* (L.) Roxb.
KAL-23	*Canarium indicum* L.
KAL-24	*Piper betle* L.
KAL-25	*Azadirachta indica* A.H.L. Jussieu
KAL-26	*Timonius timon* (Spreng.) Merr.
KAL-27	*Citrus aurantifolia* (Christm.) Swing.
KAL-28	*Alstonia scholaris* R.Br.
KAL-29	*Manihot esculenta* Crantz
KAL-30	*Alstonia spectabilis* R.Br.
KAL-31	*Ocimum americanum* L.
KAL-32	*Piper* sp.
KAL-33	*Alpinia oceanica* Burk.
KAL-34	*Cayratia trifolia* (L.) Domin
KAL-35	*Epipremnum pinnatum cv. Aureum* (L.) Engl.
KAL-36	*Hoya* sp.
KAL-37	*Derris elliptica* (Roxb.) Benth.
KAL-38	*Tridax procumbens* L.
KAL-39	*Passiflora foetida L. var. hispida* (DC. Ex Triana & Planch.) Killip
KAL-40	*Canavalia cathartica* Thouars
KAL-41	*Ipomoea pes-caprae* (L.) Sweet
KAL-42	*Mikania micrantha* H.B.K.
KAL-43	*Inocarpus fagifer* (Parkinson) Fosberg
KAL-44	*Terminalia catappa* L.
KAL-45	*Artocarpus altilis* (Park.) Fosberg
KAL-46	*Bischofia javanica* Bl.
KAL-47	*Ficus* sp.
KAL-48	*Octomeles sumatrana* Miq.
KAL-49	*Myristica* sp.
KAL-50	*Intsia bijuga* (Colebr.) O. Kuntze
KAL-51	*Artocarpus altilis* (Park.) Fosberg
KAL-52	*Sterculia shillinglawii* F. Muell.
KAL-53	*Carica papaya* L.
KAL-54	*Momordica charantia* L.
WAN-01	*Bruguiera gymnorrhiza* (L.) Lam.
WAN-02	*Pluchea indica* Less.

WAN-03 *Euphorbia geniculata* Ort.
WAN-04 *Synedrella nodiflora* (L.) Gaertn.
WAN-05 *Secamone elliptica* R. Br.
WAN-06 *Avicennia marina* (Forsk.) Vierh.
WAN-07 *Rhizophora stylosa* Griff.
WAN-08 *Aegiceras corniculatum* (L.) Blanco
WAN-09 *Acrostichum aureum* L.
WAN-10 *Acanthus ebracteatus* Vahl.
WAN-11 *Phyllanthus niruri* L.
WAN-12 *Euphorbia hirta* L.
WAN-13 *Neisosperma* sp.
WAN-14 *Acacia auriculiformis* A. Cunn. ex Benth.
WAN-15 *Physalis angulata* L.
WAN-16 *Cordiaeum variegatum* (L.) Bl. *var. moluccanum* (Decne) Muell. Arg.
WAN-17 *Jasminum* sp.
WAN-18 *Cordiaeum variegatum* (L.) Bl. *var. moluccanum* (Decne) Muell. Arg.
WAN-19 *Melochia odorata* L.f.
WAN-20 *Flagellaria indica* L.
WAN-21 *Leaucaena leucocephala* (Lam.) De Wit
WAN-22 *Syngonium angustatum* Schott.
WAN-23 *Dioscorea alata* L
WAN-24 *Melanolepis multiglandulosa* (Bl.) Reichb. E. & Zoll.
WAN-25 *Ficus benjamina* L.
WAN-26 *Ficus wassa* Roxb.
WAN-27 *Celtis latifolia* (Blume) Planch.
WAN-28 *Alpinia coerulea* (R. Br.) Benth.
WAN-29 *Leea indica* (Burm. f.) Merr.
WAN-30 *Dianella ensifolia* (L.) DC.
WAN-31 *Maniltoa steenisii* van Meeuwen
WAN-32 *Gnetum gnemon* L.
WAN-33 *Polyalthea forbessii* F. Muell.
WAN-34 *Tinomiscium petiolare* Hook. f. & Thom.
WAN-35 *Theobroma cacao* L.
WAN-36 *Diospyros maritima* Bl.
WAN-37 *Gulbia costata* Becc.
WAN-38 *Crotolaria incana* L.
WAN-39 *Erythrina variegata* L.
WAN-40 *Desmodium velutinum* (Willd.) DC.
WAN-41 *Acalypha wilkesiana f. wilkesiana* Muller
WAN-42 *Celtis* sp.
WAN-43 *Timonius timon* (Spreng.) Merr.
WAN-44 *Imperata cylindrica* (L.) P. Beauv.
WAN-45 *Conyza* sp.
WAN-46 *Alstonia spectabilis* R.Br.
WAN-47 *Vigna* sp.
WAN-48 *Mimosa pudica* L.
WAN-49 *Vernonia cinerea* (L.) Less.
WAN-50 *Dracaena angustifolia* Roxburgh
WAN-51 *Uvaria* sp.
WAN-52 *Syzygium trivene* (Ridley) Merr. & Perry.
WAN-53 *Flagellaria indica* L.
WAN-54 *Ageratum coryzoides*
WAN-55 *Imperata conferta* (Presl) Ohwi.
WAN-56 *Tagetes erecta* L.
WAN-57 *Terminalia catappa* L.
WAN-58 *Cymbopogon citratus* (DC) Stan.
WAN-59 *Derris elliptica* (Roxb.) Benth.
WAN-60 *Ficus septica* Burm f.
WAN-61 *Smilax* sp.
WAN-62 *Cordyline terminalis* (L.) Kunth.
WAN-63 *Kalanchoe pinnata* (Lamarck) Persoon
WAN-64 *Abelmoschus manihot* (L.) Medik.
WAN-65 *Phaleria sogerensis* S. Moore
WAN-66 *Ardisia* sp.
WAN-67 *Kalanchoe pinnata* (Lamarck) Persoon.
WAN-68 *Aloe vera* L.
WAN-69 *Caladium bicolor* (Aiton) Ventenat.
WAN-70 *Nypa fruticans*
WAN-71 *Ipomoea batatas* (L.) Lam.
WAN-72 *Cocos nucifera* L.
WAN-73 *Catharanthus roseus* (L.) G. Don
WAN-74 *Mangifera indica* L.

Voucher: KAL-01

Family: Myrtaceae Date: 13 Jan, 2004

Species: *Psidium guajava* L.
English name: Guava
Local name: Tuava (Kalo)



Location: Cultivated tree in Kalo, Rigo District.
10° 03' S 147° 47' E

Description: A large shrub or small tree up to 10m in height. Bark smooth. Leaves opposite. Fruit yellow when ripe with reddish pulp inside and many seeds.

Medicinal Use: 1: Eye problems (Wanigela); 2: Tonic (Kalo)
Plant part: Leaves
Mode of Application: Topical; Oral
Preparation: 1: Eye problems: Warm old leaves over a fire and rub on sore eyes. 2: Tonic: Buds eaten as needed for any general body ache. It increases blood flow.

Herbalist: Robert Biau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-02

Family: Agavaceae (Liliaceae) Date: 13 Jan, 2004

Species: *Dracaena angustifolia* Roxburgh

Location: Ornamental plant in a Kalo garden, Rigo District
10° 03' S 147° 47' E

Description: Shrub to 5m high or more, branched at the base. Leaves simple, spirally arranged, blade linear-lanceolate, 10-60 x 1-3 cm, leathery with ivory-coloured margins. Flowers intermittently during the year, flowers many, borne in clusters of one to four in terminal panicle 8-75 cm long, excluding the peduncle. Corolla with fused tepals, divided to near base into six segments 2-3cm long, yellowish white. Fruit an orange glubose berry 1.7-2.5 cm in diameter.

Medicinal Use: 1: Toothache; 2: Fatigue; 3: Swollen joints (Wanigela)
Plant part: Leaves
Mode of Application: Oral (Masticant)
Preparation: 1: Toothache: Chew and place leaf on aching tooth. Use as often as necessary, but do not swallow due to potantialy lethal effects. 2: Fatigue: When tired after carrying a heavy load, tie around waist and wash body in the river. 3: Swollen joints: Cut leaf lengthwise and wrap around the area. Leave on as long as needed.

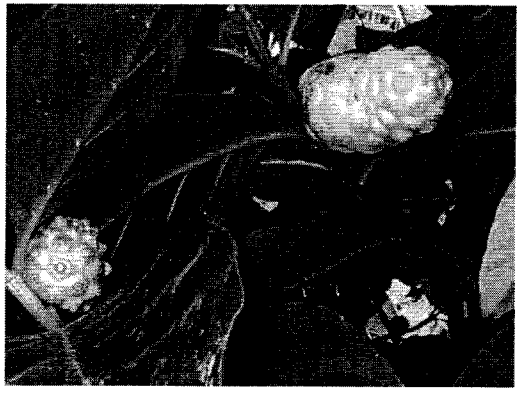
Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-03

Family: Rubiaceae

Date: 13 Jan, 2004

Species: *Morinda citrifolia* L.
English name: Noni
Local name: Nono (Kalo)



Location: Cultivated tree in residential yard, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Shrub to 3 m high. Leaves simple, opposite and decussate. Inflorescence pedunculate, axillary, swollen, green. Corolla white, tubular, 5-lobed. 'Fruit' compound.

Medicinal Use: 1: Asthma and tuberculosis; 2: Swollen joints; 3: Stomach and body aches; 4: Diarrhea.
Plant part: 1: Fruit, 2: Leaves, 3: Roots
Mode of Application: Oral, Topical
Preparation: 1: Asthma, TB, diarrhea: Chew, eat and drink the fruit and its juice as needed until better. 2: Swollen joints, body, stomach ache: Mix crushed leaves and/or shredded roots with coconut oil and massage onto swollen joints. 3: Swollen joints, body and stomach aches: Boil a handful of leaves for 10-15 minutes in a pot of water, strain and wash affected area as needed. 4: Swollen joints, body and stomach aches: Scrape a handful of roots and boil scrapings in a pot of water for 10 minutes. Cool and mix with coconut oil. Strain out the oil and massage affected area as needed.
Other ingredients: Coconut (*Cocos nucifera*)

Herbalist: Lew Guria (Kalo)
Voucher: KAL-04

Family: Verbenaceae

Date: 14 Jan, 2004

Species: *Premna dallachyana* Benth.
Local name: Kalo (Kalo)

Location: Growing in Melaleuca savanna outside Kalo, Rigo District
10° 03' S 147° 47' E

Description: Scrambling shrub to small tree, with ragged crown. Twigs grey-brown with longitudinal cracks and pale lenticels. Leaves simple, opposite, dull or glossy green above when fresh, drying blue-black, glabrous, base sometimes slightly unequal. Flowers small, with decussate branching. Calyx irregularly lobed. fruit small, green, turning black when ripe.

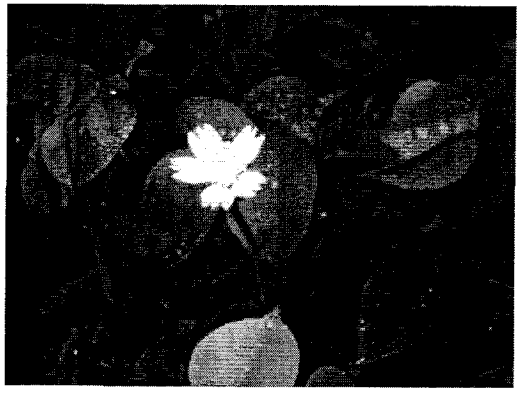
Medicinal Use: 1: Fatigue; 2: Head and bodyaches
Plant part: Leaves
Mode of Application: Topical
Preparation: 1: Fatigue: Break off a twig and whip fatigued legs to invigorate them during a long walk. 2: Head and bodyaches: Squeeze and macerate a handful of leaves and rub on affected area; on temples for headaches.

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-05

Family: Asteraceae Date: 15 Jan, 2004

Species: *Tridax procumbens* L.
English name:
Local name: Gawa (Kalo)



Location: Weed growing along garden edge, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Annual herb with a procumbant and ascending stem; ray-florets creamy white, disc-florets light yellow. Common weed.

Medicinal Use: Sores
Plant part: Leaves
Mode of Application: Topical
Preparation: Apply a handful of crushed and macerated leaves directly onto an open sore to stop bleeding and prevent infection. Secure in place with a bandage and change daily. Discontinue when sore is dry.

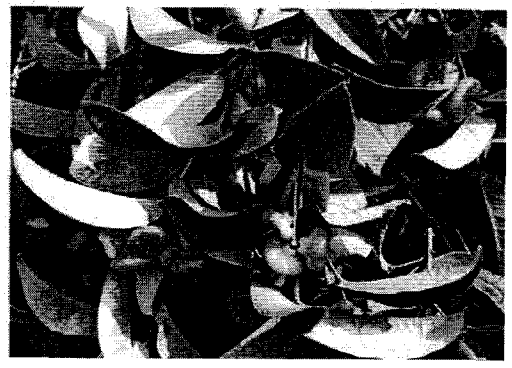
Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Note: duplicate of KAL-38

Voucher: KAL-06

Family: Myrtaceae Date: 15 Jan, 2004

Species: *Eugenia javanica* L.
Syn: *Jambosa javanica* K. Sch, *Syzygium samarangerse* Merr.
English name: Malay apple
Local name: Area (Kalo), Laulau (Tok Pisin)



http://home.hiroshima-u.ac.

Location: Cultivated tree in residential yard, Kalo, Rigo District
10° 03' S 147° 47' E

Description: A tree up to 15m high which branches near the base giving a spreading open tree. The leaves are smaller and more pointed than Malay apple and on short stalks. Flowers are about 3 wide and white. They are on leafy twigs. It produces cluster of attractive glossy pink waxy looking fruit. Fruit are 3cm long and 3-5cm wide. Trees grow on coastal deep fertile soil.

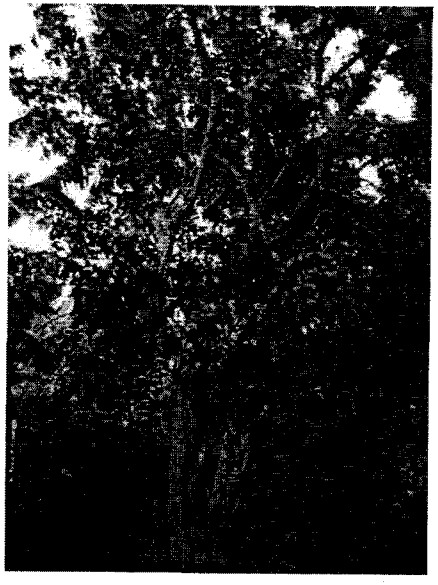
Medicinal Use: No medicinal use
Plant part: Fruit
Mode of Application:
Preparation: Food is edible and common kid's snack

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-07

Family: Bignoniaceae Date: 15 Jan, 2004

Species: *Dolichandrone spathacea* (L.f.) Schum.
Local name: Tui (Kalo)



Location: Shore of Kemp Welch River, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Medium tree, spreading, untidy, with large compound leaves, large trumpet-like white flowers and long thin pods. Seashore.

Medicinal Use: No medicinal use
Plant part: Timber
Mode of Application:
Preparation: Timber used in construction of canoes and paddles.

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-08

Family: Lamiaceae Date: 15 Jan, 2004

Species: *Ocimum tenuflorum* L.
English name: Mint
Local name: Garepepe (Kalo)

Location: Roadside weed in Kalo, Rigo District
10° 03' S 147° 47' E

Description: Violet or green flowers and small leaves. Very common.

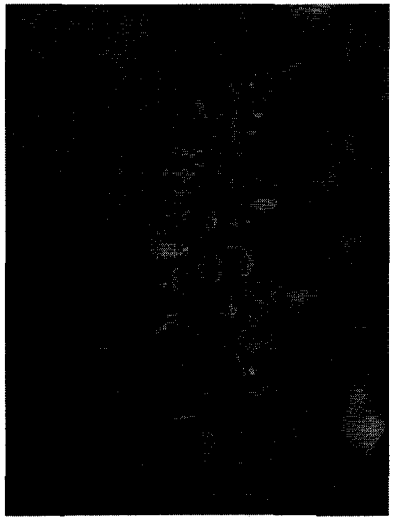
Medicinal Use:
Promote child speech (Magic)
Plant part: Herb
Mode of Application:
Preparation: Brush herb across the lips of a late-talking child to promote speech.

Herbalist: Lewa Guria
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-09

Family: Fabaceae Date: 15 Jan, 2004

Species: *Desmodium brachypodum* A. Gray.
English name: Large tick-trefoil
Local name:



www.rbgsyd.nsw.gov.au

Location: Roadside weed in Kalo, Rigo District
10° 03' S 147° 47' E

Description: Erect ascending perennial herb 30-60cm tall, stems covered with short spreading hooked hairs. Leaflets 3-5, ovate to elliptic, 1.5-7cm long, 0.7-5cm wide, drying a characteristic livid blue-green colour, obtuse, indeted or slightly acute at apex, obtuse or rounded at the base, with minute hoked hairs, particularly beneath; petioles 2-5cm long. Fruit sessile or shortly stipitate, 2-4cm long of 5-8 articles, slightly indented above at the necks, deeply indented beneath, 4-5mm long, 2.5-3mm wide, reticulately veined, covered with minute hooked hairs.

Medicinal Use: No medicinal use
Plant part: Herb

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-10

Family: Malvaceae Date: 16 Jan, 2004

Species: *Hibiscus tiliaceus* L.
English name: Beach hibiscus
Local name: Valu (Kalo)

Location: Ornamental tree in Kalo, Rigo District
10° 03' S 147° 47' E

Description: Bushy tree, to 7 m high. Young stems, petioles and pedicels grey-green and covered with very short, velvety pubescence; older stems brown with circular scar at each node. Leaves alternate, more or less circular, deeply cordate, margin crenulate; blade perpendicular to petiole. Flowers towards apex of leaf-bearing twigs. Fruit a dry capsule with 5 lines of dehiscence, the valves golden brown, pubescent; seeds numerous.

