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Thermogenic Effect of Beta-Sympathicomimetic Compounds Extracted from *Citrus Aurantium* in Humans

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SHORTENED VERSION OF THESIS TITLE

METABOLIC EFFECTS OF CITRUS AURANTIUM

Dedicated to My Parents Mrs and Mr Adam

The thermogenic effect of beta-sympathicomimetic compounds extracted from Citrus Aurantium, in humans

Mariam M Adam

Abstract

We questioned whether adrenergic amines extracted from the Seville orange *Citrus Aurantium* (CA) increases metabolic rate and enhance the thermic response to a 1.7MJ mixed meal (TEF) in lean and obese men and women; the latter had upper-body obesity, a condition associated with defective TEF, attributed to altered SNS. Nine lean (7M, 2F; BMI: 23±1 kg/m^2; waist circumference: 78±2 cm) and 13 obese (4M, 9F; BMI: 35±1 kg/m^2; waist circumference: 105±3 cm) subjects were studied. With CA: 1) RMR increased more in men vs women (94 vs 42 kJ over 5 h), independently of body composition; 2) urinary epinephrine excretion increased in both groups and dopamine only in men. A 17% lower TEF in obese subjects was no longer significant when controlled for gender. By contrast, there was an effect of gender on TEF that remained significant when adjusted for measures of obesity. Women had a lower TEF that increased to values no longer different from men with CA. CA did not affect TEF in men. CA had no cardiovascular effects. Thus, CA ingestion increased thermogenesis by 4% above RMR and enhanced the lower TEF of women by 29% (46kJ).

L'effet thermogène, chez l'humain, de composantes ß-sympathicomimétiques extraites du Citrus Aurantium

Mariam M Adam

Résumé

On a tenté de déterminer si des amines adrénergiques extraites de l'orange de Séville, Citrus Aurantium (CA), peuvent augmenter le métabolisme au repos et l'effet thermique d'un repas de 1.7MJ (TEF), chez des hommes et femmes maigres ou obèses, ces derniers présentant une obésité androïde, condition associée à un TEF diminué, vraisemblablement dû à des altérations du SNS. On a étudié 7 hommes et 2 femmes avec IMC de 23±1 kg/m^2 et une circonférence de la taille de 78±2 cm, et 4 hommes et 9 femmes avec IMC de 35±1 kg/m2 et une circonférence de la taille de 105±3 cm. L'ingestion de CA a induit une augmentation 1) du métabolisme au repos plus élevée chez les hommes vs femmes (94 vs 42kJ en 5 h), indépendamment de leur composition corporelle et 2) de l'excrétion urinaire de l'épinéphrine dans les 2 groupes et de la dopamine chez les hommes seulement. Un TEF plus bas chez les obèses de 17% n'était plus significatif une fois corrigé pour le sexe. Par contre, l'effet du sexe sur le TEF a demeuré significatif même après ajustement pour les variables de l'obésité. Les femmes ont démontré un TEF plus bas qui a augmenté à des valeurs comparables à celles des hommes avec CA. CA a eu aucun effet sur le TEF des hommes et aucun effet cardiovasculaire. En somme, la prise du CA a augmenté la thermogénèse de 4% au-dessus du métabolisme au repos et le TEF chez les femmes de 29% (46kJ).

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ABBREVIATIONS

ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BCM	Body Cell Mass
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CA	Citrus Aurantium
CNS	Central Nervous System
CT	Computerized Tomography
DIT	Diet Induced Thermogenesis
EE	Energy Expenditure
FDA	Food and Drug Advisory Board
FFM	Fat-Free Mass
FM	Fat Mass
NE	Norepinephrine
RMR	Resting Metabolic Rate
RQ	Respiratory Quotient
SNS	Sympathetic Nervous System
TEE	Total Energy Expenditure
TEF	Thermic Effect of Food
FFA	Free Fatty Acid
WHO	World Health Organisation

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Part 1: INTRODUCTION

The treatment of obesity, through the mediation of increased thermogenesis may induce a negative energy balance, and promote weight loss. A product showing a thermogenic effect, particularly in persons characterised by upper body obesity, could be a useful adjunct to weight loss therapy. Beta-adrenergic alkaloid compounds extracted from *Citrus aurantium* (CA), a bitter orange called Seville sour orange, are claimed (Fyto research Monograph) to stimulate metabolic processes, increase lipolysis and exert mild hunger suppression. For the first time, the ability of these beta - adrenergic alkaloid compounds to stimulate energy expenditure was assessed.

Obesity develops over time and once it has been established, it is difficult to treat. Indeed reports abound that recognise the limitations of many obesity treatments in achieving long term weight reduction. The health consequences associated with obesity, though result from the cumulative metabolic and physical stresses of excess weight and fat mass over a long period of time and are not fully reversible by weight loss. The proportion of the population that is either overweight or obese in developed countries is now so large that the situation is referred to as an epidemic and there is fear that health care resources to offer treatment to all people who are obese will no longer be sufficient (Bray,1998; Prentice, 2000).

Obesity results from an imbalance in the regulation of energy with energy intake exceeding energy expenditure (Plata –Salaman, 2000). This imbalance could result from alterations in ingestive behaviour, metabolism, physical activity, genetic influences, environmental influences, and neuralgic conditions (WHO, 1998). The prevalence of obesity in children and adults is increasing at an alarming rate, and the health implications

are enormous. Thirty-five percent of Canadian men and 29% of Canadian women aged 18-74 years had a body mass index (BMI) greater than 27, an index suggesting excessive adiposity (Macdonald *et al.*, 1997).

Researchers estimate that the direct costs of obesity co-morbidities represent 5.7% of the national health expenditure in the United States (WHO, 1998). In Canada, costs exceed 1.8 billion dollars per year (Birmingham *et al*, 1999). Obesity is now considered a serious chronic medical condition associated with increased morbidity and mortality linked to a heightened risk of dyslipidemia, cardiovascular disease, hypertension, stroke, diabetes mellitus, respiratory disorders and various types of cancer. Based on BMI (subject's body weight in kilograms divided by the square of his height in meters), the current World Health Organisation classification defines overweight as a BMI of 25-29.9, class I obesity as a BMI of 30.0 to 34.9, class II obesity as a BMI of 35.0 to 39.9, and class III as a BMI > 40 (WHO, 1998). Although BMI is not a correlate of the subject's fat mass, a BMI of > 30 is usually attributed to an excess of adipose tissue in most people. Environmental factors interact with the genetic background for the regulation of energy balance. Genetically predetermined abnormalities include lower rate in fat oxidation and resting energy expenditure compared with non obese persons which may lead to positive energy balance (Neel, 1998). Obesity has also been associated with a reduced thermic response to food, possibly related to low sympathetic nervous system (SNS) outflow in various tissues specifically following carbohydrate intake (Astrup, 1986). These abnormalities represent a focus point for interventions in obesity that aim at weight loss. Indeed, weight losses as little as 10% of initial weight are associated with a decrease in health risks (Markovic, 1998).

Drug treatment for obesity has been tarnished by a number of unfortunate problems (Bray, 1999). Since the first drug was used to treat obesity in 1993, almost every drug treatment that has been tried in obese patients has generated undesirable outcomes that have resulted in drug termination (*ibid*). The use of some, such as amphetamine, has been stopped because of their addictive properties. The elimination of other drugs such as fenfluramine and dexfenfluramine was also secondary to undesirable side effects. Currently, only two prescribed medications are available for the treatment of obesity: Sibutramine ^{R}, that acts on serotonergic and noradrenergic pathways and has been reported to have thermogenic properties (Hansen, 1997) and Orlistat ^{R}, an inhibitor of gastric and pancreatic lipases (Bray, 1999). A third issue in drug treatment of obesity is the perception that because patients regain weight when drugs are stopped, the drugs are ineffective (*ibid*).

There are three types of drugs currently used to treat obesity and which show effects on reducing food intake, altering metabolism, and/or increasing energy expenditure. In terms of decreased food intake, both noradrenergic receptors and serotonergic receptors have served as sites for clinically useful drugs. The stimulation of β_2-adrenoceptors, by norepinephrine (NE) or agonists such as terbutaline, clenbuterol, or salbutamol reduces food intake (Bray, 1995). This is a clinically important receptor for regulation of body weight. Another strategy in intervention could be to influence intermediary metabolism by enhancing thermogenesis, lipolysis, or inhibiting lipogenesis, and fat distribution between the subcutaneous and visceral sites.

One thermogenic approach that has been widely tested is the combined use of ephedrine and caffeine (Bray, 1999). Ephedrine stimulates thermogenesis in human

subjects (*ibid*) and caffeine is a xanthine that inhibits adenosine receptors and phosphodiesterase. Ma Huang or Ephedra is a natural source of ephedrine which also has thermogenic effect but none have been approved for the treatment of obesity (*ibid*). The sympathetic nervous system has a tonic role in maintaining energy expenditure (*ibid*). Blockade of the thermogenic part of this system will reduce the thermic response to a meal (*ibid*). NE, the neurotransmitter of the SNS may also decrease food intake by acting on $ß_2$- or $ß_3$- adrenergic receptors (*ibid*).

Sympathomimetic drugs work by a variety of mechanisms including the release of NE from synaptic granules or a direct action on adrenoceptors (Dulloo,1993). New evidence converged towards the notion that adrenergic mechanisms mediating cardiac and pressor effects differ from those mediating thermogenesis, a difference that the pharmaceutical industry has exploited to put forward a new generation of atypical adrenoceptor agonists with greater selectivity for lipolysis and thermogenesis (*ibid*). It was indeed one of these novel agonists that led to the demonstration of atypical adrenoceptors (Arch *et al* 1984) currently referred to as $ß_3$-adrenoceptors. Citrus Aurantium (CA) is a natural herbal extract containing the essential oils of Seville orange. In unpublished clinical studies, CA was found to exhibit a powerful thermogenic effect by increasing resting metabolic rate. CA is said to work primarily at the $ß_3$ receptor and therefore is not associated with undesirable side effects on heart rate, blood pressure or nervous system (Penzak *et al.,* 2001). Intakes greater than 1.65 g/day have shown to improve weight control.

Part 2: LITERATURE REVIEW

2.1 Definition and Basic Concepts

2.1.1 Thermodynamics

Humans engage in a constant exchange of energy with the environment. The human body loses energy continually to the environment in the form of heat. To sustain life, this energy must be restored. Humans derive this energy from the potential chemical energy contained in the nutrients that are absorbed from the diet. This energy is quantified by measuring the heat released (heat of combustion) when a known amount of the nutrient is oxidised in a bomb calorimeter. This heat of combustion is expressed as kcal per gram. The heat of combustion of glucose is 3.75 kcal / g; of starch is 4.1 kcal / g; of protein is 5.4 kcal / g; of fat is 9.1kcal / g and of ethanol is 7.1 kcal / g. The commonly accepted values for the useable energy contents of carbohydrate, protein, fat and ethanol are 4, 4, 9 and 7 kcal respectively. They are lower than the total free energy content because of the variability in absorption of the nutrient, the hydration of glucose, the energy remaining in the nitrogenous end products of amino acid oxidation, and the volatility of ethanol which results in some loss from the body.

When a nutrient is oxidised, part of its energy is thermodynamically obligated for conversion to heat since the heat energy content of the metabolic end products is greater than the free energy content of the initial nutrient. The amount of heat lost is not large, with approximately 95 % of ingested energy available as free energy. However, the conversion of food energy into usable high-energy compounds such as adenosine triphosphate (ATP) is not very efficient, with more than half of potentially available free

energy lost to the environment as heat. This heat loss is essential since it forms the basis of metabolic pathways and their regulators. The fraction of free energy retained in ATP is subsequently expended in four major processes: 1) Basal metabolism (Na-K-ATPase) and other ion transfer systems to maintain electrochemical gradients across the cell membrane (Himms-Hagen, 1976), the energy required for growth and repair of the structural compounds of the body (Garrow, 1981) energy expended during the inter conversion of metabolic substrates, and mechanical work performed by the cardiovascular and respiratory systems; 2) The performance of external work by muscular contraction (Flatt, 1992); Westerterp *et al.* 1985); 3) The thermoregulatory mechanisms that are designed to maintain core body temperature constant (Girardier, 1983; Himms-Hagen, 1984); 4) The thermic effect of food (Rothwell *et al.,* 1983). In most individuals, the basal metabolic rate is the single greatest determinant of overall daily energy expenditure. The basal metabolic rate is primarily dependent on the lean body mass of the subject, although age, sex and familial factors also play a role (Bogardus *et al.*, 1986). Body cell mass is reported to be the best predictor (*ibid*).

2.1.2 Energy Balance

The energy balance of the body is maintained only if the energy content of the food absorbed from the diet is equal to the energy or heat generated. When energy *in* equals energy *out*, a person is said to be in neutral or zero energy balance. If energy *in* is greater than energy *out* a person is said to be in positive energy balance and is storing energy. When energy *in* is less than energy *out*, a person is in negative balance and oxidises his or her endogenous stores to provide energy (Burszstein *et al,*.1989).

Although daily variation in food consumption and bodily energy demands may frequently produce short-term imbalances in energy economy (Bessard *et al.* 1983); it is clear that the long-term maintenance of a stable body weight depends on an extraordinary tight coupling of energy intake and output (Acheson *et al.* 1983).

2.1.3. Energy Storage

When humans ingest carbohydrate, proteins, or fat, they either oxidise them immediately and use the energy released to do work, or transform them into fat that can be stored as potential energy (Burszstein *et al.*, 1989). The body stores energy as fat and glycogen. When exogenous energy is not available, as occurs during an overnight fast, the body derives energy from its endogenous fuels (Garrow, 1978). Energy is stored mainly as fat. A normal 70 kg man has about 13 kg of fat, which can yield approximately 125,000 kcal (525000 kJ) on oxidation. These stores can theoretically allow an individual to survive for 50 days, given a daily energy requirement of 2500 kcal. A normal man has about 11 kg of protein of which 7 kg are intracellular and 4 kg are extracellular. The extracellular or structural proteins in bone and connective tissue are very stable, and are considered to be unavailable for metabolic needs. The intracellular proteins, which make up the body's active cell mass, serve many functions but are chiefly enzymes and contractile proteins; there is no storage of protein. The estimated 11 kg of protein can potentially yield approximately 45000 kcal (188,000 kJ), or a maximum of 18 days of energy at 2500 kcal/day. However, due to the essential function of protein, loss of protein leads to adverse functional consequences which are conventionally considered to be the

cause of death in long term starvation (Hoffer, 1993). In addition to fat and protein, the body contains between 1000-3000 kcal of carbohydrate stored in liver and muscles as glycogen. This is available for immediate energy needs such as during exercise but is of little long-term value by virtue of its limited supply.

2.1.4 Energy Expenditure

Energy Expenditure is the total heat output of the subject, including the equivalent of the heat of water vaporised. In practice it is rarely measured directly, but it is estimated by various methods. The immediate energy source for metabolic work in the tissues is a phosphate bond in ATP or some other high-energy phosphate compounds, which must be resynthesised if metabolism is to continue. Each substrate has a different heat of combustion and generates a different amount of heat per litre of oxygen used to burn it. The oxidation of 1 mol (180g) of glucose involves the uptake of 6 mol (134.4 litres) of oxygen, and the production of 6 mols (134.4 litres) of carbon dioxide and 6 mols (108 litres) water, with the liberation of 2.78MJ (665 kcal). Of this energy less than half (about 44%) is recovered in high–energy phosphate bonds which can be used to drive further metabolic work, and the remainder is degraded to heat. Each fuel also has a characteristic respiratory quotient (RQ), the volume of CO_2 divided by the volume of O_2 comsumed for oxidation. The RQ for carbohydrate is 1.0, for fat is 0.7 and for protein 0.8. Despite the large differences in the heat of combustion, they all produce similar amounts of energy per litre of O_2 used , 5.05 kcal for carbohydrate, 4.46 for protein and 4.74 for fat, according to the formula of Park et al, (1992)

Energy production (kcal/minute) = 4.80 * O_2 (litre / minute)

It is under these conditions that oxygen consumption can be used as an indicator of energy expenditure.

2.2 Determinants of Energy Expenditure

Energy expenditure can be divided conceptually into three major components. The largest component is the resting energy expenditure or resting metabolic rate (RMR) which accounts for up to 75 % of the total energy expenditure. Resting energy expenditure is the energy expenditure for homeostatic processes and is measured in resting post absorptive subjects after overnight fasting. The second component of energy expenditure is diet-induced thermogenesis (DIT) or thermic effect of food (TEF). The TEF accounts for 10 % of daily energy expenditure, but can vary depending on the amount of energy consumed and the nutrient composition of the diet (Fugate, 1990).The third component of energy expenditure is environment (shivering/non shivering thermogenesis), and physical activity. The total physical activity related energy expenditure of an individual not engaged in heavy work accounts for 15-20 % of total energy expenditure, but it can increase two–fold with heavy exercise.

2.2.1 Resting Metabolic Rate

Resting metabolic rate is the amount of energy expended by an individual resting in a thermoneutral environment without the effects of meal consumption, physical activity, or other physiological or mental stress (Hoffer, 1993). The value may be slightly greater than the true basal metabolic rate which, in addition to the above conditions, is measured in the morning upon awakening after 12-18 hours of rest in a comfortably warm environment, at least 12 hours after the last meal and includes the definition of conditions

with respect to circadian rhythms (Garrow, 1978). The energy expended at rest includes the costs of maintaining the biochemical and structural integrity of the body, and the costs of performing internal work, ion pumps, synthesis and degradation of cell constituents, biochemical cycles, and leakage of protons across the mitochondrial membrane (Burszstein, 1989).