Medicinal Use: 1: Body pains
Plant part: Buds and new shoots
Mode of Application: Topical
Preparation: Combine 5-6 buds with a few drops of water and a pinch of salt, and rub on affected area as a counter-irritant. It will itch, so don't scratch. Use as often as needed but preferably 2 times per day, and do not use on open wounds.
Other ingredients: Salt
Other: Stringy inner bark is woven and used as rope

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai

Voucher: KAL-11

Family: Fabaceae

Date: 16 Jan, 2004

Species: *Crotalaria pallida* Ait.
English name: Smooth rattlebox
Local name:

Location: Roadside weed in Kalo, Rigo District
10° 03' S 147° 47' E

Description: Erect much-branched annual or short-lived perennial subshrubby herb up to 1-2m tall with short hairy stems. Leaves with 3 leaflets, very variable, elliptical to obovate, 4-11cm long, 2.5-5.2cm wide, glabrous above, thinly appressed pubescent beneath; stipules small, filiform or absent. Racemes 15-30 cm long with many fairly close flowers about 13cm long; corolla yellow, the standard often veined with reddish brown. Fruit subcylindrical, 3.8-4.6cm long, 6-8mm wide, often slightly curved, puberulous or more or less glabrous. Bare gravel, roadsides, grasslands, savanna and as a weed in old cultivations.

Medicinal Use: No medicinal use

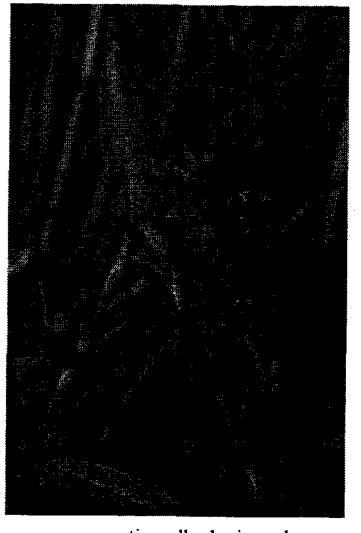
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-12

Family: Commelinaceae

Date: 16 Jan, 2004

Species: *Murdannia nudiflora* (L.) Brennan
English name: Nakedstem dewflower
Local name:



Location: Roadside weed in Kalo, Rigo District
10° 03' S 147° 47' E

Description: Creeping ascending herb; leaves narrow ovate-lanceolate acuminate; spathe boat-shaped, flowers pale blue. Common weed.

Medicinal Use: No medicinal use

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

www.nationaalherbarium.nl

Voucher: KAL-13

Family: Malvaceae Date: 16 Jan, 2004

Species: *Sida acuta* Burm. f.
English name: Spiny-headed Sida
Local name: Tepagauga (Wanigela)


blog.yam.com

Location: Weed on edge of Melaleuca savannah growing next to Kunai grass (*Imperata cylindrical* and *Phragmites* spp.), Rigo District
10° 03' S Long: 147° 47' E

Description: Subshrubby perennial herb, linear leaves and yellow flowers. Common weed.

Medicinal Use: 1: Fever, Malaria, Diarrhea, Headache, 2: Balding
Plant part: Roots
Mode of Application: Oral, Tropical
Preparation: 1: For Fever, Malaria, diarrhea or headache: Eat the roots of 2 plants raw 2 times per day until one feels better. 2: Balding: Mix roots of 2 plants with coconut oil to make a shampoo against balding.
Other ingredients: Coconut (hairwash) (*Cocos nucifera*)
Note: Safe for pregnant women to ingest for fevers.

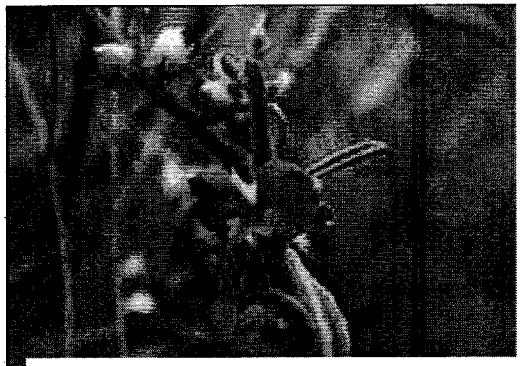
Herbalist: Robert Biau (Wanigela), Kokoa Nubu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-14

Family: Fabaceae Date: 03 Feb, 2004

Species: *Indigofera linifolia* (L.f.) Retz.

Location: Melaleuca savanna outside of Kalo, Rigo District
10° 03' S 147° 47' E


florabase.calm.wa.gov.au

Description: Usually a bushy annual herb 9-60cm high with a woody base and very numerous branches which are 2-ribbed and whitish due to close adpressed hairs. Leaves simple, linear, 1.2-4 cm long, 0.1-0.2cm wide, densely covered with adpressed hairs. Flowers about 3mm long in small axillary clusters; sandard reddish, purple or pink inside. Fruits ovoid-globose, more or less pointed, 1.5-2mm long, 1-seeded, densely white adpressed pubescent.

Medicinal Use: No medicinal use

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-15

Family: Fabaceae Date: 09 Apr, 2004

Species: *Pterocarpus indicus* Willd.
English name: New Guinea rosewood
Local name: Marawa (Kalo)


biotech.tipo.gov.tw

Location: Cultivated in a residential yard, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Tree 10-48 tall, foliage often drooping; Leaflets 5-11, alternate, ovate or ovate-oblong; petiole 4.5cm long, stipules lanceolate, 1.5cm long, falling almost at once. Flowers about 1 cm in axillary panicles, fragrant; corolla yellow or orange-yellow. Fruits almost round, 6.5-7 cm by 5.5-6 cm, a stiffly membranous wing surrounding the thickened central 1-4 seeded portion, densely pubescent when young, drying brown.

Medicinal Use: 1: Skin diseases; 2: Purgative
Plant part: 1: Leaves; 2: Buds
Mode of Application: Oral, Tropical
Preparation: 1: Skin disease: Boil a handful of leaves in 1 L of water until the water turns dark. Wash affected body part as often as needed. 2: Purgative: Consume 1 bud and it should be enough to cause vomiting.
Note: Rarely used as a medicine. Used primarily for canoe paddle construction.

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai

Voucher: KAL-16

Family: Asteraceae Date: 09 Apr, 2004

Species: *Erigeron sumatrensis* Retz.
Synonym: *Conyza sumatrensis* (Retz.) E. Walker

English name: Tall fleabane


www5b.biglobe.ne.jp

Location: Melaleuca savannah outside of Kalo, Rigo District
10° 03' S 147° 47' E

Description: Tall erect annual, up to 2 m. It can be distinguished from the very similar *C. bonariensis* by the side branches always being shorter than the main stem, and the pappus being straw-coloured to pale brown.

Medicinal Use: No medicinal use

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-17

Family: Oxalidaceae

Date: 09 Apr, 2004

Species: *Averrhoa carambola* L.
English name: Star fruit, carambola
Local name: Faiv kona (Tok Pisin)

Location: Cultivated tree in betelnut garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description: A small tree up to 12m high. The fruits are star shaped and yellow and up to 12cm long.

Medicinal Use: No medicinal use
Plant part: Fruit
Preparation: Fruit is an edible snack.

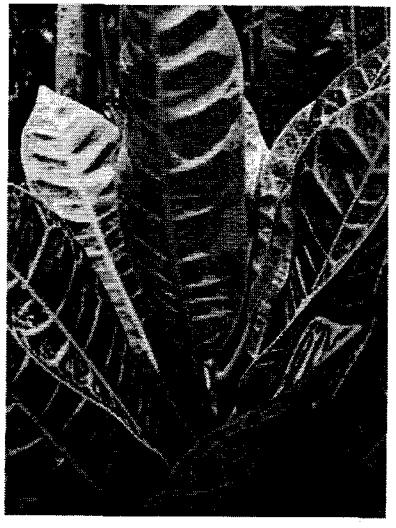
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-18

Family: Euphorbiaceae

Date: 13 Apr, 2004

Species: *Codiaeum variegatum* (L.) A. H. L. Jussieu
English name: Variegated croton
Local name: Papaka (Kalo)



Location: Cultivated shrub in betelnut garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Shrub up to 4m. Leaves simple, alternate, blade variously mottled with red, purple and yellow. Flowers borne in terminal racemes of separate male and female flowers, the male flowers many on thin stalks and bearing many white stamens, the female ones sessile. Corolla of five minute free petals. Fruit a subglobose, shallowly three-lobed capsule.

Medicinal Use: 1: Sore throats; 2: Love charm.
Plant part: Leaves
Mode of Application: Topical
Preparation: 1: Sore throats: Heat 1 leaf over a fire and apply to the throat. Reheat up to 3 times and use a new leaf every day. 3: Love charm: Reveal to the sorcerer the identity of the admired. The sorcerer will pierce 2 leaves with fingernails and places one leaf under and the other over their pillow while they sleep. After every dream, sleeping position and pillow are flipped over. The admired will dream the same dreams. Both will have a sleepless night, but at the next encounter, love should ensue.
Incantation or adjunct therapy: Chant for love charm unknown.

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai

Voucher: KAL-19

Family: Rutaceae Date: 13 Apr, 2004

Species: *Euodia anisodora* Laut. & K. Sch.
English name:
Local name: Mamata (Kalo)

Location: Cultivated shrub in betelnut garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Shrub to small tree to 6 m tall. Leaves opposite, aromatic, trifoliate (or simple), if compound, each leaflet oblanceolate, 8-10 X 15-30, or if simple, the blade lanceolate, and up to 30 cm long. Flowers small, white, fragrant, 4-parted, borne in erect panicles arising from lead axils. Fruit a 4-parted brown dehiscent follicle, with a single seed in each segment.

Medicinal Use: None.
Plant part: Leaves
Preparation: Traditional dress - Common arm-band decorations for dance dress.

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-20

Family: Moraceae Date: 13 Apr, 2004

Species: *Ficus septica* Burm. f.
English name: Fig
Local name: Loku riga (Kalo)

Location: Wild tree on border of betelnut garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Small tree, leaves ovate with a short acumen; receptacles ridged, solitary or in pairs.
Lowland and hills

Medicinal Use: 1: Tonic; 2: Swollen joints, aches and pains
Plant part: 1: Buds, 2: Leaves
Mode of Application: Oral (masticant), Topical
Preparation: 1: Tonic: Chew buds anytime, alone or with betelnut. 2: Swellings and aches: Heat leaf over a fire and apply to affected area. Reheat when cooled. Use new leaf every day, as needed. Not useful for backaches.
Note: Not commonly used, though some elderly people still use occasionally.

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-21

Family: Moraceae

Date: 13 Apr, 2004

Species: *Ficus botryocarpa* Miq.
English name: Fig
Local name: Loku kavu (Kalo)

Location: Wild tree growing in marshy conditions on border of betelnut garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Medium size tree, leaves eliptic to obovate; fruit syconia clustered on long slender, branched twigs, up to 45cm long, receptacles dark, shining green, flattish on the apex, puberulous, glandular, 1.6 x 2.6cm across; peduncle about 1 cm long subtended by a short, wide-based, hairy bract and bearing just below the receptacle a 3-partite, hairy bract.

Medicinal Use: 1: Infection and swelling, 2: Fruit promoter
Plant part: 1: Buds, 2: Fruits
Mode of Application: Topical
Preparation: 1: Infections: Crush bud between fingers and apply juice directly to a wound. Do only once and that should prevent swelling and infection. Also useful against insect bites, rashes and catfish tings. 2: Fruit promoter: Dry a few fruits and burn them at the base of a betelnut palm that is not producing nuts. The rising smoke will encourage fruiting.

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: KAL-22

Family: Fabaceae

Date: 13 Apr, 2004

Species: *Caesalpinia bolduc* (L.) Roxb.
English name: Nickerbean

www.seabean.com

Location: Thorny vine growing on the banks of Kemp Welsh River, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Woody scrambling shrub, with large bipinnate leaves that have sharp recurved hooks on the underside. Leaves 20-40 cm long, prickles scattered along leaf and pinnae rachises; pinnae 4-11 pairs, 5-20 cm long, leaflets 5-10 pairs, elliptic to oblong or ovate; petiole 5-10 mm long. Racemes axillary, often branched, 10-20 cm long, pubescent; pedicels 2-6 mm long, jointed. Sepals shorter than petals, pubescent. Petals 10-12 mm long, yellowish. Pods oblong-elliptic, covered with bristly spines; seeds 1 or 2, ovoid to globose.

Medicinal Use: 1 Ward (Magic)
Plant part: Leaves
Mode of Application: Oral (masticant)
Preparation: A few leaves are chewed to warn of impending danger or enemies. The person will feel prickles on their skin as if struck by thorns as a warning sign.

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: KAL-23

Family: Burseraceae

Date: 16 Apr, 2004

Species: *Canarium indicum* L.
English name: Canarium almonds
Local name: Galip (Tok Pisin), Manukove (Kalo)

Location: Tree growing on rainforest edge close to the banks of the Kemp Welsh River, Kalo, Rigo District
10° 03' S 147° 47' E

objectief.be

Description: Large tree, wild and cultivated for its fruit. Strong resinous smell. Young plant has large leaves.

Medicinal Use: Headaches and colds
Plant part: Leaves
Mode of Application: Inhalant
Preparation: Crush and macerate a few leaves in hands, form a cup and inhale deeply to clear sinuses. Strong resinous, peppery odour.

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-24

Family: Piperaceae

Date: 09 Apr, 2004

Species: *Piper betle* L.
Syn: *Chavica betle* Miq.
English name: Betel pepper
Local name: Daka (Tok Pisin), Anuanu (Kalo)

Location: Cultivated vine growing on betelnut palm in garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description: A woody vine which attached to trees by roots at the nodes on the main vine. The leaves can be 12cm long. Flowers are separately male and female. Male spikes are thinner and longer than female. The spikes droop.

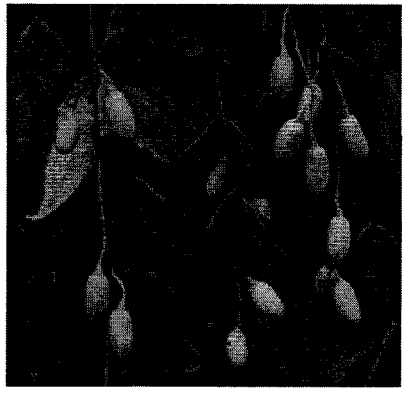
Medicinal Use: Bee stings
Plant part: Fruit
Mode of Application: Topical
Preparation: Rub fresh pepper directly on bee stings or insect bites and the swelling will be reduced.

Herbalist: Kakai Kini (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-25

Family: Meliaceae Date: 27 March, 2004

Species: *Azadirachta indica* A.H.L. Jussieu
English name: Neem



www.efloras.org

Location: Planted tree in a residential yard, Kalo, Rigo District
10° 03' S 147° 47' E

Description: An evergreen tree up to 30 m tall with limbs reaching half as wide. The shiny dark green pinnately compound leaves are up to 30 cm long; each leaf has 10-12 serrated leaflets that are 7 cm long by 2.5 cm wide. Flowers white or pale-yellow, small, scented, numerous and found in long, axillary panicles; the drupes are yellow on ripening, aromatic, oblong and smooth, with a single exalbuminous seed.

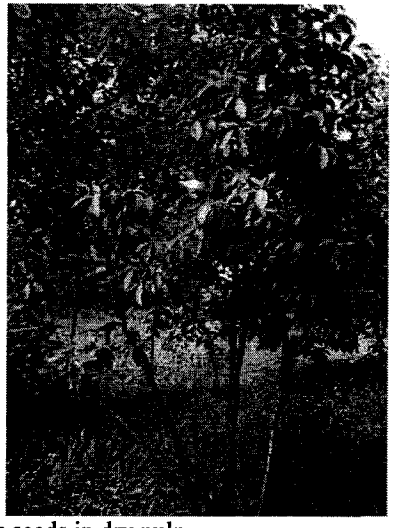
Medicinal Use: Diabetes (Wanigela)
Plant part: Leaves
Mode of Application: Topical
Preparation: Boil 4 leaves in 1 L water for about 10 minutes. Strain and drink 1 cup per day, every day. Use healthy fresh leaves.
Note: Kokoa derived the use of Neem against diabetes from it's use against malaria from friends. Has only been used in the last 4 years, encouraged by the SDA publication "Back to Eden".

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-26

Family: Rubiaceae Date: 27 March, 2004

Species: *Timonius timon* (Spreng.) Merr.
Local name: Arapa (Kalo)



Location: Planted tree in residential yard, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Small, spreading, unisexual shrub or tree, 2-6m high. Leaves simple, opposite, elliptical, tapering at both ends; margin entire. Diocious. Male flowers in cymose clusters, calyx 5-toothed, hairy; corolla white, tubular, salviform, 5-lobed, each lobe with central crest, hairy on outside, scented; anthers exserted. Female flowers solitary and somewhat larger, with 9-11 corolla lobes. Fruit smooth, ovoid, to 2 cm diameter, green, ripening pale brown, containing numerous seeds in dry pulp.

Medicinal Use: 1: Anti-venom; 2: Fatigue
Plant part: Buds and young shoots
Mode of Application: Oral (masticant)
Preparation: 1: Anti-venom: Chew a few (3-6) young shoots and buds as soon as bitten. 2: Fatigue: Chewing shoots will give energy, vitality and make one fit. Chew opportunistically while walking in the field. Chewing the shoots also acts as a ward against dangers and enemies.
Note: Duplicate: WAN-43

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai

Voucher: KAL-27

Family: Rutaceae
Date: 27 March, 2004

Species: *Citrus aurantifolia* (Christm.) Swing.
English name: Lime
Local name: Muli (Tok Pisin), Tiporo (Kalo)

Location: Cultivated tree in a residential yard, Kalo, Rigo District
10° 03' S 147° 47' E

Description: A small much branched tree up to 5m tall and with short sharp spines. Leaves are small. Narrow wings on leaf stalk.