RMR is influenced by a number of basic factors, including age, sex and body temperature (Burszstein, 1989). Much of the effects these factors have on RMR relate to differences in body composition. The overall size of an individual is one of the biggest determinates of RMR, and as such, energy expenditure increases with increasing height and weight. RMR most closely correlates with fat–free, lean body mass and body cell mass.

2.2.2 The Thermic Effect of Food

The thermic effect of food (TEF) is defined as thermogenesis induced after the ingestion of food and represents the heat released during the metabolism of nutrients (Jequier and Schutz, 1988). This type of thermogenesis, if defective, could promote significant cumulative weight gain over time (Garrow, 1978). However, though TEF is commonly represented as 7-13% of TEE, it is difficult to measure and is the least reproducible component of daily EE (Tataranni, 1995). According to Thiebaud *et al.*, (1982), TEF measured, both as short term (3h) and long term (6h), increased EE by 8-10% when energy equivalent to energy needs was consumed.

2. 2.2 a) Factors that Influence the Thermic Effect of Food

Many factors influence TEF (Tataranni, 1995). Some of these include: physiological factors (subjects' genetic background, age, physical fitness, and sensitivity to insulin), the characteristics of the test meal (palatability, nutrient composition), circadian rhythms, regulation of the SNS, gastric emptying time, alteration in hormone levels, and methodological problems in the measurement of TEF (indirect calorimetry equipment, interfering environmental factors and duration of the measurement). Of these factors, the physiological factors will be discussed in the following section. Circadian influences and methodological problems in the measurement of TEF remain the most important factors to consider.

TEF is most often measured by using ventilated-hood systems (Weststrate, 1993 and Segal *et al.,* 1992) in which TEF is assessed for a short time in response to a single meal or by respiratory chambers (Ravussin *et al.*, 1986 and Schutz *et al.,* 1984), a method that has the advantage of reproducing more physiological conditions over a longer period of time while regular meals are consumed.

The metabolic efficiency of the diet is also influenced by the time when the diet is consumed. In studies by Capani *et al* (1984) and Deuel (1927), comparing oxygen consumption in response to the same meal taken in the morning or in the evening, after a similar period of fasting, found significantly higher oxygen consumption when a protein containing meal was given in the morning. However, there was no effect of time of day with carbohydrate or fat meal. Romon *et al*, (1993) explained this decline in the TEF of protein as the day progresses, to be nocturnal insulin resistance that could lead to reduce

thermic effect of meals.

2.2.2. b) Physiological Aspects of Thermic Effect of Food

Flatt, (1978) observed that measured TEF has repeatedly been found to be larger than what would be expected by the stoichiometric energy cost of nutrient absorption, transport and storage. This observation was conducive to the concept that TEF can be divided into two parts.

1) An obligatory component which is related to the metabolic cost of processing the nutrients and that results from the energy expended for processing, absorbing and mostly storing the nutrients (Ravussin *et al*,1986; Jequier *et al*, 1988; Acheson *et al*, 1984)

2) A facultative component that is the energy expended in excess of obligatory of nutrients, such as the stimulation of the sympathetic nervous system, substrate cycling protein turnover, and sodium pumping (Acheson *et al,* 1984). Both types of thermogenesis have been considered separately for carbohydrates, proteins and lipids. Carbohydrate: Glucose-induced thermogenesis is referred to as an increase in energy expenditure induced due to glucose ingestion or infusion (Acheson *et al*, 1984; Welle *et al*,1983). Accordingly, (Flatt, 1978) the obligatory component can be considered as the cost of glucose storage as glycogen because 2 moles of ATP are utilised for each mole of glucose that is converted to glycogen.

We can calculate that the energy cost of glucose storage as glycogen is 0.20 kcal/g. Consequently the theoretical cost of converting glucose to glycogen is about 5% of the glucose energy content (Flatt, 1978; Acheson *et al*, 1983; Thiebaud *et al*, 1983;

Thiebaud *et al,* 1982). However, the actual increase in energy expenditure is well in excess of this theoretically calculated value (Shetty *et al*, 1981; Thiebaud *et al*, 1982; De Fronzo *et al,*1984; Tappy *et al*, 1986). Two main factors have been postulated to explain facultative thermogenesis of carbohydrate: insulin action and adrenergic activity

According to Rothwell and Stock (1981) insulin *per se* is a major regulator of TEF. They demonstrated that streptozotocin-diabetic rats exhibit a blunted thermogenic response to feeding compared with non diabetic control animals. It is not easy to distinguish between direct effect of insulin in stimulating thermogenesis and an indirect effect of the hormone that allows glucose to enter into the cell with consequent stimulation of intracellular glucose metabolism.

If insulin resistant obese subjects are given pharmacological amounts of insulin such as is used during euglycaemic hyperinsulinaemic clamps to increase their glucose metabolims to levels observed in controls, then a normal thermogenic response is obtained. Ravussin *et al.,* (1984) suggested that it is the cellular rate of glucose metabolism, and not the plasma insulin concentration *per se,* that is in some way related to the thermogenic response. Rowe *et al* (1981) observed that increases in glucose-induced thermogenesis tended to be greater during hyperinsulinaemia and euglycaemia than with normal levels of insulin and hyperglycaemia.

The other important factor implicated in facultative thermogenesis is the sympathetic adrenal system. Cori *et al*, (1930) have demonstrated that infusion of small amounts of adrenaline into healthy humans (using doses that produce plasma adrenaline concentrations within physiological ranges) stimulates thermogenesis and increases heart

rate and respiratory frequency.

Several studies (Acheson *et al.*, 1984; Acheson *et al.*, 1983; Tappy *et al.*, 1986; Astrup *et al.*, 1986; Schwartz *et al.*, 1988;Welle *et al.*, 1980;Thorin *et al.*, 1986) have suggested that facultative thermogenesis is mediated by the sympathetic nervous system. Factors suggested to explain the variability found in TEF studies iclude: differences in the palatability of the carbohydrate containing food ingested (LeBlanc *et al,* 1985), duration of the study after the meal (LeBlanc *et al*, 1985), and variability of thermogenic sensitivity to ß-adrenergic stimulation among subjects (Connacher et al, 1988). and the rate of administration of ß-adrenergic blockade (Vernet *et al*, 1987)

With regard to where both obligatory and facultative thermogenesis take place, Brundin and Wahren (1993) showed that there were no measurable splanchnic energy costs for absorption, processing or storage after a 75g carbohydrate load. But there was an increase in whole body EE. This demonstrates that glucose-induced thermogensis occurs exclusively in extrasplanchnic tissue. Furthermore, they suggested that glucose ingestion, by raising plasma insulin concentrations, inhibits in large part but maybe not totally, hepatic gluconeogenesis and that insulin reduces the rate of protein degradation and amino acid oxidation, two mechanisms which require energy and produce heat.

Skeletal muscle is one of extra splanchnic tissue is believed to be a site where marked stimulation of energy output takes place (Astrup *et al*, 1989; Bergstrom *et al,* 1967; Haussinger *et al*, 1991). It was demonstrated by Astrup *et al* (1989) that 60% of the TEF induced by carbohydrate takes place in muscle. Simon *et al* (1993) showed by infusing epinephrine, that skeletal muscle contributed about 40% and adipose tissue about

5% of the epinephrine-induced thermogenesis. Astrup (1989) provided evidence for a facultative thermogenesis component in skeletal muscle mediated by epinephrine via $ß_2$ adrenoreceptor. However he also suggested that there was a non-muscle component mediated through $ß_1$ adrenoreceptors induced by norepinephrine released from the SNS.

In conclusion, TEF from carbohydrate is principally due to insulin action stimulating intracellular glucose metabolism, or to activation of the sympathetic nervous system.

Proteins: As reviewed by Shetty *et al* (1981), Rubner in 1902 was the first to observe the marked thermogenic effect of protein, exceeding that of both carbohydrate and fats; and that protein provides the greatest stimulus to oxygen consumption both in animals and man. The results of the study by Brundin and Wahren (1993) indicate that whole-body oxygen uptake rose gradually to a peak level of 26% above basal level at 2 hr after protein ingestion. He demonstrated that the splanchnic proportion of whole body amino acid-induced thermogenesis amounted to 51±11%. Whereas carbohydrate intake has been demonstrated to stimulate norepinephrine activity, proteins do not alter the sympathetic nervous system (Welle *et al*, 1981).

Lipids: As observed by Todesco *et al.,* (1997), lipids are the substrates that least stimulate energy expenditure. Stimulation of energy expenditure can be accounted for by ATP consumption in the process of free fatty acid re-esterification to triglycerides, corresponding to 2% of the energy content of the fat meal (Ravussin *et al,* 1983). Fat intake, like protein intake, does not alter plasma norepinephrine concentrations (Welle *et al*, 1981; Schwartz *et al*, 1985).

2.2.3. Thermic Effect of Physical Activity The thermic effect of exercise is the energy expended above the resting level, both during and after physical activity (Blundell, 1992). Physical activity accounts for 15-20% of total daily expenditure in most individuals. The contribution of exercise to total energy expenditure depends on the intensity of the work performed and the duration of the sum of all activities over the day. There is a wide variation in the energy cost of any activity both within and among individuals, due to differences in body size and the speed and dexterity with which an activity is performed. To account for differences in body size, it is now common to express the energy costs of activities as multiples of RMR. The factors range from 1.3 to 1.7 X RMR for most activities, though values of up to 2.4 X RMR have been recorded during intense exercise. Two publications give estimates for energy costs of different activities (Ainsworth *et al.*, 1993; James *et al.*, 1990), but these should be seen as approximations rather than exact values, which can only be obtained by direct measurement.

2.2.4 Age, Gender and Neurohormonal Regulation of Energy Expenditure

Age is one of the predictors of total energy expenditure (TEE) and RMR because, as age increases, EE decreases due to decreases in FFM and increases in FM (Ravussin, *et al.*, 1986; Astrup *et al.*,1992; Owen, 1988). Gender is also a predictor of TEE and RMR because men usually have more FFM than women and because men are larger in size. For the same body size, men have a greater FFM than women (Ravussin ,1982; Astrup, 1995).

Stimulation of the SNS causes the excretion of catecholamines which indirectly

increase thermogenesis and (EE). Catecholamines such as isoprenaline, epinephrine, and Norepinephrine exert their thermogenic effect by acting on the ß-adrenoreceptors ($ß_1$,$ß_2$ and $ß_3$) (Astrup, 1995). For example, NE is a determinant of 24 hr EE once it is corrected for body composition, physical activity and thyroid hormone (Saad *et al.*, 1991).

Besides catecholamines, thyroid hormones stimulate EE. In particular, triiodothyroxine (free T_3), is the most active thyroid hormone and accounts for about 2 % of EE in premenopausal females, independent of body composition and spontaneous physical activity (Astrup *et al.*, 1992). In 24-h EE, inter-individual variation in T_3 accounted for up to 600kJ/day (Astrup *et al.*, 1995).

2.3 Methods of Measuring Energy Expenditure

At present, energy expenditure can be measured by direct calorimetry, indirect calorimetry (whole-body chamber and ventilated hood system), doubly-labelled water and labelled bicarbonate. Direct calorimetry measures energy expenditure as the rate with which heat is lost from the body to the environment. This heat is transferred through non-evaporative heat loss (radiation, convection, and conduction) and through the evaporation of water. This technique is usually a whole-body measurement made within the confines of a chamber. Measurements have also been achieved using a heat-exchanging body suit. This equipment is very expensive and few laboratories in the world use this technique.

Indirect calorimetry measures energy expenditure as the rate at which heat is produced in the body. Heat production is calculated from the rates of respiratory gas exchange (oxygen consumption and carbon dioxide production) associated with the

oxidation of the major energy-yielding substrates; carbohydrates, fats, proteins and alcohol. This is a widely used method. In whole body indirect calorimetry, the subject is kept in a sealed room or chamber which is ventilated with a constant and measured supply of fresh air. Samples of well-mixed chamber air are drawn off for continuous analysis, and comparison of the differences between oxygen and CO_2 concentration of air going in and of air going out permits calculation of the subjects respiratory exchange and hence energy expenditure.

Indirect calorimetry provides at least two pieces of information: a measure of energy expenditure from which 24 hr energy requirements can be estimated by using a factor to account for non RMR energy costs and a measure of substrate utilisation, shown by the RQ. Indirect calorimetry measures oxygen consumption (VO_2) and carbon dioxide production (VCO_2), and allows for the calculation of energy expenditure through a series of assumptions. VO_2 is the greater determinant of energy expenditure, as indicated by the de Weir equation: energy expenditure $= (3.94 * VO_2) + (1.11 * VCO_2)$.

The human body has a large heat capacity that undergoes changes in heat storage. Thus an increase in heat released by oxidative processes can be immediately assessed by indirect calorimetry. Indirect calorimetry has the advantage of a small response time. This is because the body's oxygen stores are very small and the capacity of anaerobic synthesis of ATP is limited. Since for each litre of oxygen consumed there is a known amount of heat released, the measurement of the subject 's oxygen consumption is the principle upon which indirect calorimetry is based.

2.3.1. Sources of Error in Indirect Calorimetry

As reviewed by Simonsons and De Fronzo (1990), there is no simple way, at present, of obtaining the sort of information that gas exchange measurement can provide. The technical requirements are: 1) an air–tight canopy with a constant air flow to be adjusted to give O_2 and CO_2 concentrations within the workable range; 2) sensitive and stable O_2 and CO_2 analysers for continuous sampling of the expired air; 3) a calibration routine using standard gas mixture; 4) some system to trap or condense out the moisture of the expired air line feeding into the sensors, because humidity alters fractional gas concentration and can interfere with the response of the analysers; 5) a software to store and manipulate the data in any small desktop computer.

The accuracy is checked by standard calibration which is to burn a known weight of alcohol or butane gas, to see if the result obtained by indirect calorimetry corresponds with the known thermal equivalent of the fuel. This form of calibration checks the entire system: flow rate, gas analysis and leaks. If the answer obtained agrees with the theoretical answer within 1%, the system can be used with confidence.

Indirect calorimetry is currently, the best method to measure RMR, the thermic effect of food, and the energy expended for physical activity. A first advantage of indirect calorimetry is the immediate response of oxygen consumption (measured by the method of respiratory gas exchange) in relation to the real oxygen consumption in the tissues and organs within the body. There is no delay in measuring oxygen consumption because the body has negligible O_2 stores. A second advantage of indirect calorimetry in comparison with other methods is the possibility to assess nutrient oxidation rates, when oxygen

consumption, CO_2 production, and urinary nitrogen excretion are measured concurrently.

In calculating calories generated by disappearance of substrates, a number of assumptions are inherent. First the value of protein disappearance derived from urinary nitrogen excretion may not be entirely accurate during short term measurement, since the size of the body's urea pool may change. One may account for this by measuring the change in serum urea nitrogen concentration during the period of urine collection and making a suitable correction

A second assumption is that all energy is derived from the disappearance of carbohydrate, lipid and protein although this is generally true for the healthy individual, this assumption is not correct in the presence of metabolic derangements such as lactic acidosis, diabetic ketoacidosis or ethanol consumption.

Furthermore, a change in the circulating concentration of lactic acid, ketoacid etc. may lead to the release or retention of CO_2 as plasma bicarbonate level changes. In the case of ketogenesis, each mole of ketone generated would also lead to the release of 1 mmol of hydrogen ion. Because the hydrogen ion would theoretically be buffered by the plasma bicarbonate pool, an excess amount of CO_2 would be generated and recovered in the expired air. Finally it is important to stress that the technique of indirect calorimetry actually measures the net disappearance of substrate, independent of its intermediate pathways of metabolism.

Circadian rhythms persist throughout the day: oxygen uptake falls in the early hours of the morning, whether the subject is asleep or not. In women, this circadian rhythm persists, but the oxygen uptake is consistently lower post-menstrual than it is pre

menstrual (Garrow, 1978).

2.4 Mechanisms Explaining Blunted Thermogenesis

2.4.1 Insulin Resistance

Insulin resistance is impaired physiological response insulin action (Wolfe, 1998). According to Bessesen (2001), insulin resistance is a metabolic disorder common to many diseases including diabetes, coronary heart disease, obesity and hypertension. To understand insulin resistance it is necessary to review the action of insulin in the body.

Insulin stimulates the disposal of ingested glucose in skeletal muscle and adipose tissue and decreases the production of glucose by the liver by reducing glycogenolysis and gluconeogenesis (Wolfe, 1998). In addition, insulin suppresses the release of non-esterified fatty acids from adipose tissue by suppressing lipolysis (*ibid*). As stated by Withers *et al.* (2000), there has been an explosion of new information over the past ten years about the signalling pathways involved in transducing insulin's multiple actions on different tissues. After insulin binds to its receptor on the cell membrane of the responsive cell, a variety of signalling events occurs. Autophosphorylation of the insulin receptor occurs via tyrosine residues which leads to an increase in insulin receptor kinase activity and to phosphorylation of a variety of insulin receptor substrates. These phosphorylated insulin receptor substrates then interact sequentially with downstream proteins to stimulate glucose uptake through the translocation of glucose transporters (GLUT 4) to the cell surface. Glucose uptake then causes alterations in gluconeogenesis through

changes, for example, in the expression of relevant enzymes, to increase cell growth, suppress lipolysis and increase glycogen synthesis (LeRoith and Zick, 2001).

Evidence is increasing that certain intracellular signalling pathways are more affected (resistant to stimulation by insulin) than others. This means that within a single tissue, certain insulin actions may be more resistant than others to hormone stimulation. In addition, it is not clear how genetic variation in relevant gene products along these insulin-signaling pathways might alter the relationships between nutrients and insulin action in particular tissues (Bessesen, 2001).