Medicinal Use: 1: Anti-venom; 2: Colds, coughs and flu
Plant part: 1: Fruits, 2: Leaves
Mode of Application: Oral
Preparation: 1: Anti-venom: Immediately after being bitten, eat 5 unripe fruits with the peel. Use only once. 2: Colds: Infuse a few leaves (3-4) in every cup of black tea, preferably 3 cups per day.
Incantation or adjunct therapy: None
Other ingredients: None

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-28

Family: Apocynaceae
Date: 27 March, 2004

Species: *Alstonia scholaris* R.Br.
English name: Milky Pine
Local name: Puro (Kalo)

www.townsville.qld.gov.au

Location: Wild tree on rainforest edge close to the banks of the Kemp Welch River, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Tall tree buttresses, leaves in whorls, follicles long, hanging. Wood used for canoe railing.

Medicinal Use: 1: Tonic (Kalo); 2: Weight loss (Wanigela)
Plant part: 1: Leaves, 2: Buds
Mode of Application: Oral
Preparation: 1: Tonic: Boil a handful of young leaves and buds per cup of water and prepare a decoction. Drink as often as desired. 2: Weight loss: Boil leaves in a pot of water and have a person who needs to gain weight immerse themselves in the steam (Wanigela).
Incantation or adjunct **therapy**: None
Other ingredients: None
Note: Should not be used by pregnant women

Herbalist: Gewa Kimali (Kalo), Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-29

Family: Euphorbiaceae Date: 27 March, 2004

Species: *Manihot esculenta* Crantz
English name: Tapioca, cassava
Local name: Maniota (Kalo)

Location: Common garden staple, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Woody herb, shrub or small tree to 2 m high. Sap milky. Leaves alternate, simple or palmatifid with 3-9 segments; glabrous. Petioles crimson. Flowers in yellowish racemes, lower ones female. Fruit globose, with 6 narrow wings; 3-celled.

Medicinal Use: None
Other: Tuber is a common food

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-30

Family: Apocynaceae Date: 27 March, 2004

Species: *Alstonia spectabilis* R.Br.
Local name: Goli (Kalo)

Location: Planted as a pair near the entrance to Kalo village, Rigo District
10° 03' S 147° 47' E

Description: Pagoda-shaped tree to 10-15 m high, with about 4 horizontal branches at each node. Stems square. Contains white sap. Leaves in whorls of 4, obovate, dark green above, yellow-green below, glossy; veins white, prominent. Inflorescence cymose, pedunculate, more or less hemispherical. Flowers small; corolla tubular, 5-lobed, with hairs on exposed surface of lobes, white, furry. Fruit a long, thin, pendent follicle, splitting longitudinally, green, turning brown at maturity, containing numerous seeds each with tufts of white hair.

Medicinal Use: Nightmares (Magic)
Plant part: Leaves
Mode of Application: Topical
Preparation: Boil a handful of leaves in a pot of water for 5-10 minutes, and wash the whole body of a child who has recently had a nightmare. Repeat whenever nightmares are experienced.
Note: Duplicate of WAN-46

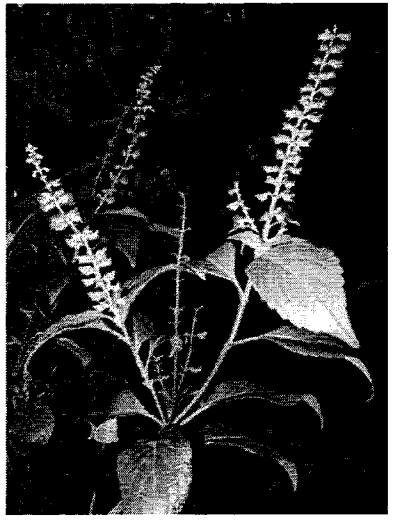
Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: KAL-31

Family: Lamiaceae

Date: 27 March, 2004

Species: *Ocimum americanum* L.
English name: Mint
Local name: Loka (Kalo)



Location: Herb growing on edge of garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Erect aromatic herb with woody base.

Medicinal Use: Body pain (Wanigela)
Plant part: Leaves
Mode of Application: Topical
Preparation: Rub crushed fresh leaves on affected area (Wanigela)

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-32

Family: Piperaceae

Date: 09 Apr, 2004

Species: *Piper* sp.
English name:
Local name: Kaugana Popa (Kalo)

Location: Vine growing on betelnut palms near betelnut garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description:

Medicinal Use: 1: Body aches; 2: Purgative
Plant part: 1: Roots, 2: Buds
Mode of Application: Oral
Preparation: 1: Body aches: Scrape roots of a single vine and squeeze juice into mouth and swallow. Has a multipurpose function, also used for diarrhea, stomach pains and pigbel. 2: Purgative: Squeeze the juice out of buds directly into mouth and swallow until vomiting occurs. Useful against poisoning.
Note: Roots and buds must be used fresh. Used by those who know about it, usually the elderly. Otherwise not commonly known.

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-33

Family: Zingerberaceae Date: 09 Apr, 2004

Species: *Alpinia oceanica* Burk.
Local name: Puramu (Kalo)

http://www.dalbergpoulsen.com

Location: Rainforest beside banana garden, up river from Kalo, about 50 m from the Kemp Welch River, Rigo District
10° 03' S 147° 47' E

Description:

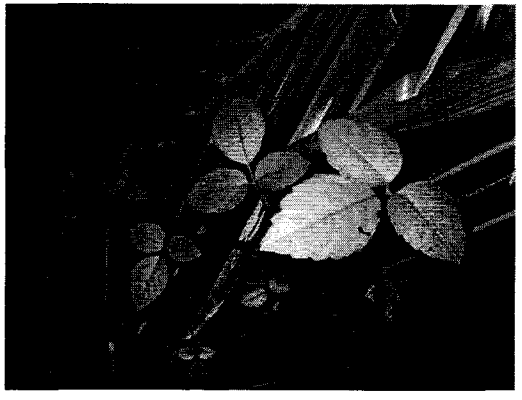
Medicinal Use: Body aches, remove fatigue
Plant part: Leaves
Mode of Application: Topical
Preparation: Rub the leaves on fatigued or aching body part. Also, the stem can be split and made to form a hoop. Put this around the neck and bathe in the river, wash and the runoff from the leaves onto the body will relieve fatigue. Alternatively, split stem to form a hoop and hang around neck, then bathe.
Other ingredients: River water

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-34

Family: Vitaceae Date: 09 Apr, 2004

Species: *Cayratia trifolia* (L.) Domin
Syn: *Cissus trifolia* (L.) Schum.
Local name: Lapia Koko (Kalo)



Location: Vine growing on sago palms near betelnut garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Small tough-stemmed herbaceous creeper. Stems and petioles minutely hairy and ridged. Leaves trifoliate, alternate, a tendril opposite each petiole or sometimes absent; leaflets softly hairy; epidermis white-dotted below. Flowers, bisexual, minute, in much branched terminal umbellate cyme, softly hairy. Calyx pale green; corolla pale green to yellowish with 4 free lobes; disk entirely surrounding ovary; style red. Fruit fleshy, 0.8 cm diameter, green.

Medicinal Use: 1: Kidney and stomach problems; 2: Swelling.
Plant part: 1: Leaves, 2: Buds
Mode of Application: Oral
Preparation: 1: Kidney and stomach problems: Chew 3-4 leaves with food or betelnut and swallow. Consume as much as needed. 2: Swelling: Boil a handful of leaves in a pot of water for 5-10 minutes. Wash swollen area 5-6 times per week until swelling goes down. At the same time, chew 1 bud while washing, 5-6 buds per week.
Note: Pregnant women should not consume.

Herbalist: Gewa Kimali (Kalo)

Voucher: KAL-35

Family: Araceae Date: 09 Apr, 2004

Species: *Epipremnum pinnatum cv. Aureum* (L.) Engl.
Synonyms: *Epipremnum aureum, Pothos aurea* Linden ex André, *Raphidophora aurea* (Linden ex A.) Birdsey, *Scindapsus aureus* (Linden ex André) Engl.
Local name: Pome (Kalo)

Location: Creeping epiphyte growing on a sago palm in mesic forest beside betelnut garden, Kalo, Rigo District
10° 03' S 147° 47' E

Description: Climber, usually on trees, leaves petiolate; blades large ovate-subcordate, to 60 cm long, apex subobtuse, to 40-45 cm broad, lateral veins slightly ascending, the tissue between these often yellow, the leaves thus mostly with irregularly spaced yellow bands and green bands on each side of the midrib. Inflorescences several together, erect; spathe cream, soon withering, navicular; spadix cream, up to 17 cm long and 3 cm in diameter, slightly shorter than spathe. Berries 1-2-seeded.

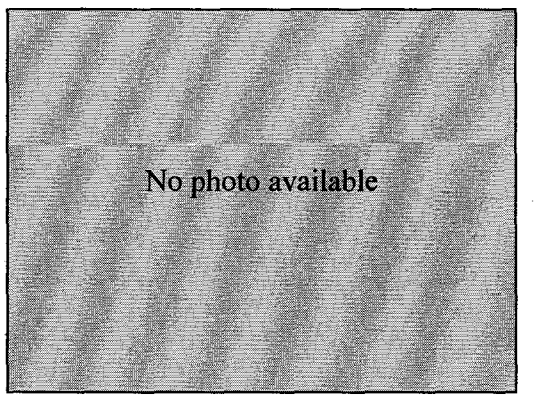
Medicinal Use: 1: Back Pain; 2: Numbness; 3: Diabetes
Plant part: 1: Leaves; 2: Stem
Mode of Application: Topical, Oral
Preparation: 1: Minor body pains: Squeeze and mash a small handful of fresh leaves and rub directly on affected area. 2: Major body pains: Cut the stem and soak it in boiling water until water colour changes. Drink 1 cup per week. The taste is very bitter, so may be taken with food.
Note: Leaves must be used fresh.

Herbalist: Gewa Kimali (Kalo)

Voucher: KAL-36

Family: Asclepediaceae Date: 09 Apr, 2004

Species: Hoya sp. (?)
English name:
Local name: Karo pola (Kalo)



Location: Creeping and climbing vine near a coconut plantation, Kalo, Rigo District
10° 03' S 147° 47' E

Description:

Medicinal Use: 1: Back Pain; 2: Numbness; 3: Diabetes
Plant part: 1: Leaves; 2: Root
Mode of Application: Topical, Oral
Preparation: 1: Backpain and numbness: Warm a handful of leaves over a fire and rub directly on affected area. 2: Back pain and numbness: Scrape the roots of 1-2 plants and infuse scrapings in boiling water for 5-10 minutes. Drink 1 cup 1-2 times per week until recovered. Tastes spicey and can be eaten with meals. 3: Diabetes: Squeeze the juice out of about 10 leaves and filter through a coconut sheath. The resulting juice won't be that much, but drink all in one swallow. Repeat once every 2 months, 6 times per year.
Incantation or adjunct therapy: Restrict dietary intake of sugar for diabetes.

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-37

Family: Fabaceae Date: 09 Apr, 2004

Species: *Derris elliptica* (Roxb.) Benth.
Local name: Imora (Kalo)

Location: Creeping Growing wild near Kalo betelnut garden on the edge of a Nypa palm swamp, Rigo District. 10° 03' S 147° 47' E

Description: Large liana 5-12m long or more. Leaflets 7-15, oblong-obovate to oblong-lanceolate, broadest towards the apex, Leaf rachis and petiole densely rusty hairy. Inflorescences, densely rusty hairy; 3-flowered fascicle stalks; pedicels usually purple 4-10mm.Fruit oblong or elliptic-oblong, 3.5-7 cm long, 1.8-2.5cm wide, 1-3 seeded, adpressed rusty pubescent to glabrescent, narrowly winged at the margins.

Medicinal Use: 1: Sores; 2: Fish poison
Plant part: Roots
Mode of Application: Topical
Preparation: 1: Sores: Scrape the roots of a single plant and mix scrapings with hot water. Let the water cool and wash open sores. Wash once a day until sores is dry. 2: Fish poison: Place scraped roots in a pool of water and wait until paralyzed fish float to the surface. Make sure to cover hair before submerging or risk hair loss.
Note: Common plant but little used, now or in the past.

Herbalist: Kala Gimora (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: KAL-38

Family: Asteraceae Date: 09 Apr, 2004

Species: *Tridax procumbens* L.
Local name: Gawa (Kalo)

Location: Weed growing along garden edge, Kalo, Rigo District.
Lat: 10° 03' S Long: 147° 47' E

Description: Annual herb with a procumbant and ascending stem; ray-florets creamy white, disc-florets light yellow. Common weed.

Medicinal Use: Sores
Plant part: Leaves
Mode of Application: Topical
Preparation: Apply a handful of crushed and macerated leaves directly onto an open sore to stop bleeding and prevent infection. Secure in place with a bandage and change daily. Discontinue when sore is dry.
Note: Duplicate of KAL-05.

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Note: duplicate of KAL-05

Voucher: KAL-39

Family: Passifloraceae Date: 06 Jun, 2004

Species: *Passiflora foetida L. var. hispida* (DC. Ex Triana & Planch.) Killip
English name: Stinking passionfruit
Local name: Patikola (Kalo), Patikwara (Wanigela)

Location: Vine growing among beach dunes of Hood Bay, Kalo, Rigo District.
10° 03' S 147° 47' E

Description: Herbaceous, foetid-smelling vine with tendrils. Stem, leaves, petioles, pedicels etc. covered in long, soft greenish or yellowish hairs. Leaves simple, 3-lobed, spirally arranged, yellowish-green, palmately nerved. Tendrils unbranched, in leaf axils. Flowers solitary in leaf axils; bracts 3, finely and deeply divided, terminating in sticky glandular points; calyx 5-lobed, with white margins and green central nerve; petals 5, white; corona filamentous, purple at base, cream at apex; ovary and stamens form a central androgynophore; stamens 5; styles 3. Fruit globose, green, maturing orange.

Medicinal Use: 1: Stomach pain; 2: Anti-venom
Plant part: Leaves
Mode of Application: Oral
Preparation: 1: Stomach pain: Chew 2-3 fresh young leaves every 3 hours until pain abates. 2: Anti-venom: Eat several young leaves soon after being bitten. No limit on the amount or duration of consumption.

Herbalist: Robert Biau (Wanigela), Kokoa Nubu (Wanigela)
Collector: Patrick Owen, Henry Urai

Voucher: KAL-40

Family: Fabaceae Date: 06 Jun, 2004

Species: *Canavalia cathartica* Thouars
Local name: Koko (Kalo)

Location: Vine growing among beach dunes of Hood Bay, Kalo, Rigo District.
10° 03' S 147° 47' E

Description: Perennial climber 3-10cm long, the stems sparsely pubescent or glabrous. Leaflets ovate, broadly elliptic, round or transversely broadly elliptic, acuminate to an acute tip or completely rounded, mostly rounded at the base, glabrous or sparsely pubescent. Inflorescences hanging, 4-12cm long; peduncle 10-22cm long. Calyx shortly pubescent, green speckled with crimson, flowers scented. Standard magenta-purple inside at first with white guide lines fading to bluish purple, paler inside; wings and keel paler, white at base, turning blue beneath. Fruit oblong or more somewhat inflated, eventually more or less dehiscent or indehiscent, each valve with a sutural rib and also with an extra rib just below it, often quite hairy when young. Seeds dark reddish or blackish brown, oblong-ellipsoid, slightly compressed, 1.4-1.8cm long, 0.9-1.2cm wide.

Medicinal Use: No medicinal use

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: KAL-41

Family: Convolvulaceae Date: 06 Jun, 2004

Species: *Ipomoea pes-caprae* (L.) Sweet
English name: Beach Morning Glory
Local name: Koko (Kalo)

Location: Growing among beach dunes of Hood Bay, Kalo, Rigo District.
10° 03' S 147° 47' E

Description: Prostrate creeper. Stems purplish. Leaves alternate, petiolate, somewhat succulent, broadly elliptical to round with noticed apex, glabrous. Flowers axillary in 1- to few-flowered clusters. Corolla trumpet-shaped, purple-red. Fruit a dry capsule, 2.5 cm diameter, splitting at the top into 4 sections when mature.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-42

Family: Asteraceae Date: 06 Jun, 2004

Species: *Mikania micrantha* H.B.K.
English name: Mile-a-minute

Location: Vine growing on driftwood, Kemp Welch River, Kalo, Rigo District.
10° 03' S 147° 47' E

Description: Perennial creeper-climber; stem slender, rooting at the nodes; leaves roughly deltoid-cordate, acuminate, irregularly and strongly toothed, palmate basal veins, 5, 5x4cm, foetid; petiole about 3cm long; inflorescence in terminal panicles, few-flowered, flowers small, white, all disc florets; fruit a black cypsela with a hairy pappus. Introduced.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-43

Family: Fabaceae Date: 07 Jun, 2004

Species: *Inocarpus fagifer* (Parkinson) Fosberg
Syn: *Gajanus edulis* (J.R. & G. Forst.) O. Kuntze, *Inocarpus edulis* J.R. & G. Forst.
English name: Tahitian chestnut
Local name: Wamara (Kalo)

Location: Side of trail in rainforest close to banks of Kemp Welch River, Kalo, Rigo District.
10° 03' S 147° 47' E

Description: A tree from 9-30 m tall with buttresses at the base. The leaves are large and reddish when young and shiny green when adult. The fruit is round and is a one seeded pod with ridges on the surface.