Pharmacological approaches to managing insulin resistance have been used for years, however approaches involving changes in diet and physical activity are attractive because of their lower cost and often fewer associated side effects (Rudenski *et al.*, 1991). However, insulin resistance is not a simple phenotype. Different tissues have different levels of sensitivity to insulin, and within a single tissue, certain insulin actions may be more or less involved in the process of insulin resistance (Bessesen, 2001).

A number of methods have been used to assess insulin sensitivity in animals and humans. These include, the homeostatic model , glucose or insulin area under the curve after ingestion of glucose, the hyperinsulinemic euglycemic clamp, measures of fasting insulin and glucose concentration, the frequently sampled graded intravenous glucose tolerance test, graded glucose infusions , and cellular and molecular studies of insulin signalling . In recent studies (Burning *et al.*, 1998) demonstrated the interdependence of skeletal muscle, adipose tissue, liver and pancreatic β cells in maintaining normal plasma glucose concentrations by removing insulin receptors from tissues in the β cell-specific

insulin receptor knockout mouse (BIRKO)(Kulkarni *et al.*, 1999), and the brain neural insulin receptor knockout mouse (NIRKO)(Burning *et al.,* 2000). These studies suggested that normal fasting plasma glucose and insulin concentrations, as well as normal glucose and insulin responses have peripheral insulin resistance in mice lacking insulin receptors in skeletal muscle after glucose ingestion. This observation demonstrated the limitations of these measures in demonstrating insulin resistance. Furthermore, the presence of mild insulin resistance, hyperinsulinemia and obesity in NIRKO mice demonstrated the important role of the brain as an insulin-sensitive organ (*ibid*). Although experimental studies using the euglycemic clamp and the oral glucose tolerance test have focussed on skeletal muscle and liver, these recent knockout experiments emphasise the critical role of the pancreas, brain and adipose tissue as insulin-sensitive organs. Insulin resistance is likely not the same in all tissues as it develops.

It is becoming increasingly clear that sequential alteration in the relative insulin sensitivities of these critical tissues may be very important in the development of disease states such as diabetes. DeFronzo *et al.*, (1992) suggested that Type 2 Diabetes Mellitus (DM) is characterised by both hepatic and extra-hepatic insulin resistance. Although defects in either insulin secretion (Porte, 1990) or insulin action (DeFronzo *et al.*,1992) can be demonstrated in DM, it is highly probable that both defects as well as a defect in the feedback effect of hyperglycemia on the release of glucose by the liver (Rudenski *et al.*, 1991) must be present to establish the full-blown syndrome.

2.4.2 The Sympathetic Nervous System

In most mammals, the sympathoadrenal system, under the control of genetic and environmental factors, regulates energy intake and EE and thus represents a target for obesity intervention (Astrup, 1995). An individual's thermogenic response to food is determined by the extent to which the sympathoadrenal system is activated (*ibid*). A low RMR for a given body composition is a factor responsible for genetically determined obesity. Furthermore, a low sympathetic activity may be another such factor but, as yet, no conclusive evidence has been found. Stimulation of the SNS by pharmacological sympathomimitic compounds suppressed appetite and increased EE through stimulation of $ß_1$, $ß_2$ and $ß_3$ receptor subtypes (*ibid.*). If stimulation is prolonged, $ß_3$- mediation of thermogenesis may predominate due to down regulation of $ß_1$, $ß_2$-receptors. The release of epinephrine and NE at pre-synaptic sites in the SNS is potentiated by the indirect action of sympathicomimetic compounds at $ß_3$ receptors. Thus, catecholamines mobilise and reduce fat stores by increasing the rate at which fat is released from body stores (lipolysis) while simultaneously increasing the metabolic rate (thermogenesis) that increase the requirements for substrate oxidation (*ibid.*).

2.4.3 Blunted Thermic Response to a Glucose Meal in Obesity and Diabetes

Sims *et al*, (1973) suggested that deliberate overfeeding in humans could precipitate insulin resistance and impaired glucose tolerance. It was observed that obese subjects with impaired glucose tolerance demonstrated a significantly lower thermic response to a meal or 75 g glucose than lean control subjects or obese subjects with

normal glucose tolerance Golay *et al*, (1982). Results from (Bray *et al*, 1979 and Golay et al, 1982) showed that obese patients with diabetes or abnormal glucose tolerance have shown a reduced thermic response to a glucose meal, an effect that may perpetuate obesity which is associated with insulin resistance and impaired glucose tolerance; impaired glucose tolerance, in turn, reduces the thermic response to food and reduction in TEF could promote obesity obesity.

As reviewed by Ravussin *et al.*, (1983) obese diabetic patients have a higher RMR/24 h/kg fat free mass (FFM) than normal weight subjects. When RMR corrected for FFM is high, energy per kg of lean body mass required to maintain body weight is also high despite a low thermic response to food. Ravussin *et al.*(1983) observed that RMR in obese women with poorly controlled type 2 diabetes or with insulin resistance is higher than in not only lean healthy control subjects but also in obese persons with normal glucose tolerance. Consequently, because of their elevated RMR, the reduced thermic response to a glucose meal in obese women with impaired glucose tolerance and diabetes was not sufficient to reduce total energy expenditure relative to other women. Hence in obese patients with diabetes or impaired glucose tolerance, we cannot conclude that obesity is perpetuated by a reduced TEF. In obese patients with insulin resistance there is high rate of protein synthesis in the post-absorptive state (Nair *et al.* 1983). As a result elevated gluconeogenesis (Ravussin *et al.* 1983) and protein synthesis (Nair *et al.* 1983) can therefore be factors responsible for an increased RMR. A reduction in these biochemical processes after a glucose meal could reduce the net increase in meal induced thermogenesis. In poorly controlled diabetic, patients lose glucose in the urine, their glucose storage is likely to be less than with improved control, this may reduce the energy

cost for glucose storage after a meal. Therefore these two factors may be responsible for a reduced thermic response to a glucose meal (Gougeon, 1996).

Both Pittet *et al.* (1976), who studied the thermic response after a glucose load and Shetty *et al* (1981) after a liquid meal, confirmed the evidence that obese people have a smaller post-prandial thermogenesis. Furthermore, subnormal thermogenesis in response to cold exposure (Blaza *et al,* 1980; Jequier *et al*, 1974) and thermogenic agents (Jung *et al*, 1979) have been described in subjects with familial obesity.

As reviewed by (Flatt, 1981), the theoretical values of glucose-induced thermic costs depend on the metabolic fate of glucose. When ingested glucose is directly oxidised, glucose induced-thermogenesis is less than 2 %, whereas the cost of converting dietary glucose to glycogen corresponds approximately to 5% of its energy content. Another pathway, lipogenesis from glucose, is energetically wasteful since in theory about 24 % of the energy content of the glucose converted into lipid is expended in the process. According to Bjorntorp *et al.* (1978), lipogenesis may represent a component of post-prandial thermogenesis after carbohydrate ingestion, but the fraction of glucose converted into lipid is small in man.

Reduced sympathetic nervous system activity is a possible factor for a reduced thermic response to a glucose meal. It was Wang *et al.* (1924) who first showed a reduced increment in post-prandial energy expenditure in obese subjects. Furthermore, Pittet *et al.* (1976), by coupling indirect and direct methods, found only a 5% rise in metabolic rate in obese subjects after eating, compared to 13% in lean controls. Similar observation of diminished thermic response to a high protein meal fed to women with

juvenile onset obesity was shown by Kaplan and Leveille (Kaplan *et al*, 1976).

Catecholamines as observed by (Himms-Hagen, 1972) are considered to be important regulators of metabolic rate. This was confirmed later by Welle *et al.* (1981) who have shown that plasma norepinephrine concentrations are increased with carbohydrate feeding. It has been suggested that SNS may play a role in the maintenance of weight stability during chronic overfeeding (Landsberg *et al.* 1978). Post-prandial NE concentrations were observed to be higher in obese subjects and correlated with the degree of adiposity. Thus, if the plasma levels of NE accurately and equally reflect SNS activity in both the obese and lean groups, the reduced thermic effect of feeding in obese subjects cannot be explained by reduced SNS activity. In this case the higher plasma NE levels in the obese subjects might be interpreted as counterregulatory hormonal responses to the impaired thermic response to eating (Schwartz *et al* 1983). Consequently, it is possible that plasma NE does not similarly reflect SNS activity in normal weight and obese subjects.

Landsberg *et al* (1978) evaluated NE turnover and found that starvation was associated with reduced and overfeeding with increased NE turnover. These results are complemented by reports of decreased NE levels among patients with anorexia nervosa (Gross *et al*, 1979).

Studies (Landsberg *et al*, 1980; Jung *et al*,1980) suggest that diet-induced thermogenesis is accompanied by changes in the activity of the sympathetic nervous system. Increased sympathetic activity has been observed after carbohydrate ingestion both in man and animals (Young *et al*, 1980). As reported by Young *et al*, (1980)

although the changes in plasma NE concentrations which have been reported are small, this process may contribute to the stimulation of energy expenditure, and do not coincide with the time course of thermogenesis. Shetty *et al* (1981) showed a greater rise in plasma NE levels and a lower thermogenic response in obese compared with lean subjects after a liquid meal. This finding complements that the sympathetic nervous system of obese subjects may respond adequately after a meal. Since a lower thermogenic response to infused norepinephrine (Hims-Hagen, 1972) has been reported both in obese and "post obese" subjects, it is possible that sensitivity to the stimulation of energy expenditure by the SNS is impaired in some obese subjects. Therefore obesity with glucose intolerance is accompanied by a decrease of the glucose-induced thermogenesis. This further suggests that insulin may be required for glucose-induced thermogenesis in man. The presence of both insulin resistance and low glucose induced thermogenesis could play a role in the development of obesity. According to Jequier (1990) the glucose not cleared from the ciculation, remains in the extra cellular space and glycemia increases. This glucose contributes as extra energy which is needed to synthesize glycogen. Thus the thermic effect is reduced after carbohydrate ingestion. However, it should be emphasised that age itself contributes to the lower thermogenic response to glucose ingestion. Presumably, insulin resistance in the obese further reduces this thermogenic response. Another factor responsible for relapse obesity could be a more blunted energy expenditure after multiple hypoenergetic diets and with evolution of obesity. In a study by Golay, (1982), basal energy expenditure failed to increase to the same level as before weight loss following relapse of body weight gain. Furthermore reduction in body weight of obese subjects with a family history of obesity was not only associated with a decrease in RMR, but also

in a blunted thermogenic response to a glucose load (*ibid*). The greater reduction in TEF in the obese after weight loss is a new observation which may be important in explaining the frequent relapse of body weight after weight loss. In post obese patients, the post glucose load energy expenditure was lower. This would suggest that formerly obese individuals, after reaching a normal weight should have to ingest less energy than weight-matched never obese individuals to maintain their new body weight. The diminution of energy expenditure and the blunted glucose induced thermogenesis which is observed over the years, after weight loss and weight relapse in obese patients, may favour the maintenance of obesity and may contribute to explain why a sustained body weight loss is so difficult to achieve in obese patients who have a long history of obesity.

2.4.4 Pathogenic Role of Visceral Fat

As observed by Kaplan (1989) and Bjorntorp(1991), upper-body obesity has a stronger association to metabolic and cardiovascular diseases than lower-body obesity. This occurs mainly because Free Fatty Acids (FFA) produced by the visceral fat depots through lipolysis in fat cells in that region are a main trigger for the complications in obesity (Bjorntorp, 1991; and Frayan, 1992). The visceral fat mass is increased in upper body obesity (Sjostrom, 1991). It is only this fat depot which has direct access to the liver through the portal system. It has been suggested that several of the unwanted effects on liver such as glucose intolerance, impaired metabolism and action of insulin, and altered lipoprotein synthesis are an outcome of excessive release of FFA into the portal circulation (Bjorntorp, 1991; Frayan *et al*, 1992; Ferranini *et al.*, 1983; Felber *et al.*, 1982). The resulting insulin resistance may, in turn, contribute to the development of

diabetes and hypertension and cause a further deterioration in lipoprotein metabolism. Studies suggest that lipolysis regulation is impaired in obesity, a finding that is less apparent in lower-body obesity than in upper-body obesity (Jensen MG et al, 1989; Martin *et al.*, 1991). This finding may be explained through the catecholamine effects which are modulated through four adrenoceptor subtypes: stimulation via β_1-, β_2 -, and β_3 adrenoceptors and inhibition via alpha $_2$ adrenoceptors (Melander *et al* 1977). It is observed that β_3 adrenoceptors, despite their expression in several human fat depots (Krief S, et al, 1993), they have little lipolytic action on the subcutaneous fat tissue but a marked lipolytic function in fat cells from the visceral region (Langin *et al,* 1991; Lonnqvist *et al.,* 1993).

It has been demonstrated *in vitro* that the lipolytic sensitivity to epinephrine and norepinephrine is impaired in the subcutaneous region in upper-body obese subjects (Mauriege *et al,* 1991; Reynisdottir, 1994). This indicates regional differences in the impact of abdominal obesity on lipolysis, i.e., increased catecholamine action in the visceral fat depot but decreased action in the subcutaneous fat depot. Furthermore, the overall rate of release after an overnight fast is higher in upper–body obese subjects than in normal or lower–body obese subjects *in vivo* (Roust *et al.* 1993). The increased lipolytic activity of catecholamines in omental fat cells of obese subjects could mainly be perdominated by the β_3–adrenoceptor, but not by the β_1-or β_2 – adrenoceptors. The potential importance of the β_3–adrenoceptor in the omental fat cells is further stressed by the fact that the sensitivity of this receptor correlated strongly with norepinephrine–induced lipolysis and fat cell volume. Neither the β_1-or β_2- adrenoceptors sensitivity nor

the alpha$_2$- adrenoceptor sensitivity correlated with these variables (Lonnqvist *et al.*, 1995). It is of interest to study catecholamine–induced FFA and glycerol release from visceral fat cells since catecholamines are the only hormones with marked acute lipolytic effects (Arner, 1992).

Compounds where action mimics that of catecholamines may stimulate greater lipolytic effects in visceral obesity. Such effects may be reflected in greater fat utilisation as indicted by a lower respiratory quotient measured by indirect calorimeter, and in an increase in free fatty acid concentrations.

2.4.5 Anthropometry and Determination of Body Composition

Various anthropometric techniques have been developed which are more appropriate to epidemiological, clinical or hospital settings (Kushner, 1992). However, the accuracy of the estimates is limited by inter and intra-examiner errors, changes in subcutaneous and visceral fat distribution between males and females of different ages, and difficulty measuring ill patients (Kushner, 1992).

There is a significant correlation between the waist-to-hip ratio and visceral adipose tissue accumulation measured by computed tomography. However the waist circumference alone was also used as it is the single and best anthropometric predictor of visceral adipose tissue accumulation (Pouliot *et al.*, 1994; Lemieux *et al.*, 1995). A waist circumference above 102 cm in men and 85 cm in women between 40 and 60 years of age has been shown to be associated with greater probability of excess visceral adipose tissue and metabolic complications (Lemieux *et al.*, 1996). In anthropometric-based models as compared to impedance-based models used to predict mean body fat for various

populations, diverse anthropometric variables are used to develop prediction equations for the assessment of body composition. The inconsistent use of anthropometric variables prevents the researcher from assessing how much each variable contributes to the accuracy of the prediction equation. Heitman (1990) compared skinfold-thickness measurement, impedance, and BMI. By developing new multivariate regression equations for all three methods, and by using, across variable approach, he showed that even if all three methods predict mean body fat equally well, the bioelectrical impedance analysis method had significantly lower variability of estimates, making it the most accurate of the simpler methods. According to Kushner (1992), bioelectrical impedance analysis (BIA) is a rapid, safe, portable, non invasive, and relatively inexpensive method for evaluating body composition in field and clinical settings. Thomasett (1962) established this method, which is based on the electrical property of biological tissues, low electrical current is passed through the subject's body, and the impedance (Z), or opposition to the flow of current, is measured with a BIA analyzer. The individual's total body water (TBW) can be estimated from the impedance measurement because the electrolytes in the body's water are excellent conductors of electrical current.

Measuring the "electrical volume " of a body compartment, such as the TBW or FFM, is based upon the principle that various biological tissues act as conductors, semiconductors or insulators. Electrical conduction in the body is ionic in type and related to the free ion content of the various salts, bases and acids, their concentration, mobility and conducting medium temperature (Hoffer, 1969).

When the volume of TBW is large, the current flows more easily through the body with less resistance (R). The resistance to current flow will be greater in individuals with

large amount of body fat, given that adipose tissue is a poor conductor of electrical current due to its relatively small water content. Because the water content of the fat free body is relatively large (73% water), fat free mass can be predicted from TBW estimates. Individuals with a large FFM and TBW have less resistance to current flowing through their bodies, compared to those having a smaller FFM.

The whole body BIA approach for estimating body fat is based on empirical relationship established by many investigators. In general, most studies find that BIA estimates are better than estimates from height and weight alone. In studies of a heterogeneous population Ht^2/R appear to be the single best predictive variable of TBW or FFM (Thomasett, 1962 and Nyboer, 1959). The accuracy of a prediction equation is determined when the equation is applied to independent samples in cross validation studies. Despite the limitation of BIA there is considerable evidence that for single frequency impedance at 50 khz the impedance index (S2/R) has a good empirical relation to whole body composition and can be used as an index of TBW, FFM, fat mass and percentage body fat. As stated by Kushner *et al.*, (1992), the accuracy and precision of BIA are affected by instrumentation, subject factors, technician skill, environment, and the prediction equation used to estimate FFM. Lohman (1992) reported that the theoretical error is estimated to be 1.8 kg if the reference method (e.g. hydrodensitometry) is error-free.