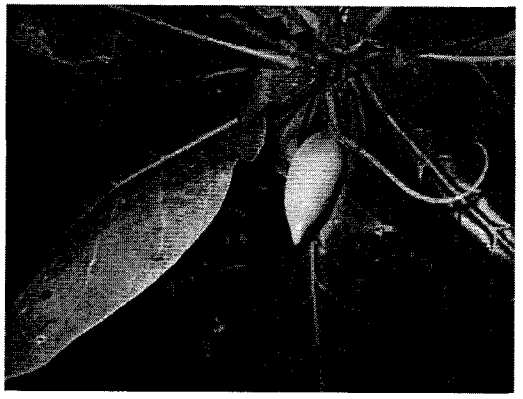
Medicinal Use: No medicinal use
Plant part: Nut
Preparation: Food: Nut is edible

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-44

Family: Combretaceae Date: 07 Jun, 2004

Species: *Terminalia catappa* L.
English name: Beach almond
Local name: Rako (Kalo)



Location: Common trailside tree growing near banks of Kemp Welch River, Kalo, Rigo District.
10° 03' S 147° 47' E

Description: Deciduous tree, to c. 8 m high with pagoda-branching, producing about 5 horizontal branches per layer. Leaves in clusters towards ends of thickened twigs and leaving prominent leaf scars; blade mid-green, turning red when old, glabrous, slightly glossy. Inflorescence an axillary raceme at end of twig brown. hairy; producing an overpowering smell of honey. Fruit 5.5 x 3-4 cm, ellipsoid, somewhat flattened, surrounded by a stiff flange, somewhat fleshy with a hard fibrous stone inside a single seed.

Medicinal Use: No medicinal use
Plant part: Nut
Preparation: Food: Nut is edible
Note: Duplicate of WAN-57

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-45

Family: Moraceae Date: 07 Jun, 2004

Species: *Artocarpus altilis* (Park.) Fosberg
English name: Breadfuit
Local name: With seeds: Mekwa (Kalo); seedless: Gunu (Kalo)

Location: Seeded breadfruit among coconut palms close to banks of Kemp Welch River, upriver from Kalo, Rigo District.
10° 03' S 147° 47' E

Description: A straight-trunked 15-20 m tall with a wide and spreading crown. The trunk, leaves, and fruit exude a milky sap when injured. Leaves spirally arranged, entire to deeply dissected, most commonly 7-9 lobed, 30-60 cm long x 20-40 cm wide; veins yellow or white. Plants are monoecious, with male and female inflorescences on the same tree. The unisexual flowers are small and inconspicuous, grouped into fleshy inflorescences. Male inflorescences appear first, and are yellow, club-shaped, and drooping. The female inflorescences are dense spikes or heads. The fruits are round, oval or oblong, 10-30 cm in diameter, yellow-green at maturity, often with a warty or spiny surface. When ripe, the flesh is white or pale yellow.

Medicinal Use: No medicinal use
Plant part: Nut
Mode of Application:
Preparation: Food: Fruit is edible

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: KAL-46

Family: Bischofiaceae Date: 07 Jun, 2004

Species: *Bischofia javanica* Bl.
English name: Bishipwood
Local name: Rimi (Kalo)

Location: Growing on banks of Kemp Welch River, upriver from Kalo, Rigo District.
10° 03' S 147° 47' E

Description: Evergreen tree, slow growing to 40-60 feet tall (12-18 m); gray to reddish-brown bark; milky sap; leaves with 3 bronzish-green leaflets, 2 to 5 inches long (5-12 cm). Dioecious. Tiny greenish-yellow flowers without petals, in clusters, in the spring. The female trees bear reddish or bluish-black, pea-shaped, fleshy, to 0.33 inch in diameter (9 mm), containing 3 seeds.

Medicinal Use: No medicinal use
Plant part: Timber
Mode of Application:
Preparation: Timber used in construction of canoes and paddles.

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-47

Family: Moraceae

Date: 08 Jun, 2004

Species: *Ficus* sp.
English name: Strangler fig
Local name: Maki (Kalo)

Location: Strangling a *Barringtonia asiatica* tree, growing on banks of Kemp Welch River, upriver from Kalo, Rigo District.
10° 03' S 147° 47' E

Description:

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

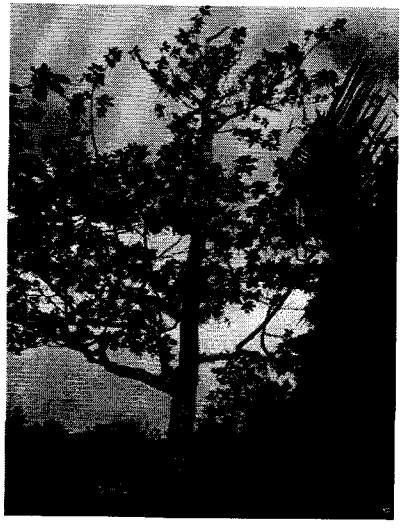
Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-48

Family: Tetramelaceae

Date: 08 Jun, 2004

Species: *Octomeles sumatrana* Miq.
English name: Erima
Local name: Gilimo (Kalo)



Location: Growing on banks of Kemp Welch River, upriver from Kalo, Rigo District.
10° 03' S 147° 47' E

Description: Tall tree up to 60 m in height and 1.5 m in diameter. The trees are of good form, and often heavily buttressed. Bark is grey white to grey brown with shallow fissures.

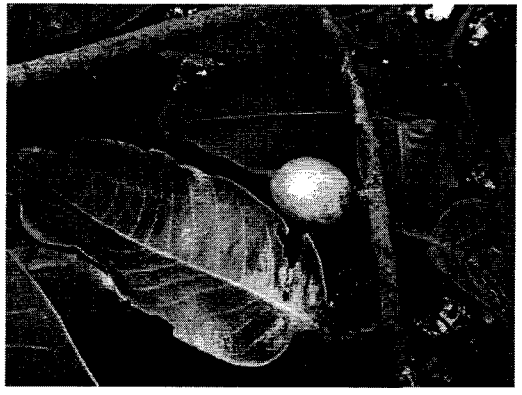
Medicinal Use: No medicinal use
Plant part: Timber
Mode of Application:
Preparation: Timber preferentially used in construction of canoes.

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-49

Family: Myristicaceae Date: 08 Jun, 2004

Species: *Myristica* sp. (?)
English name:
Local name: Kwala (Kalo)



Location: Growing Growing on banks of Kemp Welch River, upriver from Kalo, Rigo District.
10° 03' S 147° 47' E

Description:

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-50

Family: Fabaceae Date: 08 Jun, 2004

Species: *Intsia bijuga* (Colebr.) O. Kuntze
Syn: *Afzelia bijuga* (Colebr.), *I. ambroinensis* DC., *I. bijuga f. glabra* Meijer Drees.
English name: Kwila
Local name: Perila (Kalo)

Location: Cultivated tree in residential yard, Kalo, Rigo District.
10° 03' S 147° 47' E

Description: A small tree up to 42m tall. Bark usually smooth with numerous small pustular lenticels. Leaves with mostly 2 pairs of leaflets, obliquely ovate, 2.5-16.5 cm long, 1.8-11 cm wide, narrowed above to an obtuse or emarginate apex, glabrous and shiny. Flowers in dense terminal panicles or spikes up to 10cm; pedicels slender. Petals white, the claw and then the lamina turning pink or reddish. Fruits oblong, 10-28 cm long, 4-7.2cm wide, 1-8 seeded. Seeds black, flattened, oblong or cordate in outline.

Medicinal Use: No medicinal use
Plant part: Timber
Mode of Application:
Preparation: Construction

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-51

Family: Moraceae

Date: 08 Jun, 2004

Species: *Artocarpus altilis* (Park.) Fosberg
Syn: *A. communis Forst.*
English name: Kwila
Local name: With seeds: Mekwa (Kalo); seedless: Gunu (Kalo)

Location: Cultivated tree in residential yard, Kalo, Rigo District.
10° 03' S 147° 47' E

Description: Tall evergreen tree; leaves large, lobed; flowers monoecious, male inflorescence cylindrical up to 30 cm long, female globose over 30 cm across; both fleshy fruit and seeds are eaten; cultivated and wild.

Medicinal Use: No medicinal use
Plant part: Fruit
Mode of Application:
Preparation: Dietary starch supplement

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-52

Family: Sterculiaceae

Date: 08 Jun, 2004

Species: *Sterculia shillinglawaii* F. Muell.
English name:
Local name: Valina (Kalo)

Location: Rainforest tree on the outskirts of Kalo, Rigo District.
10° 03' S 147° 47' E

Description: Large tree; leaves obovate, cordate; flowers below the leaves, whitish with a maroon red centre.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-53

Family: Caricaceae

Date: 08 Jun, 2004

Species: *Carica papaya* L.
English name: Papaya, Paw paw
Local name: Popo (Tok Pisin)

Location: Cultivated in village, Kalo, Rigo District. 10° 03' S 147° 47' E

Description: Widely grown tropical fruit. Succulent, tree-like plant, 4.5-6 m tall with a stout upright, leafy stem aand milky juice. Leaves near top of stem, palmate, with seven lobes, the latter palmately divided, and up to 60 cm across. Flowers male and female usually on separate plants but sometimes hermaphrodite, sweet smelling, white; the male long-stemmed on 60-90 cm axillary racemes, female on short stems and fewer. Fruits large, oval to round, yellowish when ripe, to 45 cm long, and weighing 0.9 kg-sometimes much more; smooth skinned, fleshy, juicy, seeds black.

Medicinal Use: Malaria
Plant part: Seeds
Mode of Application: Oral
Preparation: Eat 4-8 seeds once a day until symptoms disappear

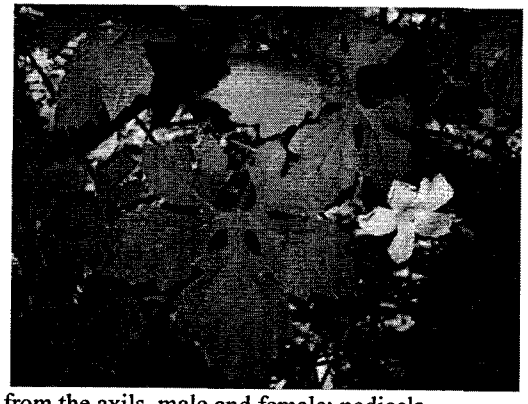
Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: KAL-54

Family: Cucurbitaceae

Date: 08 Jun, 2004

Species: *Momordica charantia* L.
English name: Bittergourd
Local name: Popo (Tok Pisin)



Location: Ornamental in village, Kalo, Rigo District. 10° 03' S 147° 47' E

Description: Perennial herbaceous climber, with tendrils, exceeding 5m on supports, or prostate; foetid-smelling. Leaves alternate, stalked, deeply lobed, with irregular teeth. Flowers solitary from the axils, male and female; pedicels slender, calyx-lobes 5, narrow; corolla yellow, 3-4cm across; in male flowers, corolla deeply 5-lobed, stamens 3; female flowers, petals free, ovary inferior, long-beaked, style trifid, stigmas mostly 2-lobed. Fruit pendulous, to 10cm long, orange with warty surface, many-seeded, finally splitting to reveal bright-red pulp, covering the seeds. Seeds flattened, light straw-coloured, about 1cm long.

Medicinal Use: The sweet red pulp is edible.
Plant part:
Mode of Application:
Preparation:

Herbalist: Gewa Kimali (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-01

Family: Rhizophoraceae Date: 18 Jun, 2004

Species: *Bruguiera gymnorrhiza* (L.) Lam.

English name: Mangrove bean
Local name: Gavera (Pigin), Garere (Motu), Kavela (Kalo, Wanigela)

Location: Swamp beside trail behind aid post, Wanigela, Abau District. 10° 03' S 148° 19' E

Description: Tree to 10m tall. Twigs bearing annular scars. Leaves in upward-pointing clusters. Blade to 15 x 6cm, coriaceous, glabrous, dark green somewhat shiny above, lanceolate, sheathing apical bud. Flowers solitary in axils. Calyx bell-shaped, red with 12-14 long pointed lobes, persistent. Hypocotyl cigar-shaped, ridged, dark green, 11 x 1cm, pendent.

Medicinal Use: Diarrhea
Plant part: Fruit
Mode of Application: Oral
Preparation: Diarrhea: Drink 2 cups of the water that has been used to boil the beans per day until regularity is restored, usually 3 days. Food: Boil fruits for about 10 min, peel, soak in salt water for 2-3 hours, boil again for 10 minutes and drain.
Note: Wanigela staple food

Herbalist: Malta Gideon (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-02

Family: Asteraceae Date: 18 Jun, 2004

Species: *Pluchea indica* Less.

Local name: Popogaro (Wanigela)

Location: Trailside behind aidpost, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Tree to 10m tall. Twigs bearing annular scars. Leaves in upward-pointing clusters. Blade to 15 x 6cm, coriaceous, glabrous, dark green somewhat shiny above, lanceolate, sheathing apical bud. Flowers solitary in axils. Calyx bell-shaped, red with 12-14 long pointed lobes, persistent. Hypocotyl cigar-shaped, ridged, dark green, 11 x 1cm, pendent.

Medicinal Use: 1: Promote child speech (Magic); 2: Hair wash, hair growth; 3: Body wash.
Plant part: Leaves
Mode of Application: Topical
Preparation: 1: Magic: To encourage a child who is a late speaker to talk, a branch with leaves is brushed across their lips. 2: Hairwash and to promote hair growth: Heat approximately 1 handful of leaves on the fire, mix with coconut oil and gratings, and wash hair as needed. 3: Body wash: Same as for hairwash, but use greater quantities to wash body. Also use as steam bath.
Other ingredients: Coconut oil (*Cocos nucifera*)

Herbalist: Robert Biau, Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai

Voucher: WAN-03

Family: Euphorbiaceae Date: 18 Jun, 2004

Species: *Euphorbia geniculata* Ort.
English name: Milkweed

Location: Trailside weed behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Erect annual herb, usually between 25 and 50cm high; stems hollow, branching if not crowded; sap milky. Leaves alternate, ovate-lanceolate, narrow to broad, with shallow teeth on the margins. Flowers separately male and female, gathered into groups in terminal inflorescences; cyathium a 5-lobed green cup, bearing a funnel-shaped gland at one side, enclosing several male flowers and 1 female flower. Male flowers, 1 stamen only; female flowers, a stalked 3-celled ovary with 3 bifid styles. Fruit 3-celled, with 1 seed in each, dehiscing explosively. Seeds grey-brown, roughly globose, pustular and ridged, about 2.5mm across. Native to tropical America.

Medicinal Use: 1: Scabies; 2: Sores
Plant part: Leaves
Mode of Application: Topical
Preparation: 1: Scabies: Boil about a handful of leaves and wrap around affected area. 2: Sores: Crush and rub fresh herbs on new sores as needed.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-04

Family: Asteraceae Date: 18 Jun, 2004

Species: *Synedrella nodiflora* (L.) Gaertn.
English name: Pig grass
Local name: Tuau loka (Wanigela)

Location: Trailside weed behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Erect annual herb, to 70 cm tall. Leaves opposite, to 10cm long, on slender winged stalks. Heads small, solitary or a few together in the leaf-axils, sessile or on peduncles tp 3cm long; bracts few; receptacle flat, paleate, the other pales about 7 x 1.5mm, the inner smaller. Flowers yellow of 2 kinds; those of the outer row (about 5) with a ligulate corolla; disc-flowers narrow, funnel-shaped. Fruit from marginal flowers flat, 4.5 x 2mm, with stiff laciniate wings and 2 awns from the top; fruit from disc-flowers narrow, more or less 3-angled, with 2-3 stiff awns. Native to tropical America.

Medicinal Use: Joint pain and broken bones.
Plant part: Leaves
Mode of Application: Oral
Preparation: To reduce joint pain or encourage speedier bone healing, eat the raw leaves of a single herb 3 times per day, until recovered.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-05

Family: Asclepiadaceae Date: 18 Jun, 2004

Species: *Secamone elliptica* R. Br.
English name:
Local name: Karo (Wanigela)

Location: Vine growing on mangroves in swamp behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Twining woody creeper with latex. Leaves yellow-green to darkish mid-green, glabrous above, variable in shape from naroowly lanceolate through broadly lanceolate to ovate, sometimes fleshy when old; venation not prominent. Flowers in axillary clusters, corolla 5-lobed yellowish. Fruit a follicle, 2-lobed but often only 1 lobe develops, green when immature turning brownish, angled or not. Seeds numerous, plumed.

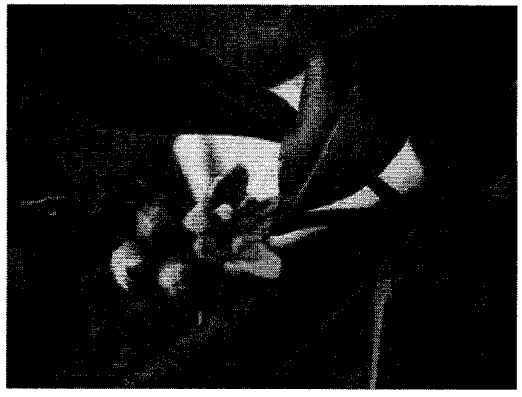
Medicinal Use: 1: Heart problems; 2: stomach ache
Plant part: Leaves
Mode of Application: Oral
Preparation: 1: Heart problems: eat 3-4 raw leaves every 3 h pr day, 5 times per day for 3 days. 2: Stomach aches: same as for heart problems.
Note: Long fruits can be eaten raw.

Herbalist: Robert Biau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-06

Family: Avicenniaceae Date: 18 Jun, 2004

Species: *Avicennia marina* (Forsk.) Vierh.
Syn: *A. intermedia* Griff, *A. mindanaunse* Elmer
English name: Grey mangrove
Local name: Tiavi guala (Wanigela)



Location: Swamp beside trail behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Hardy mangrove with slender roots that rise vertically from the mud; flowers small, orange-yellow that are sweetly scented and small almond-like fruit. Leaves opposite, grey on underside, 4-12cm long, broad or slender. The seed pods are 1.5-4cm long.

Medicinal Use: Scabies
Plant part: Sap
Mode of Application: Topical
Preparation: Use approximately 10 drops of the milky sap and mix with a small quantity of coconut oil and rub on affected area
Incantation or adjunct therapy: Wash body before use.
Other ingredients: Coconut oil (*Cocos nucifera*)
Note: Fruit is called "Waro" and used to be eaten before Kavela became a staple. Wood is a common firewood and used as tinder.

Herbalist: Lau Goloba (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: WAN-07

Family: Rhizophoraceae Date: 18 Jun, 2004

Species: *Rhizophora stylosa* Griff.
English name: Spider mangrove
Local name: Ola (Wanigela)

Location: Swamp beside trail behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Large to medium size tree, bark flaky; roots stilted, with pneumatophores; leaves entire, obovate to elliptic, apex mucronate, base cuneate, blade covered with white gladular dots above, brown lepidote beneath, 11 x 4.5cm; inflorescence axillary, in dichotomous cymes, flowers yellow; fruit roughly funnel-shaped, ferruginous-brown, with a circle of glands below the sepals; sepals 4, erect above the fruit, lanceolate, 1.3cm long; hypocotyl roughly cylindrical, pointed, slightly curved, 8cm long.

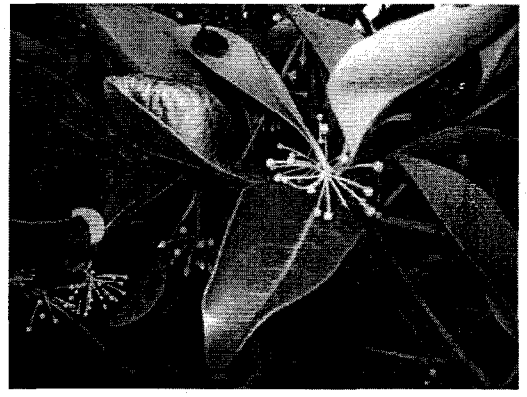
Medicinal Use: No medicinal use
Plant part: Timber
Mode of Application:
Preparation: Wood used as building material such as posts

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-08

Family: Myrsinaceae Date: 18 Jun, 2004

Species: *Aegiceras corniculatum* (L.) Blanco
Syn: *A. majus* Gaertn., *A. fragrans* König
English name: River mangrove
Local name: Kakaro (Wanigela)



Location: Swamp beside trail behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Low evergreen tre or shrub up to 6m; bark smooth, dark grey; leaves alternate, spirally arranged, stipules absent, blade coriaceous, 4-8 x 3-4cm, entire, elliptic to obovate, cuneate at the base, apex rounded to slightly emarginate. Inflorescences as simple umbels, either terminating long shoots or on short lateral shots in the axils of foliage leaves. Flowers fragrant, petals 5, white, pointed, contorted, and always twisted to the left; fruit 1 seede capsule, 5-8cm long, curved with a persistent calyx.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-09

Family: Pteridaceae Date: 18 Jun, 2004

Species: *Acrostichum aureum* L.
Syn: *Chrysodium aurem* Mett.
English name: Mangrove fern
Local name: Lape Lau (Wanigela)

Location: Swamp beside trail behind aid post, Wanigela, Abau District. 10° 03' S 148° 19' E

Description: Fertile fronds with only upper pinnae fertile, pinne up to 30, rather distant and often irregularly distributed. Rachis rounded and smooth below, decidedly grooved above with the margin of the groove acute.

Medicinal Use: No medicinal use
Plant part: Young leaves and rachis
Mode of Application:
Preparation: Food: Boil young, bright green tops for a little less than 5 minutes and drain.

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-10

Family: Acanthaceae Date: 18 Jun, 2004

Species: *Acanthus ebracteatus* Vahl.
English name: Sea-holly
Local name: Rige (Wanigela)

Location: Growing in thickets along trailside behind aid post, Wanigela, Abau District. 10° 03' S 148° 19' E

Description: Halophylic gregarious shrub up to 2m high; leaves variable in shape and size, up to 25 x 10cm, with strong midrib and 8-9 pairs of lateral veins; petiole strong, about 1.5cm long, with a strong spine at its base on the stem; inflorescence in terminal and axillary spikes, flowers large, white, enclosed in a cadacous bract and subtented by a pair of bracteoles.

Medicinal Use: Tonic
Plant part: Leaves
Mode of Application: Topical
Preparation: For general sickness or to maintain health, boil about a handful of leaves in a medium-sized pot and use as a body wash.

Herbalist: Lau Goloba (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-11

Family: Euphorbiaceae Date: 18 Jun, 2004

Species: *Phyllanthus niruri* L.
English name: Sea-holly
Local name: Rige (Wanigela)

Location: Trailside weed behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Erect annual herb, 20-50cm high. Leaves alternate in 2 rows, 5-10mm long, with narrow-triangular stipules about 1.5 mm long. Flowers small, with a greenish 6-lobed perianth, separately male and female, singly or 2-3 together from the axils; Fruit a 3-celled smooth globose capsule; dehiscent. Seeds wedge-shaped, rounded and longitudinally ribbed on the back, about 1.5mm long, light brown. Native to Malesia

Medicinal Use: 1: Diarrhea; 2: Cough, asthma, chest pain; 3: General internal illness
Plant part: 1: Roots; 2: Leaves.
Mode of Application: Oral
Preparation: 1: Diarrhea and general internal sickness: boil a handful of roots for 15-20 minutes, strain and drink the water 2 times per day for 7 days. 2: Asthma, coughs, chest pain: bring to a boil 4 leaves (or upper half of herb) combined with 5 leaves lemon grass and drink tea 2 times per day until pain is relieved.
Other ingredients: Lemon grass (*Cymbopogon citrates*)

Herbalist: Lau Goloba, Perry Otio (Wanigela)
Collector: Patrick Owen, Henry Urai

Voucher: WAN-12

Family: Euphorbiaceae Date: 18 Jun, 2004

Species: *Euphorbia hirta* L.
English name: Asthma plant, Sneezeweed
Local name: Opana (Wanigela)

Location: Growing in thickets along trailside behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Erect or spreading annual herb, to 60cm high, usuaully not much branched; stem and leaves hairy; sap milky. Leaves opposite, 2-5cm long, with toothed margins: stipulate. Flowers male and female in cyathia crowded in dense axillary inflorescences; glands very small. Fruit hairy, 3-celled, about 1.2mm across, 1 seed per cell, dehiscent. Seeds red-brown about 1mm long, irregular-oblong with some faint transverse ridges. Native of tropical America.

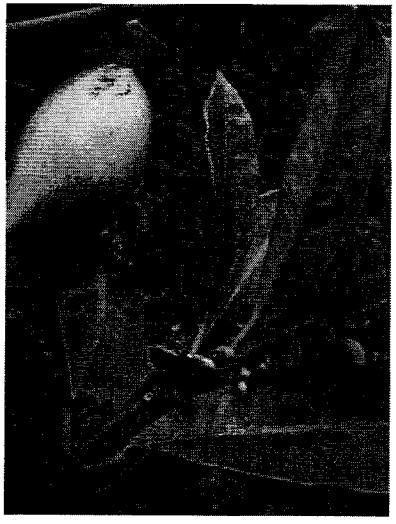
Medicinal Use: 1: Tuberculosis; 2: Diarrhea
Plant part: 1: Herb; 2: Seeds
Mode of Application: Oral
Preparation: 1: Tuberculosis: Boil a handful of leaves for 10-15 min, strain and drink the tea 2 times per day for about 7 days. After a few days wash-out period, repeat if necessary. 2: Diarrhea: Consume the seeds alone or with meals as often as needed until relieved.

Herbalist: Lau Goloba, Perry Otio (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: WAN-13

Family: Apocynaceae Date: 18 Jun, 2004

Species: *Neisosperma* sp. (?)
Local name: Gaila (Wanigela)



Location: Swamp beside trail behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Erect or spreading annual herb, to 60cm high, usuaully not much branched; stem and leaves hairy; sap milky. Leaves opposite, 2-5cm long, with toothed margins: stipulate. Flowers male and female in cyathia crowded in dense axillary inflorescences; glands very small. Fruit hairy, 3-celled, about 1.2mm across, 1 seed per cell, dehiscent. Seeds red-brown about 1mm long, irregular-oblong with some faint transverse ridges. Native of tropical America.

Medicinal Use: Sores
Plant part: Fruit
Mode of Application: Topical
Preparation: Cut fruit in half and apply the flat side and its milky sap to new sores. Fix in place with a plaster or bandage.

Herbalist: Robert Biau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-14

Family: Fabaceae Date: 18 Jun, 2004

Species: *Acacia auriculiformis* A. Cunn. ex Benth.
English name: Acacia
Local name: Kegwali (Wanigela)

Location: Solitary tree planted along road leading to Wanigela off of Magi highway, Abau District.
10° 03' S 148° 19' E

Description: Small tree t0p 6m tall. Stems green to brown, with many lenticels. Leaf-like phyllodes with several parallel longitudinal veins, dark olive-green, slightly glossy, bearing a gland near base. Inflorescence an axiliary spike; flowers very small, bright yellow, with numerous stamens. Fruits red-brown, rather wood, flat, curled, dehiscent. Introduced.

Medicinal Use: No medicinal use
Plant part: Timber
Mode of Application:
Preparation: Timber used to build canoe paddles.

Herbalist: Robert Biau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-15

Family: Solanaceae Date: 18 Jun, 2004

Species: *Physalis angulata* L.
English name: Bladder cherry
Local name: Bulubulu (Wanigela)

Location: Gravel roadside on Wanigela road on edge of Melaleuca savanna, Abau District.
10° 03' S 148° 19' E

Description: Erect, branching annual herb 30-70cm high. Leaves alternate, ovate to lanceolate, with irregular large teeth. Flowers solitary, terminal on very short branches; calyx short during flowering, afterwards growing to about 3cm long, inflated, completely enclosing fruit; corolla wide-campanulate, pale yellow, usually with 5 brownish marks inside, towards the base. Fruit a globuse berry, about 1 cm in diameter, containing numerous seeds. Seed lenticular, nearly 2mm long, light brown with finely-granular surface.

Medicinal Use: 1: Fertility charm (Magic)
Plant part: Herb
Mode of Application: Topical
Preparation: A woman who has problems conceiving can rub the herb on the groin area outside her clothings. The following night, she should conceive after relations with her husband.

Herbalist: Kanata Kokoa (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-16

Family: Euphorbiaceae Date: 18 Jun, 2004

Species: *Cordiaeum variegatum* (L.) Bl. *var. moluccanum* (Decne) Muell. Arg.
English name: Wild type croton
Local name: Kalowai (Wanigela)

Location: Field in the shade of a guava tree, Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: A shrub; branches exuding a white acrid latex; leaves obovate, entire, shining above, dull below, glabrous, rounded to emarginate at the apex, tapering at the base, blade up to 23 x 7cm, petiole up to 10cm long, lateral veins 9-11 pairs; inflorescence in long solitary, axillary racemes, flowers small. Wild species.

Medicinal Use: Toothache
Plant part: Young leaves
Mode of Application: Oral (masticant)
Preparation: Chew 1-2 young leaves on affected tooth. Chew 2-3 times per day.
Note: Duplicate of WAN-18

Herbalist: Lau Goloba (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Note: duplicate of WAN-18

Voucher: WAN-17

Family: Oleaceae

Date: 18 Jun, 2004

Species: *Jasminum* sp (?)
English name:
Local name:

Location: Vine growing on guava in field, Wanigela road, Abau District. 10° 03' S 148° 19' E

Description:

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-18

Family: Euphorbiaceae

Date: 18 Jun, 2004

Species: *Cordiaeum variegatum* (L.) Bl. *var. moluccanum* (Decne) Muell. Arg.
English name: Wild type croton
Local name: Kalowai (Wanigela)

Location: Field in the shade of a guava tree, Wanigela road, Abau District. 10° 03' S 148° 19' E

Description: A shrub; branches exuding a white acrid latex; leaves obovate, entire, shining above, dull below, glabrous, rounded to emarginate at the apex, tapering at the base, blade up to 23 x 7cm, petiole up to 10cm long, lateral veins 9-11 pairs; inflorescence in long solitary, axillary racemes, flowers small. Wild species.

Medicinal Use: Toothache
Plant part: Young leaves
Mode of Application: Oral (masticant)
Preparation: Chew 1-2 young leaves on affected tooth. Chew 2-3 times per day.

Herbalist: Lau Goloba (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Note: Duplicate of WAN-16

Voucher: WAN-19

Family: Sterculiaceae Date: 19 Jun, 2004

Species: *Melochia odorata* L.f.
English name:
Local name: Gamaeka (Wanigela)

Location: Field Road edge of Magi Highway junction Wanigela road, edge of rainforest, Abau District.
10° 03' S 148° 19' E

Description: A shrub; branches exuding a white acrid latex; leaves obovate, entire, shining above, dull below, glabrous, rounded to emarginate at the apex, tapering at the base, blade up to 23 x 7cm, petiole up to 10cm long, lateral veins 9-11 pairs; inflorescence in long solitary, axillary racemes, flowers small. Wild species.

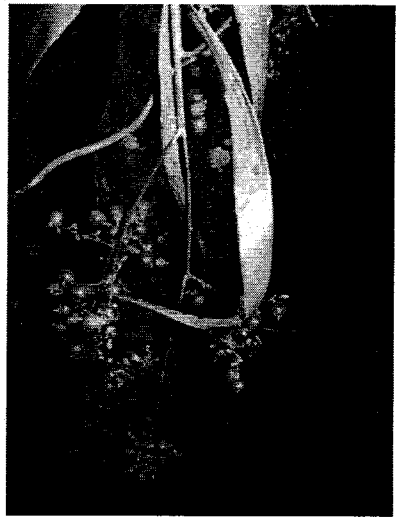
Medicinal Use: Tonic
Plant part: Leaves
Mode of Application: Topical
Preparation: For general sickness or to maintain health, boil about a handful of leaves in a medium-sized pot and use as a body wash.

Herbalist: Lau Goloba (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-20

Family: Flagellariaceae Date: 19 Jun, 2004

Species: *Flagellaria indica* L.
English name:
Local name: Magau (Wanigela)



Location: Vine growing on *Melochia odorata*, edge of rainforest on Magi Highway, junction Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Stem climbing, covered by the tubular, overlapping leaf-sheaths; leaves sessile, tapering to form tendrils at the apex; inflorescence terminal, branched, flowers small, white; fruit berries, globular, pinkish-red.

Medicinal Use: Bloody stool
Plant part: New shoots
Mode of Application: Oral
Preparation: Chew and swallow 3-4 tips (new shoots) 3 times per day until resolved.
Note: Pregnant women can use it as risk of toxicity is low. Duplicate of WAN-53.

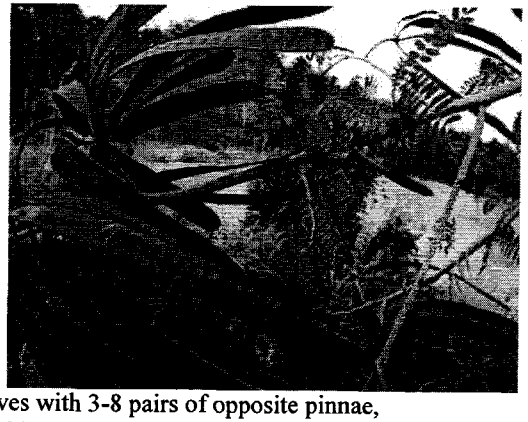
Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-21

Family: Fabaceae

Date: 19 Jun, 2004

Species: *Leaucaena leucocephala* (Lam.) De Wit
Syn: *L. glauca sensu* Benth.
English name:
Local name: Yekarata (Wanigela)



Location: Roadside tree common along Magi highway. Edge of rainforest, Abau District.
10° 03' S 148° 19' E

Description: Shrub or small tree, 0.6-9m tall with mostly densely grey puberulous or pubescent young branchlets; leaves with 3-8 pairs of opposite pinnae, leaflets in 7-17 pairs, obliquely oblong-lanceolate, 0.6-1.9 cm long, 1.5-5mm wide, acute at the apex, puberulous in the margins and sometimes the midrib. Inflorescences globose, up to 1.3cm wide in bud stage, petals pale green or white; Fruit linear-oblong, 8-18cm long, 1.8-2.1cm wide, glabrous or puberulous at margins or all over, hanging down, mostly many in a cluster and splitting down both sides.
Medicinal Use: 1: Fertility drug; 2: Antihelminthic (Pinworm)
Plant part: 1: Leaves; 2: Seeds
Mode of Application: Oral
Preparation: 1: Fertility drug: Boil a handful of leaves in a pot of water. Drink one cup weekly until conceived. 2: Antihelminthic: For pinworm, boil the seeds from 2 pods and drink the water once only.
Note: Plant will kill nearby vegetation (Allelopathy)

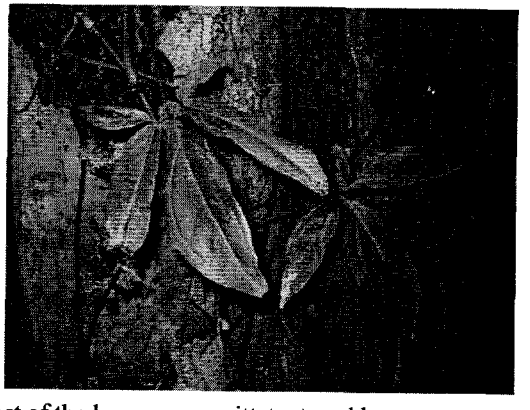
Herbalist: Lau Goloba (Wanigela)
Collector: Patrick Owen, Henry Urai

Voucher: WAN-22

Family: Araceae

Date: 19 Jun, 2004

Species: *Syngonium podophyllum* Schott.
English name: Arrowhead vine
Local name: Galagapala (Wanigela)



Location: Liana growing on *Alstonia scholaris*, edge of rainforest on Magi Highway, Junction Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Perennial vine. In the juvenile form, most of the leaves are sagittate, to subhastate, to hastate in form. Leaves will reach up to 1 foot (30 cm) long by one-third as wide with up to 2-foot (60 cm) petioles. Leaves in the adult form are pedate. Plants will reach up to 15 feet (4.5 m) in length.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-23

Family: Dioscoraceae Date: 19 Jun, 2004

Species: *Dioscorea alata* L.
English name: Wild yam
Local name: Ralala (Wanigela)

Location: Climber vine growing on *Alstonia scholaris*, edge of rainforest, Magi Highway, junction Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Plant, glabrous, unarmed, quadrangular in section, 4-winged; bulbils present; leaves subsagittate or subhastate-ovate, acuminate; female inflorescence up to 60cm long; male about 20cm, flowers small; fruit capsules winged. Many varieties.