2.4.6 Determination of Visceral Fat Tissue

As emphasised by Despres *et al.*,(1998) despite the usefulness waist-to-hip ratio to assess health risks associated with obesity this variable only provides a crude estimate of

adipose tissue distribution and does not adequately assess the absolute abdominal adipose tissue accumulation. The recent development of imaging techniques such as magnetic resonance imaging and computed tomography have allowed a more accurate measurement of abdominal fat accumulation (Despres *et al.*, 1990). These methodologies are particularly helpful to distinguish subcutaneous fat accumulation from visceral or intra-abdominal adipose tissue deposition. Several prospective studies have shown that a high proportion of abdominal fat was associated with the presence of type 2 diabetes (Ohlson *et al.*, 1985).

In a study of both men and women, visceral adipose tissue depositions of approximately 130 cm^2 measured at the levels of L4 to L5 were associated with dyslipidemia hyperinsulinaemia, and increased glycemic response to an oral glucose load, (Despres *et al,* 1993). A waist circumference above 90 cm in men and women between 40 years and 60 years of age was associated with a greater probability of finding excess visceral adipose tissue and related metabolic complications (Lemieux *et al*, 1996).

As reviewed by Krotkiewski *et al* (1983), obese women have enlarged fat depots in the abdominal *and* gluteal-femoral regions; while men accumulate fat mainly in the abdominal region. The fat is stored, in both sexes and in all regions, first by enlarging adipocytes up to a critical size (0.8μg); thereafter the number of fat cells increases. A study comparing the risk of having health disorders between men and women, showed that men had higher blood pressure, blood glucose, plasma insulin and triglyceride concentrations. This is only applicable for moderately obese subjects, women reach men's risk zone only when they become severely obese.

It is suggested that an enlarged fat depot with elevated lipolytic rates produces

large quantities of FFA. If this depot, such as the subcutaneous abdominal depot, is emptying its FFA into systemic circulation then peripheral tissues would be exposed to large amounts of FFA. Randle *et al.* (1963) have proposed that this might lead to deranged glucose metabolism in the periphery. The intra abdominal fat depot empties its FFA into the portal vein. Excess FFA in the portal vein produces hypertriglyceridaemia (Carlson *et al,* 1965). An enlarged lipolytically sensitive intra abdominal fat depot would inhibit insulin uptake by the liver, produce peripheral hyperinsulineamia, followed by a "down-regulation" of insulin receptors and insulin resistance. This, in turn, might be a first step towards the development of diabetes mellitus (Wolfe, 1998). More refined measurements of the abdominal fat mass by computerized tomography (CT) might increase the possibilities for a correct and early diagnosis of abdominal obesity and might be helpful here. According to Borkan *et al*, (1982), CT is an alternative approach that allows the precise measurement of adipose tissue areas at any site of the body and particularly the delineation of the amounts of deep (visceral) and subcutaneous fat. Such information is not possible to obtain from anthropometric measurements at the present.

As observed by Hartz *et al*, (1983), in a large survey of obese subjects with excessive upper body fat had a relative risk of diabetes that was 10-fold higher than normal women with peripheral fat accumulation. Kinetic studies have shown that obesity was associated with increased pancreatic insulin production, whereas a high proportion of abdominal fat was associated with a reduced metabolic clearance of insulin, due to a diminished hepatic insulin extraction (Pelris *et al,* 1986; Pelris *et al*, 1987.)

2.5 Physiological Rationale for the use of Adrenergic Thermogenic Drugs

2.5.1 Agents that Influence Metabolism and the Nervous System

2.5.2 Caffeine

Studies in animals demonstrate that caffeine and other methylxanthines reduce body weight and fat mass by both norectic and thermogenic effects (Dulloo and Miller, 1984). The stimulatory effects of caffeine on metabolic rate is also well established in man, both at high doses (Acheson *et al*, 1980) and in the amounts present in a cup of coffee (Dulloo *et al*, 1989). Dulloo *et al*, (1989) reported that administration of 100 mg caffeine increased the RMR of both lean and post obese human volunteers by 3-4 % and enhanced the TEF by 25-30 % in post-obese subjects, but had no significant effect in non obese subjects. Twenty-four hour energy expenditure was increased by 5 % in both obese and non-obese subjects. They also found that 100 mg doses of caffeine given every two hours for 12 hours immediately increased energy expenditure by 8-11% but had no effect on the subsequent 2 hours. These results support previous findings showing that the thermogenic effects of caffeine are short-lived (Arch *et al.,* 1987). The means by which caffeine increases energy expenditure are not fully understood; however caffeine has been shown to augment the thermogenic effects of certain sympathetic stimulants (Astrup *et al*, 1991) which in itself may suggest that caffeine potentiates these effects via adrenergic mechanisms.

2.5.3 Nicotine

Nicotine has known appetite-reducing effects and these effects are thought to be the cause for the weight gain that occurs after the cessation of smoking (Dallosso and James, 1984). Furthermore, cross-sectional and longitudinal data on energy intake and physical activity indicate that smokers do not differ from non-smokers (Fisher and Gordon, 1985: Stamford *et al*, 1984). Given the findings that smokers weigh less than non–smokers, the only remaining determinant of energy balance to account for these differences is metabolic rate. In a review by Warwick *et al.*, (1987) of 14 studies that assessed the metabolic effects of smoking, 57 % report that this was the case. The thermogenic effect of nicotine is thought to be mediated in part through autonomic nervous system activity since nicotine stimulates nicotinic acetylcholine receptors which form part of the autonomic nervous system (of which the SNS is one division) (Vander *et al.* 1985). Nicotine has also been shown to stimulate SNS activity (Cryer *et al*, 1976).

2.5 4 Thyroid Hormone

As observed by Rothwell *et al* (1982) changes in the level of feeding and the nutrient composition of the diet can profoundly affect the rate of thermogenesis, and these effects appear to involve changes in the sympathetic nervous system activity and thyroid hormone concentrations. Studies suggesting the fall in RMR during energy restriction may be related to the concomitant fall in plasma levels of thyroid hormones prompted researchers to evaluate the effectiveness of exogenous T_3 administration in fasting or dieting subjects. The results indicated that T_3 administration prevented both

the fall in its plasma levels and the decline in RMR (Welle and Campbell, 1986; Webb, 1986). Furthermore, studies revealed that with thyroid hormone therapy, both muscle-protein breakdown and body N losses increased (Gardner *et al*, 1979: Burman *et al*, 1979) such that weight loss was mainly due to a decrease in FFM.

2.5.5 Norepinephrine

Studies have shown that chronic NE treatment caused a substantial increase in plasma T_3 levels, but T_4 concentrations were not significantly affected, and this would be more consistent with an increase in the peripheral conversion of T_4 toT_3 than a direct effect on thyroid secretion (Melander *et al.*, 1977). It is well established that treatment of hyperthyroid patients with propranolol reduces the severity of the sympathomimetic symptoms, such as tachycardia, tremor, and sweating but it has also been shown to cause a reduction in serum T_3 levels (Lotti *et al*, 1977 and Verhoeven *et al*, 1977). Evidence showing stimulation of T_3 production from T_4 by catecholamines has been provided by Galton (1977). The activity of the mitochondrial enzyme α-GPD is generally considered to reflect thyroid hormone status (Lee *et al.*, 1956). Thus simultaneous changes in thyroid hormone levels, and a thyroid-sensitive mitochondrial enzyme suggest that, apart from their direct thermogenic effects on metabolism, catecholamines may potentiate their actions via an increase in peripheral thyroid hormone metabolism. One can therefore conclude that the requirements for a metabolic response are a rise in plasma T_3 and an available supply of substrates for energy metabolism. Carbohydrate feeding meets both requirements. Injecting NE is effective because it raises plasma T_3, and plasma FFA

levels presumably rise as a result of NE's lipolytic actions.

2.5.6 Ma Huang (Ephedra)

Ma Huang or ephedra has been used in China for over 5000 years as a treatment for asthma and other ailments (Chen, 1929 and Tyler, 1993). The primary active ingredients of Ma Huang are ephedrine alkaloid, a sympathomimetic agent. Recently, herbal mixtures of Ma Huang and Guarana, a source of caffeine, have been marketed in the USA as weight loss agents. The Ma Huang /Guarana combination is the herbal counterpart of the well-researched weight loss treatment of ephedrine plus caffeine. In a study by Boozer *et al* (2000) to examine its short-term safety and weight-loss effects, 72 mg Ma Huang and 240 mg Guarana were given in a double blinded, placebo controlled trial. In the treatment group, weight decreased by 3.5 kg but not in the placebo group. In addition a decrease in body fat and % body fat were 3.5 kg and 2.1 ± 3 %, respectively. Furthermore, there was a decrease in serum triglyceride (TG) levels in the active treatment group. Mean systolic blood pressure did not differ between groups at any time point. Interestingly, the weight loss and other additional beneficial responses observed in the treatment group were comparable to what was reported in studies of ephedrine and caffeine (Astrup *et al.* 1990 and Daly *et al.* 1993). However, the possibility that some of the other ingredients contained in the product contributed to these effects cannot be excluded. The above evidence supports the notion that adrenergic mechanisms mediating cardiac and pressor effects differ from those mediating thermogenesis, a difference that the phamaceutical industry has exploited to put forward a new generation of adrenoceptor agonists with selectivity for lipolysis and thermogenesis. Citrus Aurantium is one of those natural products which will be evaluated in this study.

2.5.7 Citrus Aurantium

ZHISHIN™ is trademark for the extracts of an immature citrus fruit (*Citrus. aurantium*) containing a family of indirect acting adrenergic amines (β-sympathicomimetic amines) that are claimed to facilitate utilization of energy substrates, stimulate metabolic processes, favour uptake of amino acids into muscle, increase lipolysis and exert a mild hunger suppressant action (Fyto Research Inc). The active agents present in ZHISHIN ^{MT} include synephrine, hordenine, octopamine, tyramine and N-methyltyramine, as shown in Figure 1a.

ACTIVE AGENTS in CITRUS AURANTIUM

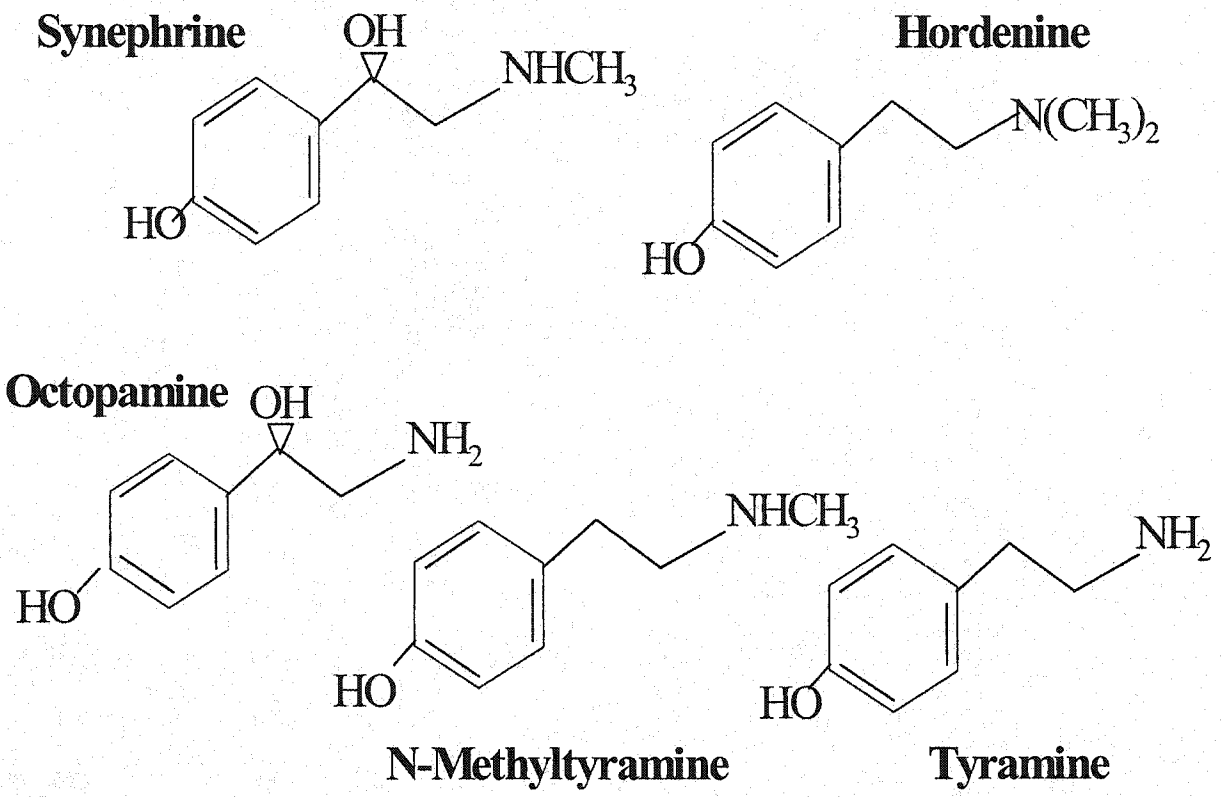

Figure 1A. The active agents present in ZHISHIN include synephrine, hordenine, octopamine, tyramine and N-methyltyramine

Since their health uses have long been known, and use in North America predates the Dietary Supplements Health and Education Act of 1994 by many years, ZHISHIN ™ does not require FDA pre approval before use.

Mechanism of action: According to Munson (1995) at the cellular level, activation of β-receptors results in stimulation of adenylate cyclase. This leads to increase in intracellular levels of cyclic adenosine monophosphate (cAMP). The precise sequence of events is as follows.

1. The β-agonist binds to the β-receptor.
2. The receptor-agonist complex has high affinity for a stimulatory guanine nucleotide regulatory protein termed the Gs protein, and binds to this protein.
3. Formation of the receptor-agonist-Gs complex facilitates the exchange of guanine diphosphate (GDP) for guanine triphosphate (GTP) on the Gs protein.
4. The Gs-GTP complex dissociates from the receptor-agonist complex and then interacts with the catalytic subunit of adenylate cyclase, promoting the conversion of adenosine triphosphate to cAMP.
5. The cAMP activates a cAMP–dependent protein kinase, which can then phosphorylate a variety of intracellular proteins, ultimately leading to a pharmacological response.
6. Feedback inhibition control is achieved by phosphorylation of receptor proteins, which results in their desensitization.

Activation of most α-2 receptors has an opposite effect, the first step being

inhibition of adenylate cyclase through a guanine nucleotide regulatory protein termed Gi. The Gi protein, by inhibiting the catalytic activity of the adenylate cyclase, leads to a reduction in cellular levels of cAMP, which decrease the activation of the cAMP-dependent protein kinases (Figure 1b).

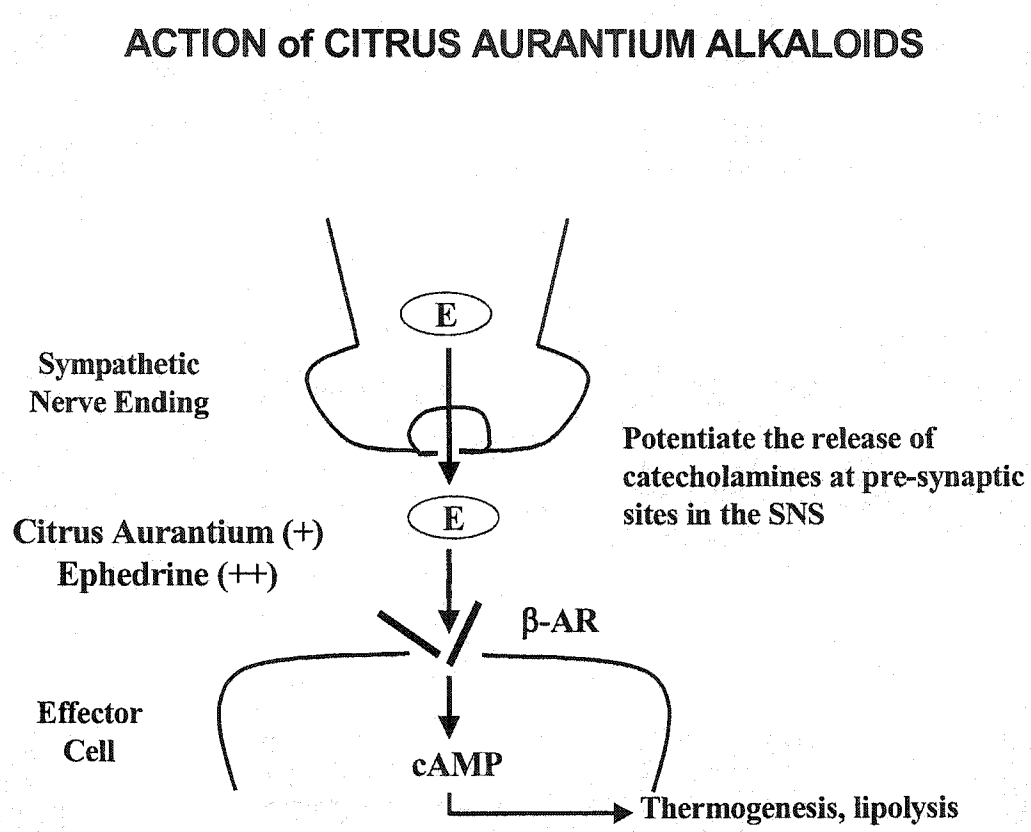

Figure 1B. Mechanistic action of sympathomimetic alkaloids. Release of Nor Epinephrine (NE) or Epinephrine (E) from the presynaptic site binds to beta-adrenergic receptors (β-AR) to enhance thermogenesis.

The effects on blood pressure, are in part due to the stimulation of α-2 receptors, where such stimulation produces peripheral vasoconstriction.