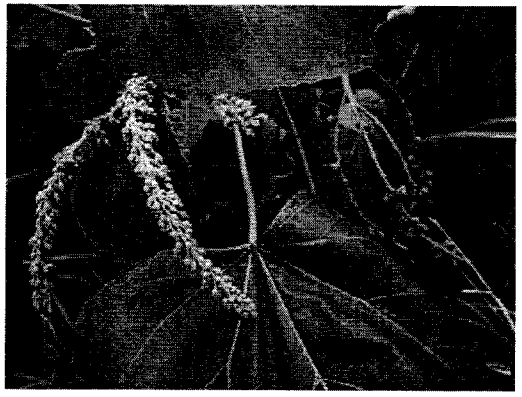
Medicinal Use: Headache, bodyache
Plant part: Leaves
Mode of Application: Topical
Preparation: Rub leaves on head or body as a counter-irritant.
Note: Plant not used locally. Ethnomedical info supplied by co-collector, Henry Urai, who uses it in Rabaul, New Britain Island.

Herbalist: Henry Urai
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-24

Family: Euphorbiaceae Date: 19 Jun, 2004

Species: *Melanolepis multiglandulosa* (Bl.) Reichb. E. & Zoll.
Local name: Vologovu (Wanigela)



Location: Small tree on edge of rainforest, Magi Highway, junction Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Medium size tree; leaves palmately-veined, roughly 3-lobed to subdeltoid, remotely and irregularly dentate, glandular stellate, on very long petioles; flowers on very long spikes, longer than the leaves; fruit small covered with grayish dust.

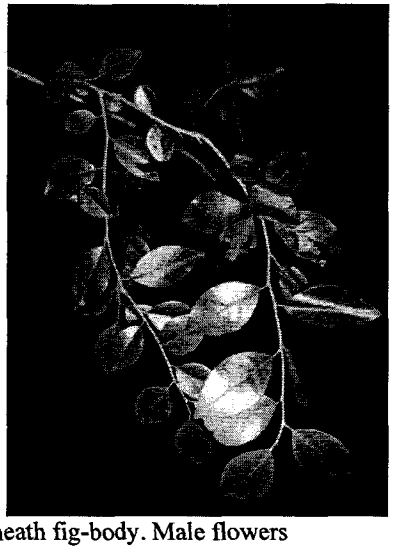
Medicinal Use: Tonic
Plant part: Leaves
Mode of Application: Topical
Preparation: Rub leaves on head or body as a counter-irritant.
Note: Timber used to build canoes

Herbalist: Lau Goloba
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-25

Family: Moraceae Date: 19 Jun, 2004

Species: *Ficus benjamina* L.
English name: Strangler fig
Local name: Kini (Wanigela); Bekal (Wanigela)



Location: Vine intertwined with lianas growing in rainforest, area south of Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Large banyan, glabrous; adventitious roots few to none. Leaves subdistichous, elliptic to ovate; lamina 3-12 cm long, 1.5-6 cm wide; Figs usually sessile, ellipsoidal, ovoid, obovoid, or rarely subglobose, 8-12 mm diam., ripening dark red; pedicel if present thick; ostiole enclosed by 3 flat apical bracts in a disc 2 mm diam., often with a rim; basal bracts 2 or 3, to 1.5 mm long, concealed beneath fig-body. Male flowers abundant, pedicellate; tepals 3. Female flowers sessile; tepals free, 3 or 4. Gall flowers pedicellate; tepals 3 or 4

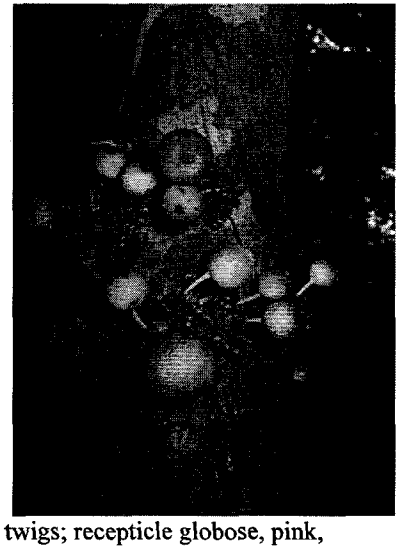
Medicinal Use: Headache
Plant part: Leaves
Mode of Application: Oral
Preparation: Chew 1-2 young leaves until pain dissipates. Use as often as needed.

Herbalist: Lau Goloba
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-26

Family: Moraceae Date: 19 Jun, 2004

Species: *Ficus wassa* Roxb.
English name: Fig
Local name: Kini (Wanigela); Bekal (Wanigela)



Location: Rainforest tree in area south of Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Shrubby tree, bark grayish, flaky, scaly, branches brown with grayish patches, grey-hairy under the young leafy parts; leafy twigs reddish-brown, scabrid, with upturned, short, stiff hairs; leaves opposite, lanceolate sinuate, remotely serrate, apex acuminate, base cuneate, scabrid; receptacles clustered at the axils and around leafy twigs; recepticle globose, pink, turning creamy-yellow, with brown dots, scabrid, 7mm across; peduncle reddish, glandular, scabrid, 6mm long.

Medicinal Use: No medicinal use
Plant part: Leaves
Mode of Application: Oral
Preparation: Chew 1-2 young leaves until pain dissipates. Use as often as needed.
Note: Rough leaves used to scrub pots.

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-27

Family: Ulmaceae Date: 19 Jun, 2004

Species: *Celtis latifolia* (Blume) Planch.

Location: Vine climbing on fig, rainforest in area south of Wanigela road, Abau District. 10° 03' S 148° 19' E

Description: Medium to large tree, bark brownish-grey, smooth, with fine cracks and pustules, young branchlets brown-hairy, older ones rough, slightly furrowed, dark brown to grey; leaves simple, alternate, oval, often asymmetrical, 3.5-7.6 cm, acuminate tip, round base, smooth margins, multiple veins arising from base. Flowers in a aillary or terminal panicle, small, yellow-green, slight odourous. Fruit a glubose drupe

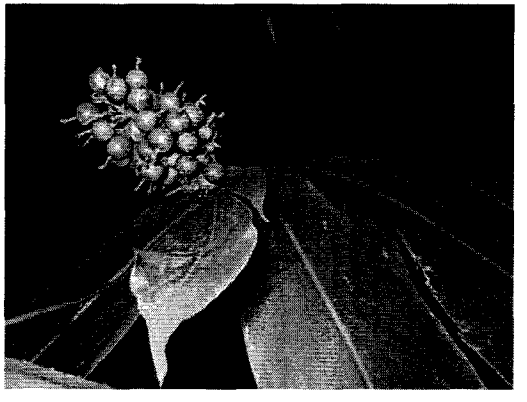
Medicinal Use: Fever
Plant part: Young roots
Mode of Application: Oral, topical
Preparation: For general sickness or to maintain health, boil about a handful of new leaves and buds in a pot of water and drink one cup 2 times per day. This is accompanied by a bodywash made with leaves in fresh water (not boiled).
Note: Rough leaves used to scrub pots.

Herbalist: Lau Goloba (Wanigela)
Collector: Patrick Owen, Henry Urai

Voucher: WAN-28

Family: Zingiberaceae Date: 19 Jun, 2004

Species: *Alpinia coerulea* (R. Br.) Benth.
Synonym: *Hellenia coerulea* R. Br.
English name: Native ginger, Common ginger
Local name: Puramo (Wanigela)



Location: Rainforest, close to edge of Melaleuca savanna, south of Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Leaves dark green, frond-like resembling various gingers grown ornamentally. Fruits bright blue, about 1.5cm wide, consisting of a brittle shell enclosing angular seeds in crisp white pulp.

Medicinal Use: Anti-venom
Plant part: Roots
Mode of Application: Oral (masticant)
Preparation: Directly after being bitten, chew 1-2 root tips and repeat 2 times per day until better.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-29

Family: Leeaceae Date: 19 Jun, 2004

Species: *Leea indica* (Burm. f.) Merr.
Syn: *Stenolobium stans* (L.) Seemann.
English name:
Local name:

Location: Rainforest, close to edge of Melaleuca savanna, south of Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Shrub with long curving branches; leaves large, compound, leaflets bipinnate except the last pair which is 3-pinnate; inflorescence opposite the upper leaves, corymbose-cymose; fruit a berry; subglobular, blue.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-30
Family: Lilliaceae (Phormiaceae) Date: 19 Jun, 2004

Species: *Dianella ensifolia* (L.) DC.
English name:
Local name: Muna (Wanigela)

Location: Rainforest, close to edge of Melaleuca savanna, south of Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Stems slightly tufted; leaves narrow, long, the margin and the midrib at the back of the leaf with teeth, rough to the touch; inflorescence in lose panicles, flowers white and light green, fruit a purple berry.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-31

Family: Fabaceae Date: 19 Jun, 2004

Species: *Maniltoa steenisii* van Meeuwen
English name:
Local name: Guara (Wanigela)

Location: Rainforest, close to edge of Melaleuca savanna, south of Wanigela road, Abau District. 10° 03' S 148° 19' E

Description: Tree 4-7.5m tall; bark mottled grey, dark brown, white or greenish with round pustular lenticels; Leaflets 2-3 pairs, elliptic to lanceolate, 3-10cm long, 1-3cm wide, obliquely acuminate, the extreme apex apiculate or obliquely emarginate, rounded or somewhat auriculate on the basal side, glabrous; leaf rachis 1.5-4cm long; Petals white or pink.

Medicinal Use: Tonic
Plant part: Young leaves, bud
Mode of Application: Oral and topical
Preparation: For general sickness or to maintain health, boil about a handful of new leaves and buds in a pot of water and drink one cup 2 times per day. This is accompanied by a bodywash made with leaves in fresh water (not boiled).

Herbalist: Lau Goloba
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-32

Family: Gnetaceae Date: 19 Jun, 2004

Species: *Gnetum gnemon* L.
English name:
Local name: Obeta Lau (Wanigela)

Location: Rainforest, close to edge of Melaleuca savanna, south of Wanigela road, Abau District. 10° 03' S 148° 19' E

Description: Small tree; leaves simple, opposite, elliptic or lanceolate; flowers in axillary spikes; fruit ellipsoid.

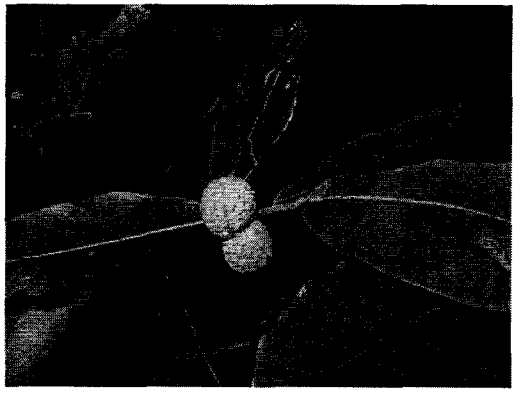
Medicinal Use: Tonic
Plant part: Leaves
Mode of Application: Topical
Preparation: For general sickness or to maintain health, boil a handful of leaves in a pot of water and wash the body as often as desired.

Herbalist: Lau Goloba
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-33

Family: Annonaceae Date: 19 Jun, 2004

Species: *Polyalthea forbessii* F. Muell.
English name:
Local name: Uria Tupa (Wanigela)



Location: Rainforest, close to edge of Melaleuca savanna, south of Wanigela road, Abau District.
Lat: 10° 03' S Long: 148° 19' E

Description:

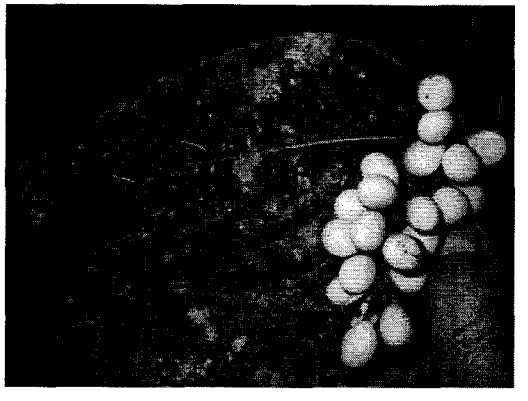
Medicinal Use: Fatigue
Plant part: Roots
Mode of Application: Oral (masticant)
Preparation: Chew roots as often as necessary to remove general fatigue.

Herbalist: Lau Goloba
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-34

Family: Menispermaceae Date: 19 Jun, 2004

Species: *Tinomiscium petiolare* Hook. f. & Thom.
English name:
Local name: Walakoko (Wanigela)



Location: Vine growing in rainforest, close to edge of Melaleuca savanna, south of Wanigela road, Abau District.
10° 03' S 148° 19' E

Description:

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-35

Family: Sterculiaceae Date: 19 Jun, 2004

Species: *Theobroma cacao* L.
English name: Cocoa
Local name: Koko
(Wanigela)

Location: Rainforest, close to edge of Melaleuca savanna, south of Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Small evergreen tree, up to 30 feet tall, with large glossy drooping leaves.
The fruit grows directly on the trunk of the tree and main branches. It has very small white flowers and the tree develops fruits after about five years. The big 8 - 12" seedpod, which is the fruit; is yellow, green or red and contains the seeds surrounded by an aromatic pulp which is formed from the seeds teguments. The fruit contains 20 - 50 flat light-brown seeds.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:
Note: Commercial crop

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-36

Family: Ebenaceae Date: 19 Jun, 2004

Species: *Diospyros maritima* Bl.
English name:
Local name: Gauguri
(Wanigela)

Location: Rainforest, close to edge of Melaleuca savanna, south of Wanigela road, Abau District.
10° 03' S 148° 19' E

Description: Small tree with few branches, dioecious inflorescence in a cluster of flowers subtended and hidden by grey-hairy bracts.

Medicinal Use: Tonic
Plant part: Leaves
Mode of Application: Topical
Preparation: For general sickness or to maintain health, boil about a handful of leaves in a medium-sized pot and use as a body wash.

Herbalist: Lau Goloba (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-37

Family: Arecaceae Date: 19 Jun, 2004

Species: *Gulbia costata* Becc.
English name:
Local name: Kolu (Kalo)

Location: Edge of rainforest and Melaleuca savanna, solitary, Abau District.
10° 03' S 148° 19' E

Description: Tall palm; leaves long, pinnate; inflorescence Areca-like, much-branched spikes; flowers creamy-white with an unpleasant scent. The large bracts are used as containers, the old inflorescence branches as brooms and the trunk, split into long lathes, for flooring.

Medicinal Use: Toothache
Plant part: Adventitious root
Mode of Application: Topical
Preparation: Chew young brown-red tender adventitious roots on affected area as often as needed. (Kalo)
Note: Fruits on panicles are eaten in Kalo as snacks.

Herbalist: Peter Ogera (Kalo)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-38

Family: Fabaceae Date: 19 Jun, 2004

Species: *Crotolaria incana* L.
English name:
Local name: Kolu (Kalo)

Location: Common in Melaleuca savanna, south of Wanigela road close to junction with Magi Highway, Abau District.
10° 03' S 148° 19' E

Description: Erect or spreading often rather bushy, annual or short-lived perennial up to 1.5-3.5m tall with hairy stems. Leaves with 3 leaflets; leaflets round, elliptic or obovate, 2.5-5cm long, 1.7-4.4cm wide, glabrous above, pilose beneath; stipules filiform. Racemes up to 30cm long with many flowers about 12mm long; corolla yellow with reddish brown or purple veins. Fruits oblong, mostly broader towards the apex, 3-4.5cm long, densely spreading hairy. Widespread in tropical America and tropical Africa.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-39

Family: Fabaceae

Date: 19 Jun, 2004

Species: *Erythrina variegata* L.
English name: Indian coral tree
Local name: Binigi (Wanigela)

Location: Small tree on edge of road leading to Wanigela, planted, Abau District.
10° 03' S 148° 19' E

Description: Deciduous tree 3-27m tall; bark grey-green or grey, furrowed; young shoots at first stellate-pubescent. Leaflets green, sometimes variegated, ovate to broadly rhomboid, acute or acuminate at the apex, truncate, rounded or slightly cordate at the base. Inflorescence dense, many-flowered. Corolla scarlet or crimson, rarely white. Fruit stipitate, sausage-shaped or elongate-cylindrical.

Medicinal Use: 1: Decrease menstral flow; 2: Body pain.
Plant part: Roots
Mode of Application: Oral
Preparation: 1: Decrease menstral flow: Scrape a handful of roots, young or old and boil in a pot of water, drink one cup of the tea 3 times per day until flow is lightened. 2: Body pains: Scrape roots (young or old), boil in a pot of water, strain and drink the tea as often and as long as necessary until pain subsides. Leaves may also be used.
Note: Pregnant women should not use. Leaves can be dried and stored for later use.

Herbalist: Lau Goloba (Wanigela)
Collector: Patrick Owen, Henry Urai

Voucher: WAN-40

Family: Fabaceae

Date: 19 Jun, 2004

Species: *Desmodium velutinum* (Willd.) DC.
English name:
Local name: Mu (Wanigela)

Location: Common trailside weed behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Deciduous tree 3-27m tall with fluted bole and much branched crown; trunk and branches thick and sappy; bark grey-green or grey, furrowed; young shoots at first stellate-pubescent. Leaflets green, sometimes variegated, ovate to broadly rhomboid, acute or acuminate at the apex, truncate, rounded or slightly cordate at the base, at first sparsely stellate-pubescent tomentose, soon almost glabrous. Inflorescence dense, many-flowered. Corolla scarlet or crimson, rarely white. Fruit stipitate, sausage-shaped or elongate-cylindrical, 4-13-seeded, slightly constricted between the seeds.

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Herbalist:
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-41

Family: Euphorbiaceae Date: 19 Jun, 2004

Species: *Acalypha wilkesiana f. wilkesiana* Muller Argoviensis
English name: Beefsteak plant
Local name:

Location: Ornamental tree in banana garden, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Shrub to 4m high. Leaves simple, alternate, blade usually ovate or nearly round to kidney-shaped, margins toothed. Flowers inconspicuous, unisexual, in separate axillary spikes, surrounded by bracts. Corolla absent, the calyx of tiny inconspicuous sepals. Fruit a capsule.

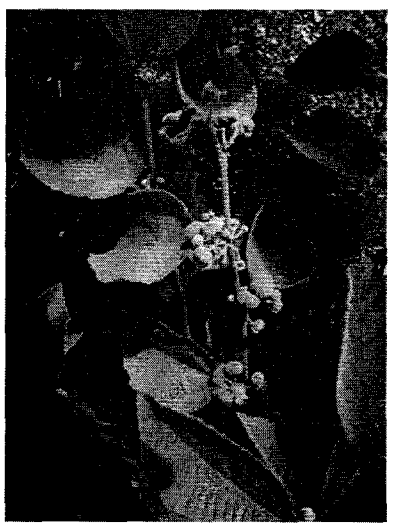
Medicinal Use: Peptic ulcer, stomach ache
Plant part: Leaves
Mode of Application: Oral
Preparation: Boil 4-6 leaves until water turns black, strain and drink 4 times per day for 1 day only.
Note: Pregnant women can use it; in fact can facilitate delivery when consumed during labour

Herbalist: Robert Biau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-42

Family: Ulmaceae Date: 19 Jun, 2004

Species: *Celtis* sp. (?)
English name:
Local name:



Location: Tree growing on roadside of Wanigela road on edge of Melaleuca savanna, Abau District.
10° 03' S 148° 19' E

Description: Shrub to 4m high. Leaves simple, alternate, blade usually ovate or nearly round to kidney-shaped, margins toothed. Flowers inconspicuous, unisexual, in separate axillary spikes, surrounded by bracts. Corolla absent, the calyx of tiny inconspicuous sepals. Fruit a capsule.

Medicinal Use: Anti-venom
Plant part: Leaves
Mode of Application: Oral
Preparation: Chew 4 fresh leaves as soon as bitten, 2 times per day for 1 week.
Note: Pregnant women should not use.

Herbalist: Robert Biau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-43

Family: Rubiaceae Date: 19 Jun, 2004

Species: *Timonius timon* (Spreng.) Merr.
Local name: Keagoli (Wanigela)

Location: Small tree growing in Melaleuca savanna, Abau District.
10° 03' S 148° 19' E

Description: Small, spreading, unisexual shrub or tree, 2-6m high; female trees rather more robust than males. Young stems softly hairy. Leaves simple, opposite, at twig apices, elliptical, tapering at both ends, pale yellow-green or dull green above, paler below, usually softly hairy on underside, especially on veins, glabrescent; margin entire. Diocious. Male flowers in cymose clusters, calyx 5-toothed, hairy; corolla white, tubular, salviform, 5-lobed, each lobe with central crest, hairy on outside, scented; anthers exserted. Female flowers solitary and somewhat larger, with 9-11 corolla lobes and sterile stamens deep in tube, stigmas protruding. Fruit smooth, ovoid, to 2 cm diameter, green, ripening pale brown, containing numerous seeds.

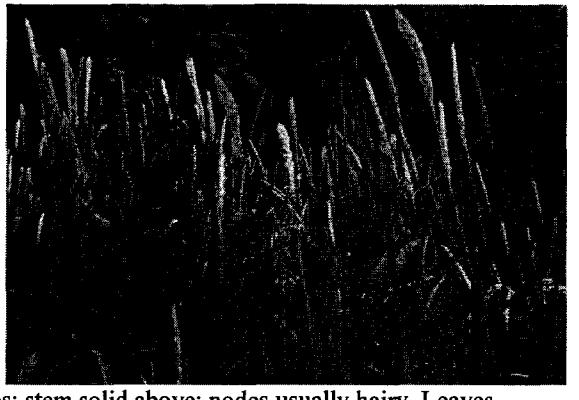
Medicinal Use: Malaria, headache, high blood pressure
Plant part: Leaves
Mode of Application: Oral
Preparation: Eat as much as desired for 3 days.
Note: Only use when bark has turned black, indicating maturity.

Herbalist: Robert Biau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: WAN-44

Family: Poaceae Date: 19 Jun, 2004

Species: *Imperata cylindrica* (L.) P. Beauv.
English name: Kunai grass
Local name: Kunai (Tok Pisin), Kura-kura (Motu), Mu (Wanigela)



Location: Small tree growing in Melaleuca savanna, Abau District.
10° 03' S 148° 19' E

Description: Robust perennial grass to 2m high, with stout rhizomes; stem solid above; nodes usually hairy. Leaves mostly from near the base, flat, broad, erect. Panicle more or less contracted, to 30cm long. Spikelets alike, 1-flowered, in long- and short-pedicelled pairs; long white silky hairs from the callus and glumes; glumes 4.5mm long, with membranous tips; lemmas much shorter, membranous, transparent; stamens 2. Grain ellipsoid, less than 1mm long, free.

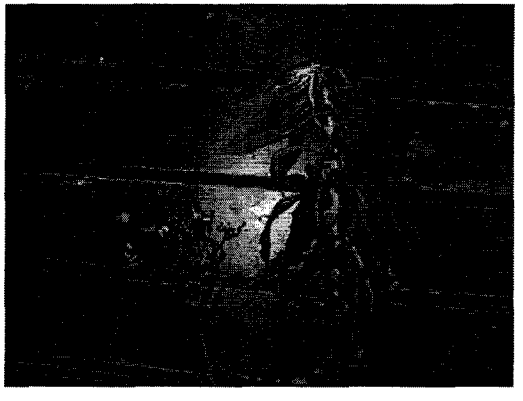
Medicinal Use: Sores
Plant part: Leaves
Mode of Application: Topical
Preparation: Chew leaves into mulch and stick to an open sore. Secure in place with a bandage and change daily. Remove once sore is dry.

Herbalist: Robert Biau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-45

Family: Asteraceae Date: 19 Jun, 2004

Species: *Conyza* sp. (?)
English name:
Local name: Leavaka (Wanigela)



Location: Grassland weed from private property, Korela, Abau District. 10° 03' S 148° 19' E

Description:

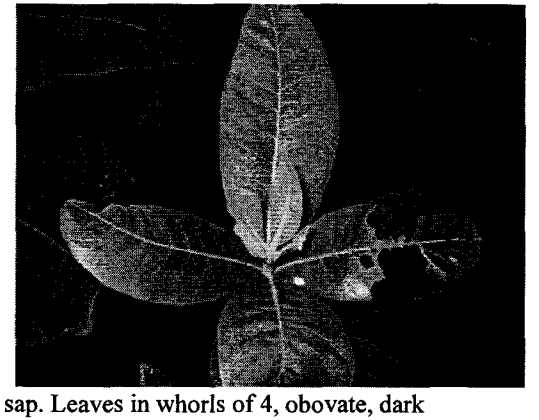
Medicinal Use: 1: Fertility drug; 2: Toothache
Plant part: 1: Leaves, 2: Stem
Mode of Application: Oral
Preparation: 1: Fertility drug: Combine a handful of leaves, 3-4 plants of *Physalis angulata* and a handful of *Cymbopogon citratus* leaves and boil 15-20 minutes. Strain and drink 1 cup daily until conception is successful. Both woman and man should drink the tea. 2: Toothache: chew stem on affected area as often as necessary until pain subsides.
Incantation or adjunct **therapy**: After both partners have consumed the tea, should try to consummate.
Note: Wellin Otio, Perry's wife obtained the fertility recipe from her Seventh Day Adventist church group. Always use the herbs fresh; cannot dry and preserve.

Herbalist: Perry Otio, Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-46

Family: Apocynaceae Date: 21 Jun, 2004

Species: *Alstonia spectabilis* R.Br.
Local name: Wiya (Wanigela)



Location: Tree growing on roadside of Wanigela road on edge of Melaleuca savanna, Abau District. 10° 03' S 148° 19' E

Description: Pagoda-shaped tree to 10-15 m high, with about 4 horizontal branches at each node. Stems square. Contains white sap. Leaves in whorls of 4, obovate, dark green above, yellow-green below, glossy; veins white, prominent. Inflorescence cymose, pedunculate, more or less hemispherical. Flowers small; corolla tubular, 5-lobed, white, furry. Fruit a long, thin, pendent follicle, splitting longitudinally, green, turning brown at maturity, containing numerous seeds each with tufts of white hair. Monsoonal scrub and savanna woodland.

Medicinal Use: Asthma, strong cough
Plant part: Buds and young leave
Mode of Application: Oral
Preparation: Eat 2-3 young leaves with buds with a sweet drink to disguise its bitterness 3 times/day for 1 week.
Note: Pregnant women should not consume due to possible toxicity to fetus. Duplicate of KAL-30.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: WAN-47

Family: Fabaceae

Date: 21 Jun, 2004

Species: *Vigna* sp.
English name:
Local name: Walowalo (Wanigela)

Location: Common trailside weed behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description:

Medicinal Use: Rashes, Ringworm, Itching
Plant part: Leaves
Mode of Application: Topical
Preparation: Boil a large quantity of leaves in water and wash affected area as much as necessary.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-48

Family: Fabaceae

Date: 21 Jun, 2004

Species: *Mimosa pudica* L
English name:
Sensitive plant
Local name: Titona (Wanigela)

Location: Common trailside weed behind aid post, Wanigela, Abau District.
10° 03' S 148° 19' E

Description:
Decumbent shrub, the stems 0.3-1.5cm long; very prickly. Leaves bipinnate, sensitive; pinnae in 2 pairs arising close together so that the arrangement appears palmate. Flowers in globose heads; calyx minute, corolla about 2 mm long; stamens 4, purplish-pink. Pods bristly on the margins, finally breaking into 1-seeded joints, which fall away from the unbroken marginal sutures. Seed 2.5mm long, light brown with a finely-granular surface. Native of tropical America

Medicinal Use: War charm (Magic)
Plant part: Herb
Mode of Application: Topical
Preparation: Rub plant all over hands. When the herb is then hung over a fire, invincibility will be granted in the upcoming battle.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-49

Family: Asteraceae

Date: 21 Jun, 2004

Species: *Vernonia cinerea* (L.) Less.
English name:
Local name: Moruvelolo (Wanigela)

Location: Common trailside weed behind aid post, Wanigela, Abau District.
Lat: 10° 03' S Long: 148° 19' E

Description: An erect herb with heads of violet mauve florets.

Medicinal Use: Promote child spech (Magic)
Plant part: Herb
Mode of Application: Topical
Preparation: Brush fresh herb on the lips of a late-speaking child to encourage talk.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-50

Family: Liliaceae

Date: 21 Jun, 2004

Species: *Dracaena angustifolia* Roxburgh
Local name: Waragoiro (Wanigela)

Location: Rainforest north of Wanigela road, midway between Magi Highway and Marshall Lagoon, Abau District.
10° 03' S 148° 19' E

Description: Shrub to 5m high or more, branched at the base. Leaves simple, spirally arranged, blad linear-lanceolate, 10-60 x 1-3 cm, leathery with ivory-coloured margins. Flowers intermittently during the year, flowers many, borne in clusters of one to four in terminal panicle 8-75 cm long, excluding the peduncle. Corolla with fused tepals, divided to near base into six segments 2-3cm long, yellowish white. Fruit an orange glubose berry 1.7-2.5 cm in diameter.

Medicinal Use: 1: Toothache; 2: Fatigue; 3: Swollen joints
Plant part: Leaves
Mode of Application: Oral (masticant)
Preparation: 1: Toothache: Chew and place leaf on aching tooth. Use as often as necessary, but do not swallow due to potentially lethal effects. 2: Fatigue: When tired after carrying a heavy load, tie around waist and wash body in the river. 3: Swollen joints: Cut leaf lengthwise and wrap around the area. Leave on as long as needed.
Note: Duplicate of KAL-02

Herbalist: Kokoa Nabu (Wanigela)

Voucher: WAN-51

Family: Annonaceae

Date: 21 Jun, 2004

Species: *Uvaria* sp. (?)
English name:
Local name: Viks (Wanigela)

Location: Vine climbing of Pterocarpus indica in rainforest, north of Wanigela road, midway between Magi Highway and Marshall Lagoon, Abau District.
Lat: 10° 03' S Long: 148° 19' E

Description:

Medicinal Use: Fever, Headache, Colds, Bodyaches
Plant part: Inner bark, wood
Mode of Application: Topical, inhalant
Preparation: Wood has a strong balm smell that is therapeutic as a steam bath, inhalant or ingestible.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-52

Family: Myrtaceae

Date: 21 Jun, 2004

Species: *Syzygium trivene* (Ridley) Merr. & Perry.
English name:
Local name: Gauguri (Wanigela)

Location: Rainforest tree north of Wanigela road, midway between Magi Highway and Marshall Lagoon, Abau District.
10° 03' S 148° 19' E

Description:

Medicinal Use: Sore muscles
Plant part: Leaves
Mode of Application: Topical, inhalant
Preparation: Crush fresh leaves and rub on affected area, usually legs.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-53

Family: Flagellariaceae Date: 21 Jun, 2004

Species: *Flagellaria indica* L.
English name:
Local name: Magau (Wanigela)

Location: Vine growing on *Syzygium trivene*, rainforest north of Wanigela road, midway between Magi Highway and Marshall Lagoon, Abau District.
10° 03' S 148° 19' E

Description: Stem climbing, covered by the tubular, overlapping leaf-sheaths; leaves sessile, tapering to form tendrils at the apex; inflorescence terminal, branched, flowers small, white; fruit berries, globular, pinkish-red.

Medicinal Use: Bloody stool
Plant part: New shoots
Mode of Application: Oral
Preparation: Chew and swallow 3-4 tips (new shoots) 3 times per day until resolved.
Note: Pregnant women can use it as toxicity is low. Duplicate of WAN-20

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-54

Family: Asteraceae Date: 21 Jun, 2004

Species: *Ageratum coryzoides* L.
English name:
Local name: Tuau loka (Wanigela)

Location: Growing in shadows of banana trees in garden, Melaleuca savanna north of Wanigela road, midway between Magi Highway and Marshall Lagoon, Abau District.
10° 03' S 148° 19' E

Description: Erect herbs with heads of white flowers in dense terminal corymbs.

Medicinal Use: White spots
Plant part: Herb
Mode of Application: Topical
Preparation: Rub on white spots on skin as often as necessary.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-55

Family: Poaceae

Date: 21 Jun, 2004

Species: *Imperata conferta* (Presl) Ohwi.
English name:
Local name: Lalaka (Wanigela)

Location: Smaller than kunai grass, ubiquitous in Melaleuca savanna, Abau District.
10° 03' S 148° 19' E

Description: Similar to *I. cylindrical*, erect with a strong rhizome; leaves radical; culm with glabrous nodes.

Medicinal Use: No medicinal use.
Plant part: Grass
Mode of Application:
Preparation: Agricultural: Rub Place grass in a freshly dug hole before planting a banana tree and the tree will grow faster.

Herbalist: Kokoa Nabu (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-56

Family: Asteraceae

Date: 21 Jun, 2004

Species: *Tagetes erecta* L.
English name: French Marigold
Local name: Garopalawa (Wanigela)

Location: Ornamental herb at Wanigela aid post garden, Abau District.
10° 03' S 148° 19' E

Description: Herb, annual, erect to 1m high with strongly scented, gland-dotted foliage; leaves simple to nearly pinnately compound, alternate to opposite on the same plant, deeply pinnately cut into 4-15 pairs of narrowly elliptic lobes with toothed margins; flowers in large, terminal, bell-shaped heads on a long stalk inflated at the top and surrounded by a cup-like series of bracts. Ray florets several in one series or double-flowered, with an obovate corolla limb usually 1-4 cm long, orange to yellow. Disk florets many, yellow to orange.

Medicinal Use: Boils, sores
Plant part: Flowers and leaves
Mode of Application: Topical
Preparation: Crush and mix 2 flowers and 2 leaves and apply to an unbroken boil. Secure in place with a bandage and change daily until the boil bursts.

Herbalist: Verron Lobo (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: WAN-57

Family: Combretaceae Date: 21 Jun, 2004

Species: *Terminalia catappa* L.
English name: Beach almond
Local name: Okari (Tok Pisin), Rakua (Wanigela)

Location: Behind Wanigela aid post, Abau District.
10° 03' S 148° 19' E

Description: Deciduous tree, to c. 8 m high with pagoda-branching, producing about 5 horizontal branches per layer. Leaves in clusters towards ends of thickened twigs and leaving prominent leaf scars; blade mid-green, turning red when old, glabrous, slightly glossy. Inflorescence an axillary raceme at end of twig brown. hairy; Fruit 5.5 x 3-4 cm, ellipsoid, somewhat flattened, surrounded by a stiff flange, somewhat fleshy with a hard fibrous stone inside a single seed. Widespread coastal tree in Asia and Polynesia, introduced elsewhere.

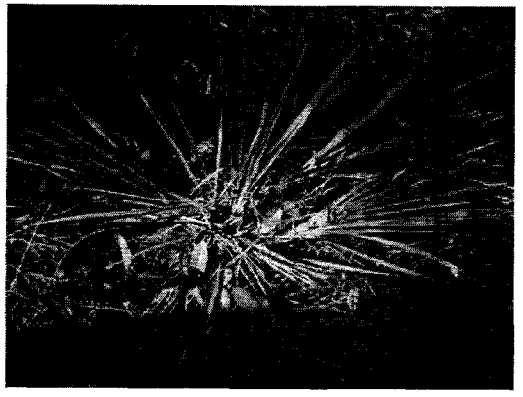
Medicinal Use: Memory loss, headache
Plant part: Leaves
Mode of Application: Topical
Preparation: Boil 6-7 young leaves for 15-20 minutes in a clean pot with a tight lid. Cool, strain and drink all the water in a single day. Repeat daily with fresh leaves for 1 week.
Note: Duplicate of KAL-44

Herbalist: Verron Lobo (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: WAN-58

Family: Poaceae Date: 21 Jun, 2004

Species: *Cymbopogon citratus* (DC) Stan.
English name: Lemongrass
Local name: Tulau (Wanigela)



Location: Behind Wanigela aid post, Abau District.
10° 03' S 148° 19' E

Description: A coarse clumpy grass about 1m high. Leaves have a rough edge, 1m x 1cm. Rarely produces flower. Lemon smell produced when leaves are crushed.