The metabolic results of adrenoceptors activation also include effects on lipolysis and thermogenesis. In the case of lipolysis, activation of α-2 receptors inhibits the process, while activation of β-receptors (believed to be of β-3-subtype) stimulates lipolysis and at the same time, possibly in part due to increased availability of substrate to induce a thermogenic effect.

Citrus Aurantium (CA) is available commercially as an herbal dietary supplement that promote weight loss; extracted from the fruit/rind of the immature Seville (sour) orange. It is a natural source of the sympathetic amines m-synephrine (phenylephrine) and octopamine (Calapai *et al.,* 1999).

In animal studies, both isolated synephrine and CA extract have been shown to raise blood pressure in animal studies (Calapai *et al*,1999 & Huang *et al* 1995).

In another study (Calapai, 1999) in rats, repeated oral administration of two CA extracts resulted in dose-dependent mortality (10%-50%) secondary to cardiovascular toxicity. Accordingly, (Scott *et al,* 2001) it is suggested that compounds containing CA may be harmful to individuals with cardiovascular conditions such as hypertension or dysarrythmias.

In a study (ibid), in humans, in a two way cross over design, subjects ingested freshly squeezed Seville orange juice (SOJ). SOJ contained 56.9 ± 0.52 ug/ml which is 13-14 mg of synephrine). SOJ had no significant effect on cardiovascular indicies such

as systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate in 12 healthy subjects.

2.6 The Sympathoadrenal System in Experimental Human Obesity

As observed (Landsberg *et al*, 1985) in both animals and humans, since a lowered metabolic rate secondary to diminished SNS activity predisposes to the development of obesity, and since energy restriction suppress sympathetic activity, a role for treatment with adrenergic thermogenic drugs is clearly physiological, since it addresses the underlying physiological alteration and is associated with improved energy balance. In humans, since the evidence for diminished SNS in obesity is not compelling, treatment with adrenergic agonists is more properly considered pharmacological (Saad *et al.*1991). Those individuals with lower metabolic rates would nonetheless benefit from adrenergic thermogenic drugs even if the thermogenic defect was not related to a lowered level of sympathetic nervous system activity. In this case, the adrenergic thermogenic agent is used as a pharmacologic agent to increase metabolic rate in a non-specific fashion. Adrenergic agonists may be partially useful in this regard since tachyphylaxis develops to the cardiovascular effects, but not to the thermogenic effects of adrenergic agonists (Scheidegger *et al*, 1984). Thermogenic drugs used in this fashion in obese would increase the range of caloric intakes over which energy balance could be maintained. When used in conjunction with decreased energy intake, weight loss would result.

Compelling evidence in studies by (William *et al*, 1991) suggested, that an additional situation in which adrenergic thermogenic drugs may prove useful is in the weight gain in smoking cessation which is associated with a prompt and sustained fall in

the excretion rates of both NE and E approximately 17 and 30 % respectively. There is, therefore, a potential rationale for the use of thermogenic adrenergic agents in the prevention of weight gain following smoking cessation.

The search for effective and safe sympathetic stimulants has been directed at two main levels (i) the development of novel ß-agonists selective for thermogenesis, and (ii) the evaluation of drugs already in clinical use for other purposes (e.g. Ephedrine) which could conceivably increase the release of catecholamines to levels that enhance thermogenesis without significant cardiovascular effects.

2.7 Physiological Response to Dietary intake and SNS Activity in Humans

The observation that dietary intake exerts important effects on the activity of the sympathetic nervous system (Landsberg *et al*, 1985) coupled with the well recognised effects of the SNS on energy production and expenditure Landsberg *et al,* (1984) have suggested a potential role for diminished SNS activity in the development and /or maintenance of the obese state. The fact that energy restriction suppresses the SNS has obvious and important implications for the effectiveness of low energy diets in the treatment of obesity (Welle, 1995).

It is believed that fasting, or energy restriction, suppresses the SNS (Young *et al,* 1977) while overfeeding exerts a stimulatory effect. The stimulatory effect of feeding is exerted by carbohydrates since these nutrients increase SNS activity even when total energy intake is not increased (Young *et al*, 1977; Walgren *et al*, 1987; Schwartz *et al*, 1983). Furthermore dietary intake exerts similar effects on sympathetic activity in

humans, as demonstrated by NE kinetic studies showing increases in plasma NE appearance rate (O Dea *et al*, 1982) and urinary NE excretion (Young *et al,* 1984).

Adrenal medullary activity, on the other hand, is increased by fasting in both humans (Young *et al,* 1984), and rats (Kaufman *et al*, 1989). In normal human subjects, urinary NE excretion decreases during a brief fast while urinary epinephrine is increased. Insulin plays an important role in the mediation of dietary effects on the SNS. Evidence has accumulated demonstrating that insulin–mediated glucose metabolism, in critical hypothalamic neurons is sensitive to both glucose and insulin, and initiates changes in central sympathetic outflow in response to alterations in nutritional status (Landsberg *et al*, 1985). Interestingly, the physiological significance of diet-induced changes in SNS activity appears to relate to changes in energy production. Sympathetic activity contributes importantly to dietary thermogenesis, serving as an important link between dietary intake and changes in metabolic rate. It has been suggested that diet–induced changes in sympathetically–mediated thermogenesis have evolved in mammals to conserve energy during periods of famine, on the one hand, and as an adaptation to a subsistence diet low in protein, on the other (Landsberg *et al*, 1983). By increasing the range of energy intakes over which energy balance might be maintained, those individuals with a more substantial capacity for dietary thermogenesis would be able to maintain energy balance by dissipating excess fuel storage in adipose tissue and triglyceride stores (ibid).

As suggested by Rauvussin *et al* (1988), a reduced rate of energy expenditure contributes to the pathogenesis of obesity. Since individuals vary in the energy intake over which they can achieve energy balance, those individuals with a more efficient

metabolism, manifested as a lower metabolic rate or diminished capacity for dietary thermogenesis, may be said to have a "thrifty" metabolic trait. Under conditions of famine, such individuals would have a survival advantage; when food is plentiful, however, such individuals would be liable to the development of obesity. As a response to evolutionary pressure, such a trait might establish a stable representation in the gene pool. Genetic differences in metabolic efficiency, interestingly, have been conclusively demonstrated by overfeeding studies in twin-pairs (Bouchard *et al,* 1990). Therefore agents that increase metabolic rate, might be viewed as overcoming the impairment in energy dissipation imposed by the presence of genetic "thrifty" traits. An example of a "thrifty " trait has parenthetically, been reported among Pima Indians (Ravussin *et al*, 1988); those individuals and families with the lowest resting metabolic rates were more likely to develop obesity.

2.8 Effects of ephedrine and aspirin on energy expenditure

It is believed that obesity may be due to a dysfunction of the sympathetic nervous system (SNS) and for this reason various SNS active agents have been studied for their effect on reducing obesity. Ephedrine, a thermogenic drug, has been shown to be one of the most effective drugs used in this regard in a variety of animal models. Additionally, the effect of drugs for treating asthma, which contain ephedrine, methylxantines (MX) and aspirin, have been tested separately in animal experiments. These experiments lead to the discovery that both MX and aspirin act to enhance the thermogenic effects of ephedrine in various animal models (Dulloo *et al*, 1988).

Subsequent to these investigations with animals, studies were conducted with

humans which examined the interaction between ephedrine and MX and between ephedrine and aspirin (Horton *et al*, 1991). These studies examined the effect of ephedrine (30mg) and aspirin (300mg) on the thermogenic response to a liquid meal (250 kcal) in 10 lean (mean BMI: 21) and 10 obese women (mean BMI: 32). The resting metabolic rate of each subject was measured by indirect calorimetry prior to each of the following treatment : meal only (M), meal plus ephedrine (ME) and meal plus ephedrine and aspirin (MEA). Also monitored, was the rise in metabolic rate, which was measured for 160 minutes following the meal, meal and ephedrine, meal, and ephedrine and aspirin. It was found that the rise in metabolic rate after the meal was lower in the obese than the lean. Ephedrine was shown to reduce the difference in thermic response and the combination of ephedrine with aspirin was shown to normalise the thermic response of the obese subjects as compared to that of the lean subjects. The mean percentage rise in metabolic rate for the lean and obese groups was for M: 16.8, 11.4; ME: 20.6,16.1 (NS); MEA: 21.6, 20.6 (NS), respectively.

Not all experimenters have observed the lower diet-induced thermogenesis in the obese and post obese. What may partly explain the discrepancy is the method of administration of the meal, which may be given as a standard dose or on a per unit body weight basis (D'Allessio *et al,*. 1988; Bukkenns *et al.*, 1991; Throne *et al.,* 1990). Both approaches are valid, because meals or drug capsules tend to be of standard size in practice.

The mechanism of action of ephedrine and aspirin on metabolic rate has been suggested to be as follows: aspirin inhibits the biosynthesis of prostaglandin and may

therefore inhibit the prostaglandin-mediated inhibition of NE release, which enhances the effect of ephedrine on sympathetically mediated thermogenesis (Dulloo *et al,* 1987). The different response of the lean and the obese to the effects of the ephedrine and aspirin combination may be explained by the differential receptor response to ephedrine in the obese as compared to the lean. Receptor response would be maximal in the lean and therefore not susceptible to further stimulation by aspirin as seen in the obese.

In conclusion, these studies indicate the importance of metabolic rate in the development and treatment of obesity. These studies also demonstrate the efficacy of the combination of ephedrine with aspirin in raising RMR in humans. This combination was shown to significantly increase TEF in obese women but not in lean and was more effective than ephedrine alone.

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PART 3: RATIONALE FOR RESEARCH PLAN

3.1 Overall rationale

The attempt to identify strategies aimed at the prevention of weight gain and obesity and effective at treating obesity is valid. Factors contributing to the increasing use of alternative methods for weight control include the rising prevalence of obesity, recognition that excessive adiposity increases risk of morbidity and the limited efficacy of conventional weight loss treatment. Preparations that contain extracts from CA, claimed to have sympathomimetic properties are among the most popular dietary supplements in use today for weight control. Thus the need to investigate their effects on energy metabolism.

3.2. Hypotheses

Alkaloids extracted from *Citrus Aurantium* (I) increase resting metabolic rate, when taken orally in a capsule form with water and (II) enhance the thermic effect of a mixed meal in lean and in obese subjects, the latter characterised by upper body obesity.

3.3. Objectives

The objectives of the study are: (I) to measure the thermic response to *Citrus Aurantium* extracts consumed orally in a capsule form, during rest, over time, in obese subjects characterised by upper body (android) obesity and in lean controls; (II) to measure the thermic effect of a mixed meal in the same subjects consumed with water only and (III) with addition of five capsules containing 1.65 g of *Citrus Aurantium* extracts.

Part 4: METHODOLOGY

4.1 Study Design

The study design is summarized in Figure 1C. A randomised crossover design was conducted in order to compare the thermic effect of a mixed meal taken with water and in some with five empty capsules as placebo with that of the same meal taken with water and five capsules of *Citrus Aurantium*. The studies were done in subjects at rest. Metabolic rate was measured for at least 280 minutes. The thermic effect of water with or without the capsules was compared to baseline resting metabolic rate in a subgroup of lean and obese subjects.

4.2 Study Population Inclusion /Exclusion Criteria

The study population consisted of obese persons of BMI > 30 kg/m^2, characterised with upper body adiposity as indicated by waist circumferences >102 cm in men and > 85 cm in women, aged 18-60 years. All subjects were to be devoid of health problems. Their suitability for the study was assessed by a complete medical history and examination by a physician and laboratory tests (HIV, Hep B Ag, ECG) and were non-smokers and taking no medication. There was an attempt to match groups of subjects for age and gender. Individuals with major complications of hepatic, cardiovascular, renal and pulmonary dysfunction and diabetes were excluded. A brief diet history was performed to ensure that the subjects were not consuming atypical diets and were weight stable for at least the preceding six months.

4.3 Subject Recruitment

Subjects were recruited from a population referred by the Royal Victoria Hospital (RVH) Metabolic Day Centre or as volunteers in response to an advertisement in a local newspaper or on hospital bulletin boards after approval by the RVH Service des communications (Appendix 1). Subjects were informed as to study protocol, the implication of their participation, as well as potential risks. It was made clear that the process of indirect calorimetry presented no health risk, the total volume of blood drawn represented less than 90 mL. Each subject signed a consent form (Appendix 2) approved by the RVH Department of Medicine Human Ethics Committee.

4.4 Procedures

All subjects were asked to refrain from eating and drinking, except water, after 20:00 hour the evening before each experiment and to arrive at 8.00 am by car and elevators, thus avoiding strenuous efforts, at the McGill Crabtree Laboratory located in the RVH. The protocol as shown in figure 1C consisted of three visits: 1) visit one for the study of the thermic effect of a 418 Calorie meal with placebo or CA ,visit two for the study of the effect of 5 Citrus Aurantium capsules and visit three for the second study of meal with placebo or CA depending upon study one. Participants were allowed to acclimatise appropriately in bed by staying recumbent and resting for at least 30 minutes in a quiet and thermoneutral environment. Afterwards, the subjects remained in a supine position and completely at rest in a bed while breathing under the plastic hood. To avoid confounding effect of changes in SNS activity and metabolic rate during the menstrual

cycle, the studies in women were done in the early follicular phase.

Brachial blood pressure {systolic blood pressure (SBP) and diastolic blood pressure (DBP)} measurements were obtained with a manual sphygmomanometer. Heart rate (HR) was measured by palpating the radial pulse. At baseline and after ingestion of placebo or CA, SBP, DBP and HR were measured at 60 minutes interval over the next 5 hours.

4.5 Resting Metabolic Rate and Meal Ingestion

Metabolic rate was measured by indirect calorimetry to determine oxygen consumption and carbon dioxide production using the Deltatrac metabolic monitor ventilated hood indirect calorimeter (Sensormedics, Anaheim, CA). This was with a room temperature averaging 22° C and a barometric pressure of 750mm Hg. Subjects were asked to breathe under a plastic canopy for 20 minutes, and the average of the last 15 minutes was used to calculate 24-h resting energy expenditure according to the de Weir equation (Weir JB, 1949). (Energy expenditure = $(3.94xVO_2) + (1.11xVCO_2)$). In an awake, alert subject at rest, the energy expenditure derived by indirect calorimetry most closely reflects RMR (Feurer ID, 1984). The thermic responses were determined as the absolute increase in metabolic rate from the measured baseline RMR values. The mixed meal was presented in the form of two chocolate flavoured food bars (Power 8R, Bariatrix International, Lachine, Quebec), each containing 209 Calories (874 kJ) each. The macro nutrient content of the meal and vitamin and mineral composition are given in Table 1. As a percentage of total energy content, 53 % was derived from carbohydrate, 29 % from protein and 18 % from fat. The 5 capsules of Zishin ^{TM} contained 1.65 gms of alkaloids such as Synephrine, Hordenine, octopamine, N-Methyltyramine Tyramine.

Each white capsule contains 480 mg of a proprietary blend of Citrus Aurantium Sevile orange whole immature fruit extract 1:10 (ZISHIN ™, Paullina Cupana (Guarana seed extract 1:4) Panax Ginseng (Ginseng root extract 1:25), Ginkgo Biloba (Ginko, leaf extract 1:100)

The subjects remained awake in a supine position at all times during the collection of data. Indirect calorimetry was performed for at least 280 minutes, a time when values in most subjects had returned to baseline. Data were collected for 40 minutes with a 20-minute break per hour during which time values were extrapolated from data collected before and after the break to give a smoothing adjustment. The thermic response was defined as the absolute increment in metabolic rate after the meal under the assumption that the basal RMR measured before the meal would not change significantly if a meal or CA had not been consumed. The thermic effect was calculated from the area under the curve above resting values. Two studies in which the subjects were given only water for the same period of time as used in the thermic studies were done to verify if RMR would show any significant change compared with what had been measured at baseline.

4.6 Blood sampling and glucose determinations

Blood was withdrawn in the post absorptive state after recumbence for at least 20 minutes and after a catheter with a three-way stopcock was inserted in an antecubital vein. Ten mL of venous blood was sampled prior to consumption of the test meal at (t=0) and thereafter at 30, 60, 90, 120, 180, 240, 300, and 360 minutes in some studies and transferred into a vacuum-sealed test tube containing heparin. After centrifugation, plasma was extracted for measurement of glucose concentrations using the Beckman Glucose Analyzer 2 and the Beckman glucose reagent kit (No.671640). The Beckman

Glucose Analyzer 2 determines glucose by means of the Oxygen Electrode. A precise volume of sample is manually pipetted into the enzyme reagent in a cup containing an electrode that responds to oxygen concentration. Solid state electronic circuitry determines the rate of oxygen consumption, which is directly proportional to the concentration of glucose in the sample. A front-panel digital meter provides direct readout in milligrams of glucose per 100 millilitres (Sterling, 1969).

The special merits of the methodology implemented by the analyzer include glucose specificity and directly usable results available within five minutes after collecting a specimen. The chemistry is free of interference from agents used to prevent glycolysis or coagulation, and will determine the glucose concentration, even in uremic, turbid, or hemolyzed specimens. Limitations of methodology: the only known interference is encountered in test samples drawn from subjects receiving intravenous infusions of starch or starch derivatives, e.g. hydroxymethyl starch, used as plasma extender. These substances give a slight positive response in the analyzer. The rest of the plasma was put into a test tube containing roughly 10-mg sodium meta-bisulphite, for subsequent determination of serum catecholamine concentration if judged necessary. The vein was kept patent by a minimal infusion of 150 mmol/L NaCl.

4.7 Urine collection

Urine was collected during each of the sessions for analysis of catecholamine (Epinephrine, Norepinephrine and Dopamine). Prior to measurement of the 20-minutes baseline RMR, "a pre-study" urine sample was obtained from a subgroup of subjects that represented the total urine collection of the previous night, against which we compared urinary catecholamines measured in urine collected during the period of the TEF

measurement. Determinations were corrected and expressed per hour. Aliquots of 10 mL from each collection was acidified with HCl to a pH of 3, and stored at –70°C until HPLC was used to determine dopamine, NE and E concentrations in the RVH endocrine laboratories.

4.8 Anthropometry and Body Composition Measurement

Anthropometric measurements included height (cm), weight (kg) and body circumferences (cm). These measurements were taken before the start of the first session in the fasted state after voiding. The subjects were weighed in light clothing without shoes. Body Mass Index (weight in kg /height in m^2) was calculated. Waist circumference was assessed as the smallest point between the lower rib and the iliac crest. The hip circumference was measured at the widest point in the great trochanter and buttocks area (Kissebah, 1985). To reduce the measurement error, the average of two recordings was taken as the measurement for each circumference. Waist-to-hip ratio was calculated to assess the distribution of body fat.

Fat-free mass and percentage body fat were measured in each participant by the bioelectrical impedance analysis method, using a 4-terminal bioimpedance analyzer (BIA-103, RJL Systems, Detroit, MI). The procedure and anatomical sites for placement of electrodes were as specified by Lukaski et al. (1986). We chose this equation because it has the same margin of error as that of Kushner (1992).

4.9. Statistical Power and Analysis

A minimum of 12 obese subjects with upper body obesity were required in order to provide a 95 probability of detecting a difference of 8 Calories in metabolic rate with an intra-individual standard deviation of 6.4 Calories. The data for treatment and control group were compared using repeated measures analysis of variance. Since the study involved making repeated observation on each subject over time, the analysis is two factors (treatment and time analysis of variance with repeated measures on one factor (time). Intra individual variation was measured using this measure. Student's t test for paired data was used when appropriate. In each regression model, the variability in the dependent variable that is not accounted for by predictor variable was calculated as described by Bogardus, (1986). Stepwise multiple regression analysis with test of equality of regression lines was also used. (Jennrich, 1981). The relationship between different variables was evaluated using Pearson-product moment correlation and multivariate regression. Statistical analyses were carried out with the SPSS/PC statistical package version 10 (SPSS Inc, Chicago, IL). Results are reported as means and standard error of the mean (SEM). Results were considered statistically significantly different if P- values were < 0.05.

4.10 Resources and Calendar of Progress

The McGill Crabtree laboratory is equipped with a metabolic room, laboratory facilities and trained technicians. The alkaloid compound and food bars have been developed by a food industry (Bariatrix International Inc.)

The product has been on the market in the USA for over 4 years. One study with 8

obese subjects, reported in abstract form, showed no significant changes in blood pressure, heart rate, electrocardiograms (Colker CM, 1999) The test dosage of the compound has been determined in previous protocols undertaken by the company. Preliminary studies have confirmed that this compound is well tolerated, safe and appeared to enhance weight loss.

4.11 Ethical Considerations

Potential subjects were informed about the risks and benefits of the study, and their signed consent to participate to the study was obtained. The protocol was reviewed and approved by the Ethics committee of the RVH before subjects were recruited.

4.12 Significance

An increase in energy expenditure through the mediation of thermogenesis, associated with the intake of Citrus Aurantium Extract, may induce a negative energy balance, promote weight loss and be of clinical significance as an adjunct in the treatment of obesity.

Part 5: RESULTS

5.1 Subject Characteristics

Twenty-two subjects were studied: 13 obese persons, which included 9 women and 4 men, and 9 lean persons, which included 2 women and 7 men. Their baseline characteristics are shown in table 2. There was a significant difference in proportion of women between groups (P=0.03) as determined by Chi-Square.

When both lean and obese groups were compared using independent sample test to see whether there was any significant difference between any of the measured variables, mean baseline RMR was not significantly different between the two groups (P= 0.40). There was no significant difference in waist-to-hip ratio and FFM. Compared to the lean group, the obese group was 31% older (P<0.05), 17 % heavier (P<0.05), and their BMI was 21.5% higher; waist and hip circumferences were significantly greater (P<0.001). Waist circumferences in obese men ranged from 102 to 117 cm and in obese women from 87 to 120. Fat mass was 51% greater and percent body fat, 36 % (both: P<0.05. Compared with lean group. In lean persons, waist circumferences ranged from 67 to 87 cm. Pulse rates and diastolic blood pressure did not differ between groups, but systolic blood pressure was significantly higher in the obese group (P<0.05). Mean baseline fasting plasma glucose (FPG) was higher in the obese subjects (P<0.04). TSH, measured only in the obese subjects, was within the normal range.

When comparisons were made between obese and lean men, significant differences were observed in weight, BMI, waist circumference, fat mass, % BF and FPG (P<0.005). When comparisons were made between obese and lean women, there were

significant differences in age, weight, BMI, waist circumference, W: H ratio, FFM, fat mass and % body fat ($P<0.05$).

5.2. Resting Metabolic Rate

The coefficient of variation of RMR was $5.2 \pm 0.6\%$ in the obese and $4.3 \pm 0.4\%$ in the lean group. We compared individual RMR values measured twice i.e. before the two thermic effect of meal studies to that of the 6 predictive equations shown in Table 3 to verify the accuracy of the estimates. The results are given in Table 4 a, b. There was no significant difference between the RMR measured before the thermic effect of the meal without CA and that measured before the meal plus Ca (Table 4 a). In the obese women, the Harris & Benedict and the WHO equations overestimated measured RMR ($P< 0.05$) by 9.8 and 10.7% respectively and that of Bernstein underestimated RMR by 9.8%. In the obese men, only the Bernstein equation was significantly different from measured RMR and underestimated it by 14 % ($P < 0.05$).

In lean men, the Harris & Benedict, WHO and James equations overestimated measured RMR by 7 % and Bernstein's underestimated it by 15 % ($P<0.05$). In lean women, the number of subjects was too small to reach significance (Table 4 b).
The results obtained from the Owen and Mifflin equations did not differ from those given by measuring RMR.

The results of the Pearson Product Moment Correlation's performed between RMR and the variables measured in our subjects are given in Table 5. RMR was significantly associated with FFM, height, and weight, W: H ratio, waist circumference

and gender ($P<0.05$). RMR was not related to % body fat, hip circumference, BMI, fat mass, and FPG (Table 5a). When data were analyzed separately by gender, RMR correlated significantly with FFM, weight, FPG, W: H ratio and fat mass in women and, with weight, FFM, BMI, waist and hip circumferences and FPG in men (Table 5a).

Data of all subjects (n=22) were analyzed using stepwise multiple regression analysis. FFM and weight explained 71% of the variation in RMR (Table 5b). Independent factors entered into the equation included gender, weight, height, and waist circumference, W: H ratio and FFM. FFM alone explained 65% of the variability in RMR in this group.

Data were analyzed separately by gender, using stepwise multiple regression analysis and all the variables that correlated significantly with RMR. In women,using weight, W: H ratio, FFM, fat mass and FPG as independent variables, only FFM entered the equation and explained 61% of the variation in RMR (Table 5c). In men, using weight, waist and hip circumferences, FFM, BMI and FPG as independent variables, only weight entered the equation and explained 61% of the variation in RMR (Table 5d).

5.3 Thermic Effect of Citrus Aurantium

The results of the two studies done in two female subjects looking at the effect on RMR of water alone using 750 mL given in three portions i.e. after initial RMR and at breaks 1 and 2 of the duration of the thermic effect studies and those of 12 women and 8 men looking at the effect on RMR of the capsules alone are reported in Figure 2. Water had no effect on metabolic rate. By contrast, the intake of 5 capsules of CA with 240 mL of water by all subjects showed a significant increment in energy expenditure compared with baseline RMR (Figure 2) and water. The increment above RMR with CA was

significant in obese and lean subjects when data were analyzed separately as shown in Figure 3A. The response did not differ between groups. The increment was also significant when data were analyzed separately for women and men as shown in Figure 3B. However the increment was significantly higher in men, even after adjusting for fat mass (P=0.043). The increment induced by CA alone reached on the average 0.25 kJ per minute or 65 kJ for 280 minutes. It amounted to 42 kJ in women and 94 kJ in men, on the average.

Figures 3C and D show the RQ response to CA alone. In obese and lean (Figure 3C) there was an effect of time that did not differ between groups. In both sexes (Figure 3D), there was a significant effect of time ($P < 0.001$) with the intake of CA, by repeated measures ANOVA. We found a trend ($P = 0.069$) towards a greater initial increase in RQ in men compared with women.

5.4 Thermic Effect of Meals ± Citrus Aurantium

The thermic effect of the meal with or without CA in all subjects combined expressed as % above RMR % of the energy content of the meal and kjoules spent over 280 minutes is shown in Figure 4. The thermic response to the mixed meal without CA was 13.4 % above RMR and represented 9.6% of the energy content of the meal and 167kJ over 280 minutes. When CA was consumed with the meal, the thermic effect was significantly greater than without CA whether the response was expressed in % above (P=0.036) RMR, in % of energy content of the meal ($P =< 0.05$) and in kjoules per 280 minutes (P=0.05).

TEF of the meal alone was correlated with sex, age, body composition, FPG and RMR. Percent above RMR correlated significantly ($P<0.05$) with weight, BMI, hip

circumference, FFM, fat mass, % body fat, FPG and RMR; % energy content of meal correlated significantly (P>0.05) with gender, height, BMI, hip circumference, FFM, fat mass and % body fat; kJ per 280 minutes correlated significantly (P<0.05) with gender, height, hip circumference, fat mass and % body fat. Table 6 shows the results of stepwise multiple regression analysis for TEF. Weight only entered the equation and explained 32 % of the variation in TEF as % above RMR; gender only entered the equation and explained 43 % of the variation in TEF expressed as % of energy content of the meal and 40% of the variation in TEF expressed as kJ per 280 minutes.

We did Univariate Analysis of Variance to assess the effect of body composition and gender on the thermic of meal and that of adding CA to it. We found that there was a significant effect of gender on TEF that remained significant when cnotrolling for body fat and other variables of body composition.

Figure 5 shows the response to meal in kJ per minute over time which tended to be lower in women but not significantly. Figure 6 shows a similar thermic response to meal with added CA in both groups. Figure 7 compares the thermic response in women with and without CA. Adding CA increased significantly the increment in energy expenditure above RMR in women while it had no effect in men as shown in figure 8.

Tables 7 and figures 9 A and B show the TEF results with and without CA in subjects grouped according to gender.

Figure 9A reports results as % above RMR and % of energy content of meal and Figure 9B those as kJ per 280 minutes. The lower thermic response to the meal in women when expressed as % of meal and in kJ per 280 minutes increased significantly with CA and was no longer different from that of men.

5.5 Respiratory Quotient (RQ)

The basline fasting respiratory quotients (RQ) and the RQ responses to meal ± CA for lean and obese subjects is presented in Figure 10. Baseline RQ did not differ between studies in the same subjects (Figures 10A and B). However, there was a trend for basline fasting RQ to be higher in obese subjects compared with lean (0.80±0.01 versus 0.83±0.01, P=0.02) (Figures 10C and D). The RQ response from 0 to 280 minutes to meal without CA was not different between obese and lean (P=0.23) but from 0 to 64 minutes, the response was significantly higher in obese (P=0.046) and reached a peak of 0.89 at 32 minutes (Figure 10C). However the overall response (area over the curve) was the same in both groups. When both meal and CA were given, the RQ response did not differ significantly between groups (P= 0.93) as shown in Figure 10D.

5.6 Responses of Plasma Glucose Concentrations

Post absorptive plasma glucose concentrations and those in response to TEF are shown in Figure 11. Post absorptive glucose concentrations were significantly greater in the obese group compared to the lean but increased similarly in response to meals ± CA in both groups.

5.7 Hemodynamic Effects of Citrus Aurantium

Table 8 shows baseline values and the effect of meal with or without CA on pulse rates, and on systolic, diastolic blood pressure and mean arterial blood pressure. There was no difference in baseline values between groups. There was no significant effect of the intake of meal and of CA on those variables for the duration of the study in lean and in obese subjects.

5.8 Effect of Citrus Aurantium on Urinary Catecholamine Excretion

Table 9 gives the results of the urinary excretion of norepinephrine, epinephrine and dopamine from complete urine collection done during overnight period of at least 6 hours (total urine collected upon rising) and that done after CA ingestion, during the study of the thermic effect of CA, a period of at least 5 hours. The results are expressed per hour and compare obese with lean subjects and men with women. Overnight fasting determinations were as follows: urinary nor epinephrine excretion did not differ between groups and did not change significantly with CA ingestion; urinary epinephrine was significantly greater ($P=0.050$) in lean and in men ($P=0.031$) and increased significantly ($P<0.01$) in all groups with CA remaining greater in lean and in male compared with obese and female subjects; urinary dopamine was significantly greater in obese subjects compared with lean ($P=0.022$) and was also significantly greater in women at baseline ($P<0.05$), it increased significantly ($P<0.008$) in lean and in male subjects with CA.

Part 6: DISCUSSION

6.1 Effect of Citrus Aurantium Alone

The main question addressed by this study was whether administration of Citrus Aurantium would modify RMR and increase TEF in lean and obese men and women subjects. Our results showed that 1.625g of extracts of CA, containing 35mg of alkaloids that included 16 mg synephrine and 6.1 mg octopamine, taken orally in one acute dose with water, increased energy expenditure beyond baseline RMR measurements by 4 %, independently of age and body composition. This thermogenic effect may explain in part the decrease in body weight reported in overweight subjects receiving CA extracts combined with caffeine and St. John's Wort daily for 6 weeks, compared with placebo or no medication (Colker, 1999). All subjects in that study were instructed to follow an 1800 kcalorie diet.

Other products have been studied as thermogenic drugs for the treatment of obesity. Ephedrine, for instance, had no effect on glucose-induced thermogenesis expressed as % of the energy content of the glucose load (Astrup, 1986). However, its chronic intake over 3 months was associated with an elevation of metabolic rate by 10% compared with a control group and a decrease in RQ reflecting greater lipid oxidation during a glucose load. These findings were associated with elevations in plasma epinephrine concentrations both at fasting and during the glucose load suggesting that the increase in RMR be related to that in epinephrine. Epinephrine has been reported to be a potent thermogenic hormone (Sjostrom, 1983)

We have tried to measure catecholamines in urine to reflect acute changes in response to CA. However the results corrected per hour obtained from the urine collected

overnight compared with that collected during the 4 hour-study after ingestion of the capsules may have been affected by other factors such as stress of coming to the laboratory, that of getting up to void during the study and contribute to the difficulty of drawing any conclusion from our results. Furthermore, the results provided by RVH endocrine laboratory for epinephrine excretion were reported as not detectable in 3 samples and very low in concentrations in 2 others in fasting and post CA samples and these may be difficult to interpret. Still we found significantly urinary epinephrine excretions in response to CA in lean subjects and in men greater epinephrine excretion concomittant with greater energy expenditure post CA are in agreement with finding in men that show a 10 % increase in systemic $VO_{2\ in}$ response to a 2 hr epinephrine infusion (Jensen *et al.*, 1996), suggesting that CA may have thermogenic effects

We also found a significant increase in urinary dopamine but in men only. These responses are in accordance with the increments observed in RMR with CA which were greater in men compared with women (Figure 3B). There was no effect on urinary norepinephrine excretion. Astrup (1985) found no change in norepinephrine concentrations with ephedrine treatment that enhanced thermogenesis.

We studied normotensive subjects and found that the oral intake of 5 capsules of CA in the dosage given, did not affect pulse rates nor blood pressure up to five hours after ingestion (Table 8). The absence of effect was seen whether CA was taken alone or with a meal. CA extracts and isolated synephrine have been reported to increase blood pressure in rats in a dose-dependent fashion that could lead to cardiovascular toxicity (Calapai G 1999, Huang 1995). Penzak *et al* (2001) found no change in cardiovascular indices measured in 12 healthy subjects with the intake of 240 mL of Seville orange juice

containing 14 mg of synephrine, an amount comparable to what was given in this study. Blood pressure was measured every hour for 5 hours after Seville orange juice ingestion.

In studies in healthy volunteers by Thomas *et al* (1991), and overweight subjects by colker 1998 adding caffeine to synephrine did not contribute to cardiovascular effects either.

Our results confirm that CA administered in that dosage may be safe when given to normotensive subjects. However, pressor and cardiac effects of synephrine have been reported to be potentiated in the presence of MAO inhibitors (Kaufer, 1993), and should be avoided in subjects taking those as well as in persons with severe hypertension or tachyarrhythmias. It is also conceivable that hemodynamic alterations such as elevations in total peripheral resistance, which went undetected in this study, may have been observed with the use of more sensitive instrumentation (i.e. impedance cardiography vs sphygmomanometry).

6.2 Thermic effect of Meal

The duration of the TEF measurement that we chose to do has been shown to be sufficient to cover at least 90% of the total TEF (Westsrate *et al.*, 1989). Values for oxygen consumption had returned to baseline by the end of the study in most subjects.