Medicinal Use:
Plant part: Leaves
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-59

Family: Fabaceae Date: 21 Jun, 2004

Species: *Derris elliptica* (Roxb.) Benth.
English name:
Local name: Imora (Kalo, Wanigela)

Location: Growing beside private residence, Korela, Abau District.
10° 03' S 148° 19' E

Description: Large liana 5-12m long or more. 7-15, oblong-obovate to oblong-lanceolate, broadest towards the apex, 2-42cm long, 2-8cm wide, rounded to acuminate at the apex, cuneate to rounded at the base, mostly densely hairy on both surfaces but particularly in the ventation beneath, becoming much less hairy with age. Leaf rachis and petiole densely rusty hairy. Inflorescences 11-26cm long, densely rusty hairy; 3-flowered fascicle stalks; pedicels usually purple 4-10mm.Fruit oblong or elliptic-oblong, 3.5-7 cm long, 1.8-2.5cm wide, 1-3 seeded.

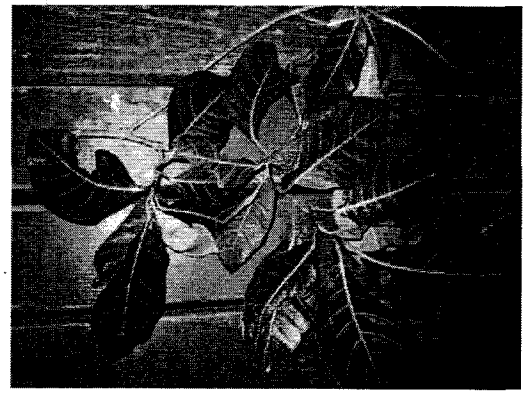
Medicinal Use: 1: Sores; 2: Fish poison
Plant part: Roots
Mode of Application: Topical
Preparation: 1: Sores: Scrape the roots of a single plant and mix scrapings with hot water. Let the water cool and wash open sores. Wash once a day until sores is dry. 2: Fish poison: Place scraped roots in a pool of water and wait until paralyzed fish float to the surface. Make sure to cover hair before submerging or risk hair loss.

Collector: Patrick Owen, Henry Urai

Voucher: WAN-60

Family: Moraceae Date: 21 Jun, 2004

Species: *Ficus septica* Burm. f.
English name: Fig
Local name: Hati Hati (Koari)



Location: Tree on private property, Korela, Abau District.
10° 03' S 148° 19' E

Description: Small tree, leaves obovate with a short acumen; receptacles ridged, solitary or in pairs.

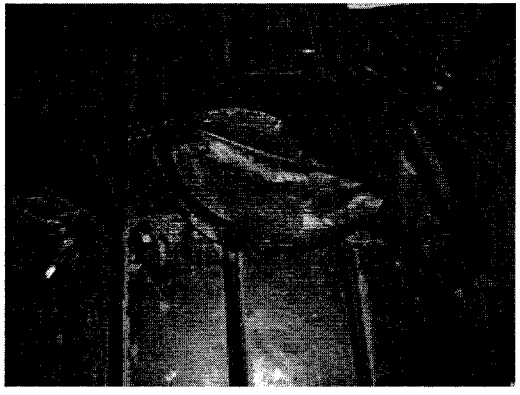
Medicinal Use: Boils, sores
Plant part: New shoots
Mode of Application: Topical
Preparation: Wrap new shots on unbroken boils or new sores. Keep in place with a bandage and replace daily.

Herbalist: Perry Otio (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-61

Family: Smilacaceae Date: 21 Jun, 2004

Species: *Smilax* sp.
English name:
Local name: Keilobe (Koari)



Location: Growing in rainforest beside private residence, Korel, Abau District.
10° 03' S 148° 19' E

Description:

Medicinal Use: Stingray stings
Plant part: Stem
Mode of Application: Subdermal
Preparation: Cut the stem segment between two leaves and heat it over a fire. A drop of sap will from on one end. Like a straw, blow into the opposite end and force bubbling liquid sap directly into sting site. Use once only.

Herbalist: Perry Otio (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-62

Family: Agavaceae (Liliaceae) Date: 21 Jun, 2004

Species: *Cordyline terminalis* (L.) Kunth.
English name: Ti-plant
Local name: Waragoiru (Wanigela)

Location: Ornamental in Wanigela, Abau District.
10° 03' S 148° 19' E

Description:

Medicinal Use: No medicinal use
Plant part:
Mode of Application:
Preparation:

Herbalist: Salome Tanau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-63

Family: Crassulaceae Date: 21 Jun, 2004

Species: *Kalanchoe pinnata* (Lamarck) Persoon
English name: Life plant, air plant
Local name: Niabele (Wanigela)

Location: Ornamental in Wanigela, Abau District. 10° 03' S 148° 19' E

Description: Herb succulent, 1.0 - 1.5m high with pith-filled stems somewhat woody at base. Leaves simple, opposite, ovate to elliptic, margins purple, crenate. Flowers many, pendulous, in terminal panicles. Corolla of fused petals, red, tubular, 4-5.5 cm long, 4-lobed about one-third its length. Fruit of four narrowly ovoid follicles 1-1.5cm long from separate ovaries.

Medicinal Use: Body aches
Plant part: Leaves
Mode of Application: Topical
Preparation: Mix 4 leaves with the oils and gratings of 1 coconut, rub on skin as a body wash. Thereafter, wash with salt water by swimming in the sea or lagoon.
Incantation or adjunct therapy: Salt water swim
Other ingredients: Coconut (*Cocos nucifera*)

Herbalist: Ruana Laba (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-64

Family: Malvaceae Date: 21 Jun, 2004

Species: *Abelmoschus manihot* (L.) Medik.
Syn: Hibiscus manihot L.
English name:
Local name: Aibika (Tok Pisin); Tu (Koari)

Location: Garden in Korela, Abau District. 10° 03' S 148° 19' E

Description: A branched shrub up to 2m or more high. Plants can last for a year or for several years. Leaf shapes and colours vary. Old plants produce a hibiscus type flower.

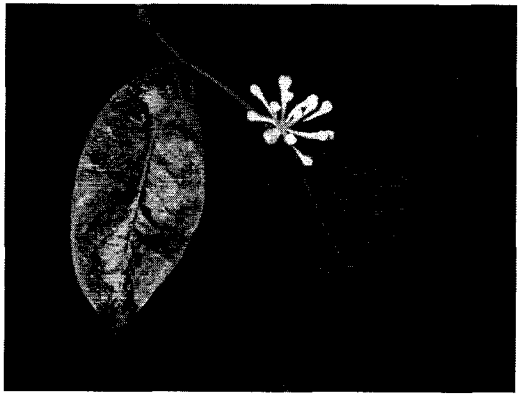
Medicinal Use: Facilitates labour
Plant part: Leaves
Mode of Application: Topical
Preparation: Boil leaves in a pot of water and wash mother-to-be daily for the 40 weeks leading to delivery. Also rub leaves directly on skin.
Other ingredients: Coconut (*Cocos nucifera*)

Herbalist: Ruana Laba (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-65

Family: Thymeleaceae Date: 16 Aug, 2004

Species: *Phaleria sogerensis* S. Moore.
English name:
Local name:
Location: Rainforest near WAN-51, Wanigela, Abau District.
10° 03' S 148° 19' E



Description:

Medicinal Use: Unknown
Plant part:
Mode of Application:
Preparation:

Herbalist: Ruana Laba (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-66

Family: Myrsinaceae Date: 16 Aug, 2004

Species: *Ardisia* sp. (?)
English name:
Local name:

Location: Rainforest near WAN-51, Wanigela, Abau District.
10° 03' S 148° 19' E

Description:

Medicinal Use:
Unknown
Plant part:
Mode of Application:
Preparation:

Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-67

Family: Crassulaceae Date: 16 Aug, 2004

Species: *Kalanchoe pinnata* (Lamarck) Persoon
Syn: *Bryophyllum calycinum*
English name: Life plant, air plant
Local name: Imoaita (Wanigela)

Location: Ornamental in Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Herb succulent, 1.0 - 1.5m high with pith-filled stems somewhat woody at base. Leaves simple, opposite, ovate to elliptic, margins purple, crenate. Flowers many, pendulous, in terminal panicles. Corolla of fused petals, red, tubular, 4-5.5 cm long, 4-lobed about one-third its length. Fruit of four narrowly ovoid follicles 1-1.5cm long from separate ovaries.

Medicinal Use: 1: Sores; 2: Chest pain; 3: Constipation
Plant part: Leaves
Mode of Application: Topical; oral (masticant)
Preparation: 1: Sores: Warm leaf by a fire or on coals and apply to sores. Rewarm as needed, secure in place with a bandage and change daily. Remove once the sore has dried. 2: Chest pain: Chew 1 leaf per day, swallow juice but spit out fibers. 3: Constipation: After 2-3 day of having no bowel movements, eat 2-3 leaves and should have an effect soon after.

Herbalist: Rakana Raka, Malta Gideon (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-68

Family: Agavaceae Date: 16 Aug, 2004

Species: *Aloe vera* L.
Syn: *Aloe barbadensis* Miller.
English name: Aloe
Local name: Aloe

Location: Ornamental in Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Perennial herb, succulent with short thick stem. Leaves simple, arranged in a basal rosette, succulent, sessile, blade

Medicinal Use: Cuts, burns, insect bites, skin inflammations
Plant part: Leaves
Mode of Application: Topical
Preparation: Rub sap on affected area.

Herbalist: Rakana Raka, Malta Gideon (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-69

Family: Araceae

Date: 16 Aug, 2004

Species: *Caladium bicolor* (aiton) Ventenat
Syn: *Caladium hortulanum* Birdsey
English name: Angel wings, elephant's ear, heart of Jesus
Local name: Kiolau (Wanigela)

Location: Ornamental in Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Perennial, stemless, 50cm or more in hight, arising from a globose rhizome. Leaves simple, alternate, blade pentate, arrowhead- or heart-shaped. Flowers many, borne tightly packed in a cylindrical spadix 6-10cm long on a short stalk, surrounded by a slightly longer, ovate, whitish spathe atached at the base. Fruit a pear-shaped white berry.

Medicinal Use: Tuberculosis, coughs or tonic
Plant part: Leaves
Mode of Application: Oral
Preparation: Combine an equal quantity of leaves with lemongrass (Tulau), acalypha (Geroro) and hibiscus (Wabuwabu). Dry them in the sun and prepare a decoction that can be taken 3 times (08:00, 12:00, 20:00) per day for as long as needed.
Other ingredients: Acalypha (*Acalypha wilkesiana f. wilkesiana*), Lemongrass (*Cymbopogon citratus*), Hibiscus (*Hibiscus rosa-sinensis*)

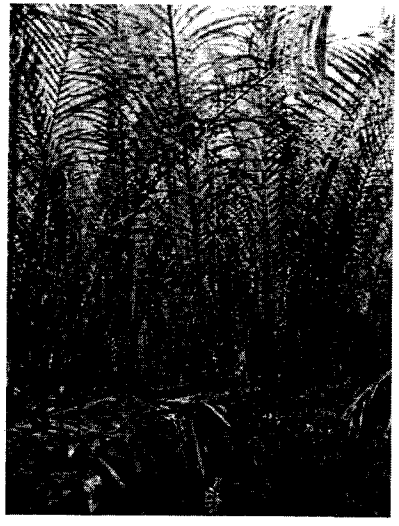
Herbalist: Salome Tanao (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: WAN-70

Family: Arecaceae

Date: 16 Aug, 2004

Species: *Nypa fruticans* Wurmb.
English name: Nypa palm
Local name: Pili (Kalo), Kalamo (Wanigela)



Location: Edge of mangrove forest, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Perennial, stemless, 50cm or more in hight, arising from a globose rhizome. Leaves simple, alternate, blade pentate, arrowhead- or heart-shaped. Flowers many, borne tightly packed in a cylindrical spadix 6-10cm long on a short stalk, surrounded by a slightly longer, ovate, whitish spathe atached at the base. Fruit a pear-shaped white berry.

Use: Leaf midrib used for broomstick
Medicinal Use: Asthma
Plant part: Leaf midrib
Mode of Application: Topical
Preparation: Cut off both ends of a dried midrib obtained from a broom. Sharpen the thinner end and burn the tip. Poke the asthmatic in the middle of the big toe of any foot and their asthma will go instantaneously.

Herbalist: Wari Rauli (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-71

Family: Convolvulaceae Date: 16 Aug, 2004

Species: *Ipomoea batatas* (L.) Lam.
English name: Sweet potato
Local name: Kaukau (Tok Pisin)

Location: Edge of mangrove forest, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Root crop which produces long creeping vines. Leaves are carried singly along vine. At the end of the vine, trumpet-shaped flowers grow. Underground tubers vary in shape, colour, texture according to variety.

Use: Common staple
Medicinal Use: Broken bones
Plant part: Tuber
Mode of Application: Topical
Preparation: Scrape off the skin from one side of the tuber and apply it to the area with a broken bone or fracture to speed healing. Secure in place with a bandage and replace daily.

Herbalist: Wari Rauli (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-72

Family: Arecaceae Date: 16 Aug, 2004

Species: *Cocos nucifera* L.
English name: Coconut
Local name: Kokonas (Tok Pisin)

Location: Cultivated throughout all coastal habitats.
10° 03' S 148° 19' E

Description:. Along-lived plant with a single trunk, 20-30 m tall, bark is smooth and grey, marked by ringed scars left by fallen leafbases. Leaves pinnate,4 to 6 m long, linear-lanceolate. Inflorescences arising at leaf axils and enveloped by a carinate spathe, unbranched spadices; female flowers borne basally, male flowers at apex. Flowers bear lanceolate petals, 6 stamens and an ovary consisting of 3 connate carpels. Fruit is a 1-2 kg drupe with a thin, smooth, grey-brownish epicarp, a fibrous, 4-8 cm thick, mesocarp and a woody endocarp.

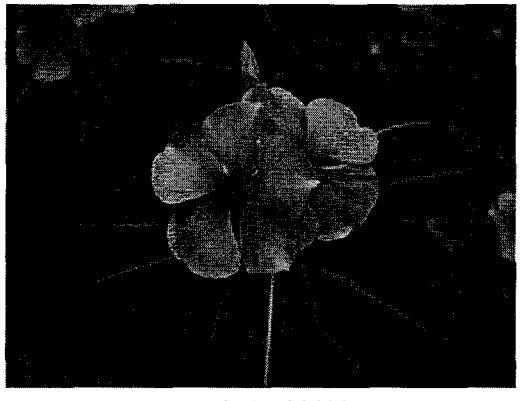
Medicinal Use: Cancer or other internal illness
Plant part: nut shell
Mode of Application: Oral
Preparation: Split 3 coconuts, scrape both sides clean and dry the 6 halves in the sun. Once dry, burn each one at a time. Mix ashes with water until pasty. Strain and drink 3 cups of the water 3 times per day as long as needed up to several years.

Herbalist: Verron Lobo (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen

Voucher: WAN-73

Family: Apocynaceae Date: 16 Aug, 2004

Species: *Catharanthus roseus* (L.) G. Don.
English name: Coconut
Local name: Kokonas (Tok Pisin)



www.fmnh.helsinki.fi

Location: Potted plant, Wanigela, Abau District. 10° 03' S 148° 19' E

Description: Subshrub, perrenial, to 70cm high with milky sap. Leaves simple, opposite, blade elliptic to oblanceolate, 3-9cm long, glossy green. Flowers in pairs at the leaf axils but appearing terminal. Corolla of fused petals, salverform, tube 2-3cm long, limb 3-5.5cm wide with 5 spreading, rounded lobes, rose-purple or white. Fruit a pair of cylindrical pod-like segments 2-3.5cm long, fuzzy and longitudinally grooved.

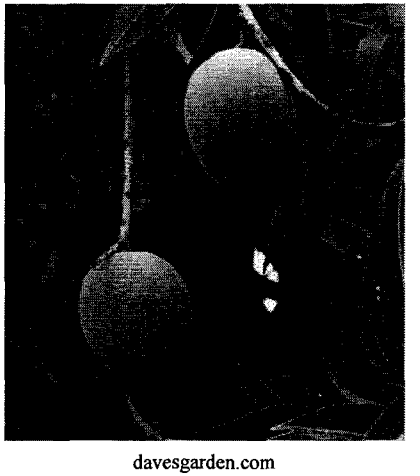
Medicinal Use: Diabetes
Plant part: Leaves
Mode of Application: Oral
Preparation: Boil 20 leaves in 1 L water as a decoction. Drink 4 cups per day for 3 days. Use especially when blood sugar level is high.

Herbalist: Salome Tanao (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen
Taxonomist ID: Pius Piskaut

Voucher: WAN-74

Family: Anacardiaceae Date: 16 Aug, 2004

Species: *Mangifera indica* L.
English name: Mango
Local name: Waiwai (Wanigela)



davesgarden.com

Location: Cultivated, Wanigela, Abau District.
10° 03' S 148° 19' E

Description: Evergreen trees 10-40 m tall, crown large and spreading. Leaves leathery, oblong-lanceolate, 5-32 cm long, 1.5-10 cm wide, glabrous, margins usually undulate, apex acute to long-acuminate, petioles 1-8 cm long. Sepals 2-3 mm long, pilose; petals greenish white or tinged purple, 3.5 mm long, the tips recurved; stamens (1-) 4-5, only 1-2 fertile. Drupes asymmetrical, green with yellow spots or yellowish green to yellowish orange, at maturity sometimes with a purple to red blush, oblong-subreniform, 5-15 cm long, 6-8 cm thick, mesocarp orange, thick, and juicy

Medicinal Use: Scabies in children
Plant part: Inner bark
Mode of Application: Topical
Preparation: Scrape a handful amount of inner bark and boil in 1 L water. Wash affected area 2 times per day for 2 days.
Incantation or adjunct therapy: Wash body before use

Herbalist: Robert Biau (Wanigela)
Collector: Patrick Owen, Henry Urai
Botanist ID: Patrick Owen