6.2.1 Effect of Obesity

Results of post prandial thermogenesis have been controversial, some showing a lower response in obese compared with lean subjects (Pittet *et al.*, 1976 ; Kaplan *et al.*, 1976;

Shetty *et al.*, 1981; Golay *et al.*, 1982, Schwartz *et al.*, 1983; Schutz 1984), while others failing to do so. (Sharief *et al.* 1982, Welle *et al.* 1983, Ravussin *et al.* 1984). Still, convincing data show an association between TEF and visceral adiposity in women (Vansant 1989, Van Gaal 1994). Armellini *et al.* (2000) investigated the relationship between body composition and TEF using a more precise method (computed tomography) to distinguish various body fat compartments and indirect calorimetry to measure TEF during 6 hours and studied 21 men, 55 premenopausal women, and 19 post menopausal women separately. They found that visceral adipose tissue depot and subcutaneous fat were positive predictors while fasting insulin and FFM were negative predictors. of TEF and explained 25% of its variation in premenopausal women. A negative effect of insulin on TEF would concur with findings of low TEF in insulin-resistant and type 2 diabetic subjects (Ravussin, 1997; Gougeon, 1996). We measured fasting insulin in only 3 subjects but FPG, which was higher in the obese subjects, correlated negatively with TEF expressed as % above RMR ($r = -0.53$, $p=0.028$, data not shown in results) indicating an effect of insulin resistance on TEF. However, with multiple regression analysis performed in the whole group, FPG did not enter the equation (Table 7). Leenen *et al.* (1992) also found in their study of 78 obese subjects a significant correlation between TEF and visceral adipose tissue, measured by magnetic resonance imaging, but only in women. We assessed visceral fat only by measuring waist circumference and W: H ratio and found a significant negative correlation between waist circumference and TEF as % above RMR but not as % of meal energy content or in absolute value kJ per 280 min. In our population, all measurements reflecting greater body mass were negatively related to TEF as % above RMR. Weight was the only

predictor of TEF as % above RMR and was a negative predictor. These results are explained by the fact that the greater body mass relates to higher RMR which becomes the denominator in the calculation of TEF as % above RMR: The greater weight = greater RMR = a lower TEF as % above RMR. Our study could not support an effect of body composition and body fat distribution on TEF expressed as % of energy content of meal and absolute value, in response to the same energy content in the meal. Others (Armellini *et al.*, 2000) who have reported an effect of body fat compartments on TEF had given a nutrient load calculated on the basis of fat-free mass and included subjects with diabetes such that in their study the men had greater TEF because of greater energy intake in meal than women. Our population of obese subjects had normal glucose tolerance but may have had insulin insensitivity of glucose with upper body obesity. The latter was not measured.

6.2.2 Effect of Gender and CA

By contrast our results show that for a similar energy intake, women have a lower TEF than men, which remains significantly lower when adjusted for body composition. Westsrate *et al.* (1989) found no sex difference in the thermic effect of a mixed meal which provided 25% of daily individual energy intake or 2.1 MJ in women and 2.6 MJ in men. TEF was about 8% of the energy content of the meal. 24-hr studies done in a respiratory chamber showed a lower TEF in women than in men, an expected result by virtue of a lower total energy intake in women. However, when adjusted for energy intake, there was no gender difference (Tataranni *et al.*, 1995).

Gender differences in TEF were reported by Leenen *et al.* (1992). They found that in premenopausal women but not in men, TEF was greater with visceral fat accumulation. Vansant *et al.* (1989) also found higher glucose-induced thermogenesis in women with upper body obesity which they explained by elevated plasma insulin concentrations that would induce greater thermogenic responses via stimulation of SNS, an explanation that is not in accordance with findings of low TEF in insulin-resistance states (Ravussin, 1997).

Correlations have been reported between visceral adiposity and fasting insulin concentrations (Leenen *et al.*, 1992) but not always with greater TEF, especially in men (Van Gaal., 1990, Leenen *et al.*, 1992). In our study, men had a greater TEF than women even when adjusted for body composition and measures of fat distribution. Adding CA, claimed to stimulate SNS, to meal increased TEF significantly only in women, and independently of their waist circumference. It is conceivable that an absence of measurable effect of CA on TEF in men was mainly because TEF was already maximal. In women, CA added to meal, increased energy expenditure by ~ 46kJ, compared with added placebo, the same increment in energy measured when CA was given alone without a meal in women. This suggests that CA with meal had no synergistic effect, and was simply additive.

What other reasons could explain the differences between sexes in their thermic response to a meal? Differences in sex steroid concentrations could play a role. It has been suggested that estradiol could have an effect on energy expenditure (Vansant *et al.*, 1992). Individuals characterised by usual low energy intake had lower thermic responses to meal and ephedrine (Morgan, 1982). Although not measured directly, based on

measured RMR and using the factorial method, it is conceivable that our group of women had habitually lower energy intake than our group of men. The lower thermogenic response to a meal observed in women compared with men was independent of body fatness and do not support an effect of obesity reported by others (in response to norepinephrine (Jung ,1979) or to glucose and insulin in men (Ravussin, 1983) characterized by insulin resistance and/or type 2 diabetes mellitus. Our obese subjects had significantly higher post-absorptive glycemia compared with lean but their values were within the normal range. They were also characterized by upper body obesity, as indicated by large waist circumferences but fasting insulin concentrations measured in some (data not reported) were not indicative of hyperinsulinemia and glucose tolerance tests were normal (data not reported). RMR has been reported to be elevated in subjects with poorly-controlled diabetes possibly due to enhanced hepatic glycogenesis (Ravussin, 1983) and protein turnover (Gougeon, 2001), did not differ between lean and obese.

Gender differences in glucoregulation were reported in response to intense exercise (Marliss *et al.*, 2000). Women cleared glucose more slowly than men, when adjusted to FFM, which lead to greater concentrations in glucose and insulin during recovery after intense exercise. In this study, there were no gender differences in plasma Norepinephrine and Epinephrine responses to intense exercise. Others (Gustafson *et al.* 1982) have found a lower catecholamine response during a handgrip strength test in women versus men, despite similar FFA increases.

Others have reported gender-based difference in catecholamine action. Jensen *et al.*, (1996) have shown a greater upper body free fatty acid release in normal weight adult women than in men in response to epinephrine.

Lipid mobilization has also been shown to be more sensitive to catecholamines in women than in men (Wahrenberg *et al* 1991), which is consistent with our findings of a lower initial RQ increment in response to CA in women than in men, indicating a greater use of fat as substrate in response to the induced thermogenesis. However, we have not measured plasma FFA concentrations in this study, a variable that could have shed some insight on gender effects. The greater fat mass and lesser FFM that characterize women, and a lower fasting RQ shown in other studies (Friedlander *et al.*. 1998) but not in ours, may explain a lesser response of muscle glycogen and a greater one of lipid with CA, indicated by a lesser increment in RQ initially in women.

Our study reinforces the importance of correcting for gender in the study of metabolism. Our group of women represented 11 subjects with a wide range of BMI, and waist circumferences that still could not result in statistically significant effects of body composition on TEF and/or the response to CA. Studies with a large number of subjects might detect an effect. Indeed, obese women with upper-body obesity have been reported to show lipolytic catecholamine resistance secondary to the presence of a low density (70% reduction) in β_2-adrenoceptors compared to non obese women (Reynidottir *et al.*, 1994). It has been suggested that chronic hyperinsulinemia could have down-regulatory effects of β_2-adrenoceptors. We do not have data on fasting insulin in this study on all subjects. The few we have showed no hyperinsulinemia.

6.3 Resting Metabolic Rate

RMR did not differ significantly among the three studies done in each subject.

We report data on Table 4a for two RMR determinations. This confirms that the technique used was reliable and precise, able to reproduce the same measurement in the same person with an acceptable margin of error. The difference between studies was not different from zero. When we compared our values to those of predicted equations (shown in table 3), we found that the equations of Owen and Mifflin gave RMR values that did not differ significantly from ours. We also found that Harris and Benedict equation, as has been reported by others (Daly *et al,* 1985; Garrel *et al,* 1996) overestimated RMR by up to 10%. This indicates that body fat, a less energy consuming tissue, increases in proportion to total body weight with weight gain such that linear equations that use weight, height, gender and age as variables overestimate RMR (Heshka *et al*, 1993). Bernstein's equation underestimated by a large percentage (up to 15%) measured RMR, with the smallest difference being for obese women. This is understandable as this equation was derived from an obese population.

We found that FFM and weight were the only variables that were significant independent predictors of RMR and explained 71% of the variation in RMR. In women, only FFM and in men only weight entered the model and explained 61% of the variation in RMR. RMR correlated with other variables shown in Table 5, such as body circumferences, BMI and FPG.

Waist circumference, an index of visceral fat, correlated with RMR. Others have shown, but only in women, that there is an association between visceral fat and RMR (Leenen *et al.*, 1992), indicating greater RMR with greater abdominal obesity, the latter may be associated with insulin resistance. The same reasoning could apply to the association with FPG in men. Higher glucose concentrations were found in the obese

who had greater body weight such that FPG no longer was a predictor of RMR in multiple regression analysis entering body weight and FFM as factors.

PART 7: CONCLUSIONS

Allison and Heymsfield, (1998) questioned whether there was wisdom in using alternative "natural" approaches for the treatment of obesity in this post fen-phen era, a time when prescription drugs amount to two (Sibutramine and Orlistat) and obese persons and their physician show concern about their long term effects. Obese persons may welcome alternative therapies that can support them as they struggle at reducing their food intake and attempt at increasing physical activity. Ephedra sinica (Ma Huang) has been shown to be a stimulant herb. Ephedra tea was used by the body guards of Genghis Khan to stay awake (Allison, 1998). It is now available mostly as an herbal weight loss product but with recognised potentially fatal side effects which has limited its use and made it illegal in Canada. This indicates that there is no assurance of complete safety of a drug or a dietary supplement and that double-blind placebo-controlled randomized clinical trials are needed to identify as many side effects as possible if we are to weigh the risk-benefit ratio of their use. Sufficient data must be available to justify their recommendation and classify them as effective and safe products. This study is an attempt at providing data on a dietary supplement the immature seville orange in (Citrus Aurantium) sold for weight loss. This study does not measure effects on appetite and food intake nor does it assess appropriate dosage for effectiveness, for chronic use. Dose-response studies remain to be done. Our data indicate that women may respond better to such a product than men, when it is combined to food. Whether CA is a useful adjunct to

weight loss therapy would require well-designed clinical trials. Its safety in the long term also has to be established. We found that CA had a small but measurable thermogenic effect compared with water and increased baseline RMR by 4% and that it enhanced the thermic effect of a meal in women, by 29% or 46kJ. Extrapolated to a day, it represents 200 kJ (50kcal) and to a year 4.3 MJ (18250 kcal) or a weight loss of > 2kg.

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McGill Nutrition & Food Science Centre

The benefits of participating in this study:
I will receive a monetary compensation of $120.00 for completing the two sessions. It is hoped that the information obtained will lead to the advancement of scientific knowledge.

The risks of participating in this study:
The risks involved in consuming the mixed diet and the citrus extract are minimal. Risks with blood sampling are considered to be minimal when carefully selected patients are studied in this manner in hospital. The intravenous catheter is the standard kind used. There might be slight pain or discomfort while introducing it, and risks of bruising with blood test. The amount of blood drawn represents less than a blood donation.

Confidentiality:
If I agree to participate, the information collected and analysed will be kept confidential. My name will not appear on any of the questionnaires nor will it be used in any reports or publications.

Information:
I can contact Dr. R. Gougeon at **842-1231, local 5011**, if I have any further questions about the study or require any explanations.
Should I have any questions about my rights as a research subject, I may contact the Patient Representative at **842-1231, local 5655**.

I have read and fully understand the implication of my participation in this study. I understand that I am free to refuse to participate or to withdraw from the study at any time for any reason, and that my decision will not affect my care at this institution.

I, _____ consent to be a subject in this project.
(Please print)

Signature_____

Dated at Montreal, this_____ day of_____, 2001.

Investigator _____
(Signature)

APPENDIX 1

Faites-vous de l'embonpoint?

Le Centre de Nutrition et des Sciences de l'alimentation de l'université McGill

est présentement à la recherche de personnes obèses pour une étude de l'effet d'un extrait d'orange de Séville sur la dépense d'énergie

Vous pouvez participer si vous avez moins de 65 ans
vous souffrez d'obésité
vous êtes en bonne santé
vous êtes non-fumeur

Votre participation comportera:

1) un examen médical, un test de sang et d'urine, un électrocardiogramme et une radiographie.

2) deux journées à l'hôpital pour mesurer votre composition corporelle et votre consommation d'énergie à la suite d'un repas pris avec ou sans extrait d'orange .

Une compensation monétaire vous sera offerte.

Si vous avez d'autres questions ou désirez de plus amples renseignements, veuillez contacter la Dr Gougeon au 842-1231 poste 5011
Si vous êtes intéressé à participer à cette étude, veuillez contacter Miriam, du lundi au vendredi de 9h à 17h, au 842-1231, poste 5011

8421231 ext .5011 Miriam

842-1231 ext 5011 Miriam

842-1231 ext.5011 Miriam

842-1231 ext.5011 Miriam

842-1231 ext.5011 Miriam

842-1231 ext.5011 Miriam

842-1231 ext.5011 Miriam

842-1231 ext.5011 Miriam

842-1231 ext.5011 Miriam

842-1231 ext.5011 Miriam

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Centre de nutrition et des sciences de
l'alimentation de l'universit[illegible]

Effet thermogène d'agents extraits de Citrus Aurantium

FORMULE DE CONSENTEMENT

La docteure R. Gougeon m'a invité(e) à prendre part à une étude des effets d'un extrait d'un agrume (C. Aurantium) sur mon métabolisme de base, quand pris seul ou avec un repas. Le but de cette étude est de déterminer si ce produit augmente la dépense énergétique et si c'est le cas, peut être utile dans le traitement de l'obésité.

Ma participation à l'étude peut inclure:

1) un examen médical comprenant un interrogatoire, un examen physique, des analyses de sang et d'urine.

2) que je vienne à l'hôpital deux jours séparés, le matin après 12 heures de jeûne, avec abstention de tabac et café; seulement l'eau est permise.

3) que l'on prenne ma pression sanguine et mon pouls, les mensurations de ma taille et mes hanches et la mesure de ma composition corporelle à l'aide de l'impédance bioélectrique, instrument inoffensif et sans risque, qui consiste en l'apposition d'électrodes sur ma main et mon pied pendant que je suis couché. Ceci dure au plus 5 minutes.

4) qu'on mesure mon métabolisme de base au repos à chaque session. Pour ce faire, je devrai respirer sous un capuchon de plastique qui couvre ma tête et mes épaules pendant au moins 20 minutes. Ensuite, à deux reprises, on mesurera l'effet thermique d'un repas. Pour ce faire, je consommerai deux barres au chocolat, avec capsules de C. Aurantium (5 X 325 mg) ou 5 capsules placebo, et resterai sous le capuchon pendant 6 heures ou moins avec pauses de 20 minutes toutes les heures.

5) Les repas sont sous la forme de barres qui contiennent 400 Calories, 53% de l'énergie vient des glucides, 29% des protéines, 18% des lipides.

6) durant les études mon pouls et ma pression sanguine seront pris à toutes les heures.

Bénéfices

Pour ma participation à cette étude, je recevrai une compensation monétaire de 120,00$. Le montant reçu sert à défrayer toutes dépenses encourues suite à des inconvénients reliés à ma participation. Je reconnais toutefois que les connaissances tirées de cette étude pourront contribuer à l'avancement de la science.

Risques associés

Les risques associés à la consommation des repas et à la prise d'échantillons de sang sont minimes puisque la sélection des sujets est faite avec soin. Il y aura risque d'ecchymose lors des prises de sang mais la quantité de sang donnée représente la moitié d'un don de sang habituel. Les résultats obtenus seront traités de façon confidentielle et il sera impossible de m'identifier lors de leur publication.

On répondra à toutes les questions que je pourrais avoir concernant les résultats de l'étude en appelant le **Dr. R. Gougeon au 842-1231 poste 5011**. Si j'ai des questions concernant mes droits en tant que sujet pour étude, je peux composer le **842-1231, poste 5655**.

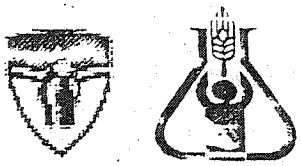
J'ai lu et comprends ce en quoi consiste ma participation à l'étude. Je sais que je peux me retirer de l'étude en tout temps quelle qu' en soit la raison et que ma décision n'affectera pas les soins que je reçois à cette institution.

Je _____ consens à participer à cette étude

Montréal, le _____ 2001.

Signature _____

Investigateur _____
(Signature)



McGill Nutrition & Food Science Centre

CONSENT FORM

Thermogenic Effect of Compounds extracted from Citrus Aurantium

I have been invited by Dr. R. Gougeon to participate in a study on the effect of an extract of a citrus fruit (C. Aurantium) on my metabolic rate, both when consumed alone and with a mixed meal. The purpose of this study is to determine whether this extract may increase energy expenditure and be useful as an adjunct in the treatment of obesity.

My participation in this study will include the following:

1) I will initially have a thorough medical examination, including routine blood and urine tests, an electrocardiogram and a chest x-ray.

2) I will be asked to come to the hospital on two separate mornings after a 12-hour overnight fast, where I will be asked to refrain from smoking, eating and drinking (especially coffee) , except water.

3) The taking of my blood pressure and pulse, my weight and the circumference of my waist and hips. My body fat content will also be assessed by bioelectrical impedance analysis, a non-invasive, harmless method consisting of having an electrode placed on each of my hand and foot while a reading is taken.

4) At each session, that will last 6 hours or less, I will rest in bed for 20 minutes and will then have my metabolic rate measured for 20 minutes. This consists of breathing under a clear plastic canopy that covers my head and shoulders.
I will afterwards, consume either the extract (C. aurantium) of a citrus fruit in a capsule form (5 capsules of 325 mg each) with water or 5 placebos, with a mixed meal in the form of a chocolate flavored food bar. At each session, after having consumed either of these, my metabolic rate will continue to be measured until it returns to baseline (for up to 340 minutes). During this time the canopy will be removed for 20 minutes at each hour.

5) The mixed meal will be provided in the form of a food bar which contains 400 calories of energy, consisting of 53% carbohydrate, 29% protein and 18% fat.

6) As well my pulse rate and blood pressure will be measured every hour.

APPENDIX 1.

Are you overweight ?

McGill Nutrition and Food Science Centre

is presently recruiting overweight persons to study the effect of an extract from Seville orange on energy expenditure

You may be a candidate if you are younger than 65
if you are overweight
if you are in good health
if you do not smoke

Your participation will include

- A medical exam, routine blood and urine tests, an electrocardiogram and an X- ray
- Two separate days in the hospital for measurement of your body composition and energy expenditure during a meal taken with or without the extract from Seville orange

A small compensation will be offered

If you have any further question about the study or require any explanation please contact Dr Gougeon at 842-1231 local 5011.

If you are interested in participating in the study please contact Miriam Monday to Friday from 9 am to 5pm at 842-1231 ext. 5011

842-1231 ext.5011 Miriam
842-1231 ext5011 Miriam
842-1231 ext.5011 Miriam
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Figure 1A. **ACTIVE AGENTS in CITRUS AURANTIUM**

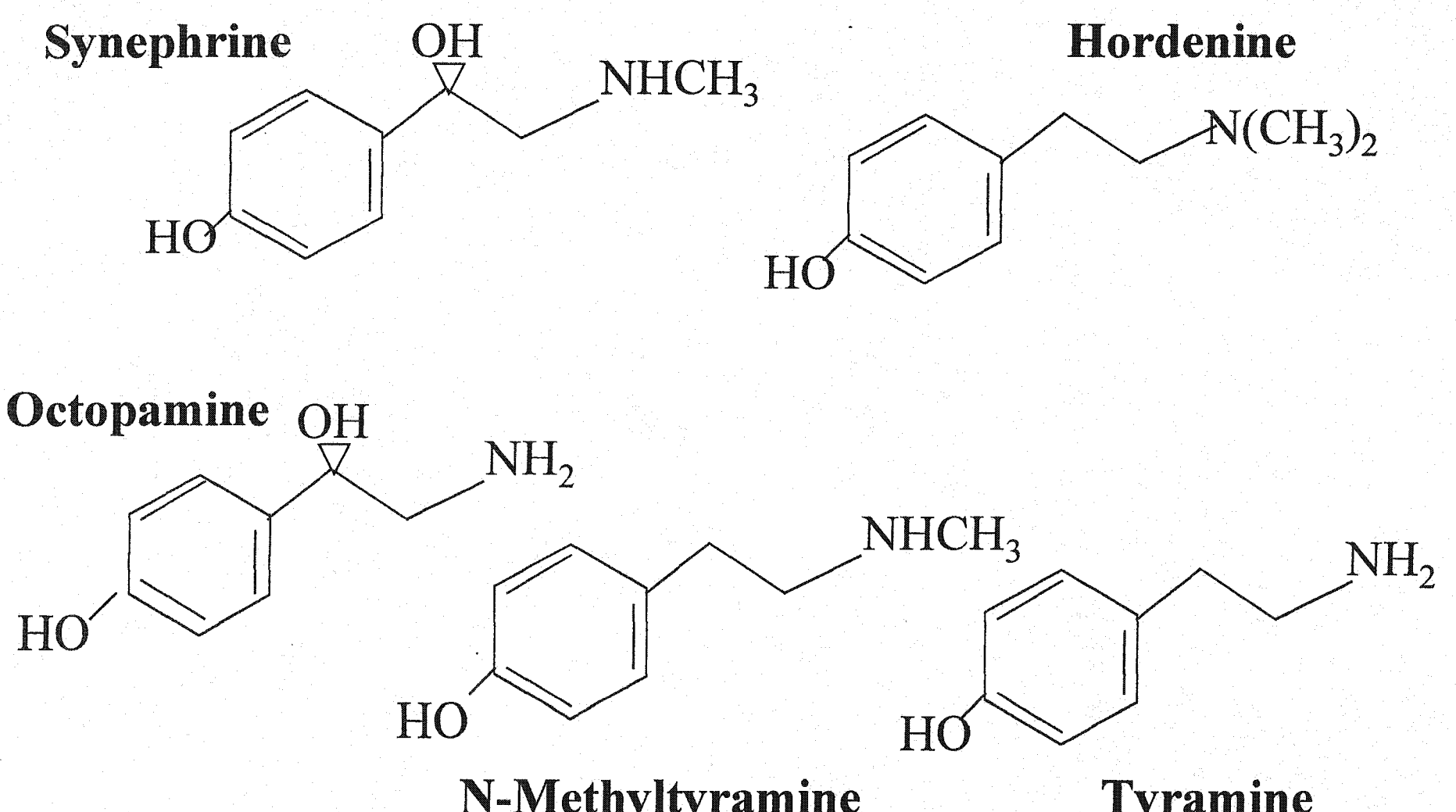


Figure 1B. **ACTION of CITRUS AURANTIUM ALKALOIDS**

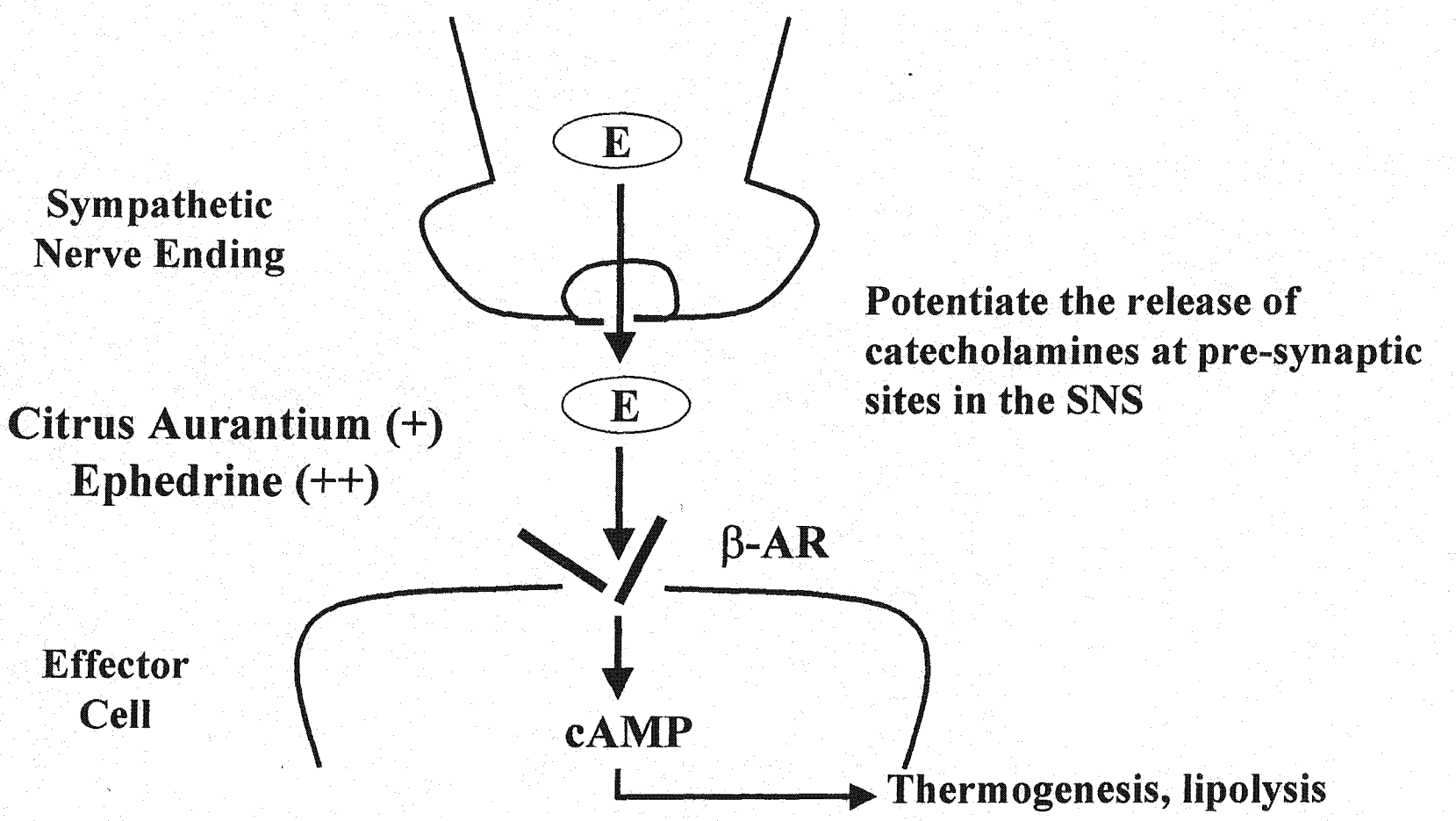


Figure 1C.

EXPERIMENTAL PROTOCOL

	Session1 Meal ± CA	Session2 CA alone	Session3 Meal ± CA
Body composition	Yes	No	No
RMR measurement	20 minutes	20 minutes	20 minutes
test meal	1.75MJ (418kcal) mixed meal	5 capsules (1.625g) Citrus Aurantium extract	mixed meal and Citrus Aurantium extract
TEF measurement	280 minutes	280 minutes	280 minutes
Study urine	Before RMR and at every break during TEF		
Blood sampling	Yes	No	Yes
	0', 30', 60', 90', 120', 180', 240', 280'		0', 30', 60', 90', 120', 180', 240', 280'
Pulse rate and BP	Before RMR and at every break during TEF		

Figure 2.

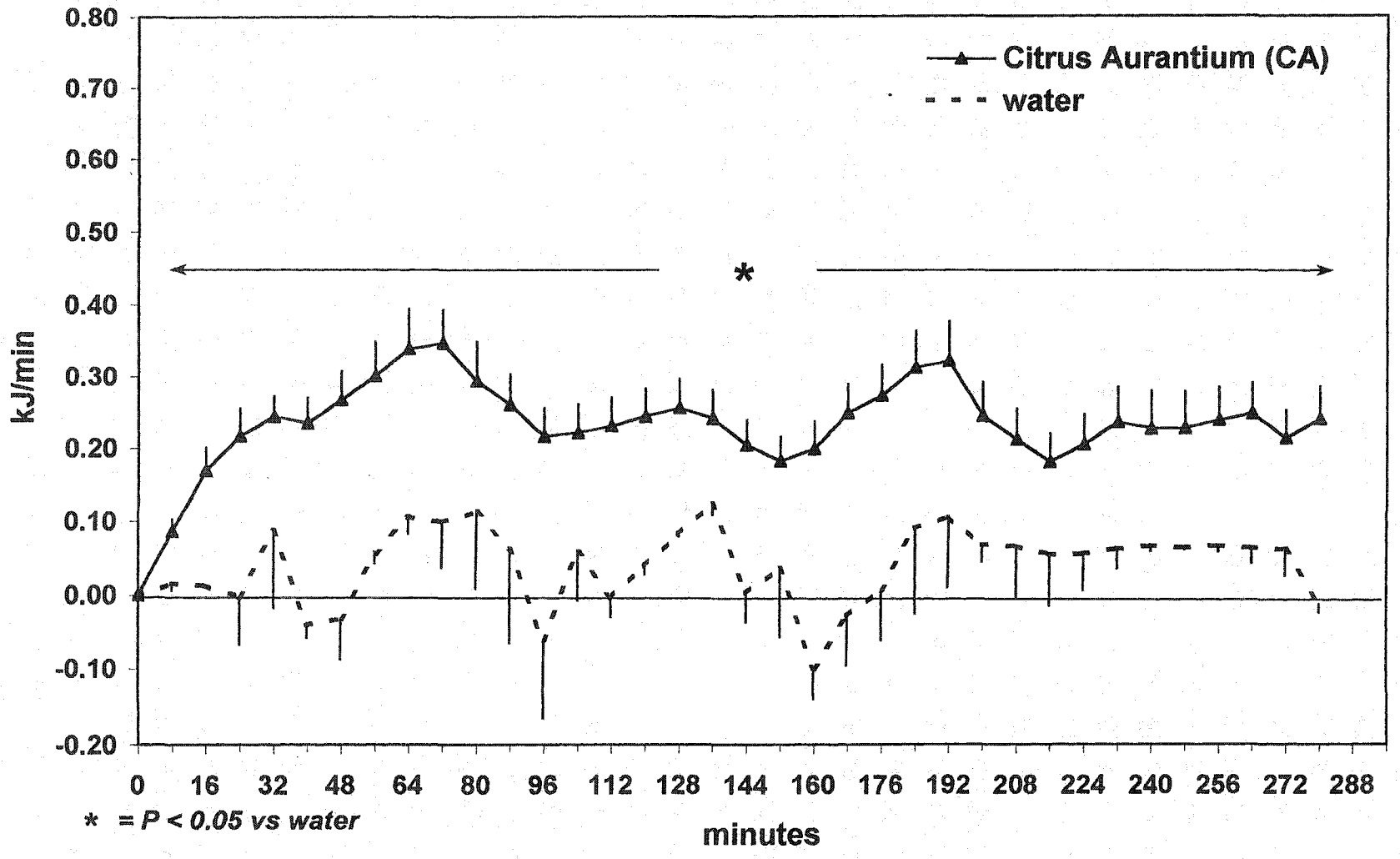

INCREMENT above RMR
Citrus Aurantium (CA)
water
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
kJ/min
*
0
16
32
48
64
80
96
112
128
144
160
176
192
208
224
240
256
272
288
minutes
* = P < 0.05 vs water

Figure 3.

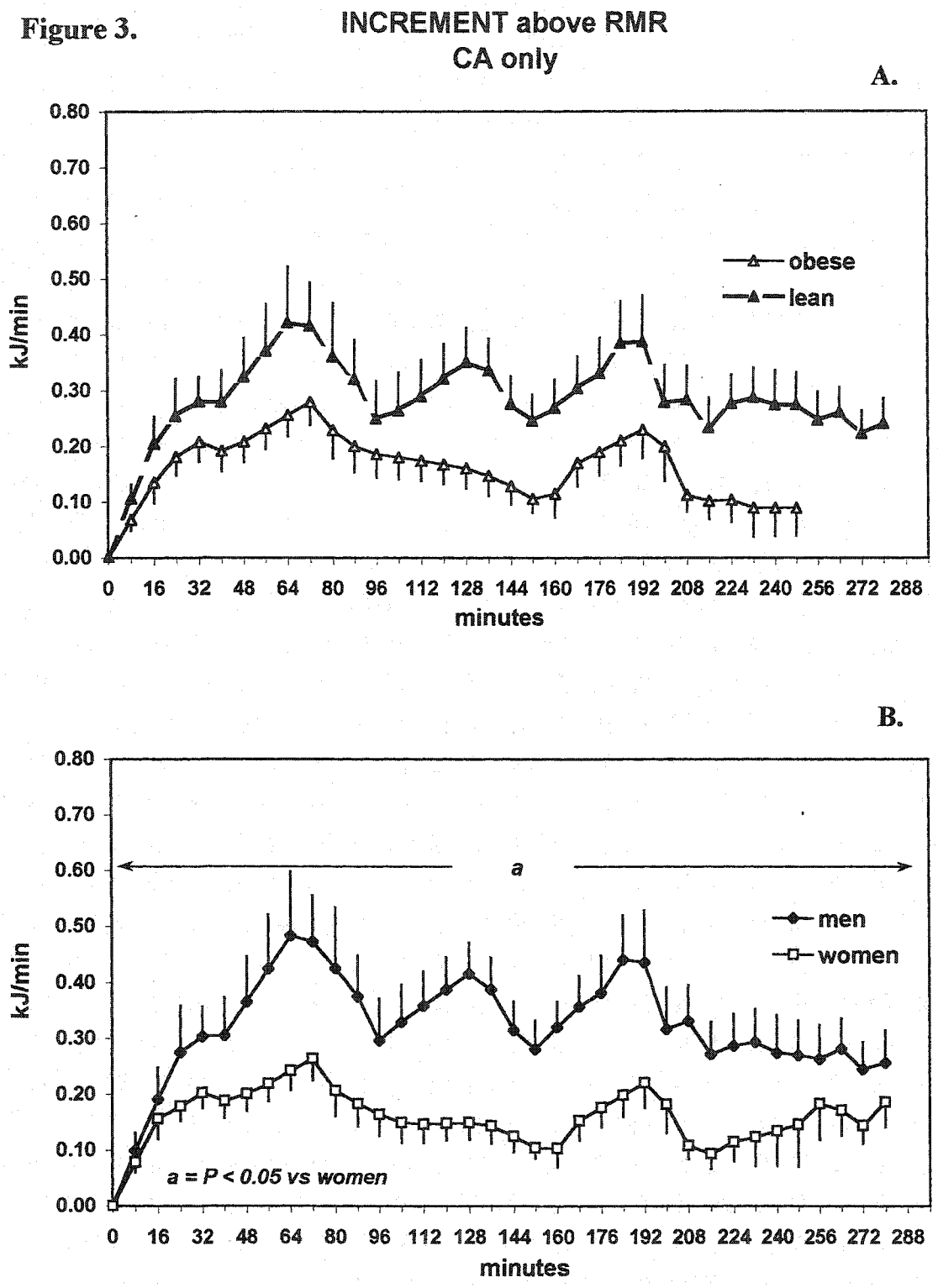

INCREMENT above RMR
CA only
A.
kJ/min
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
obese
lean
0 16 32 48 64 80 96 112 128 144 160 176 192 208 224 240 256 272 288
minutes
B.
a
men
women
a = P < 0.05 vs women

Figure 3.

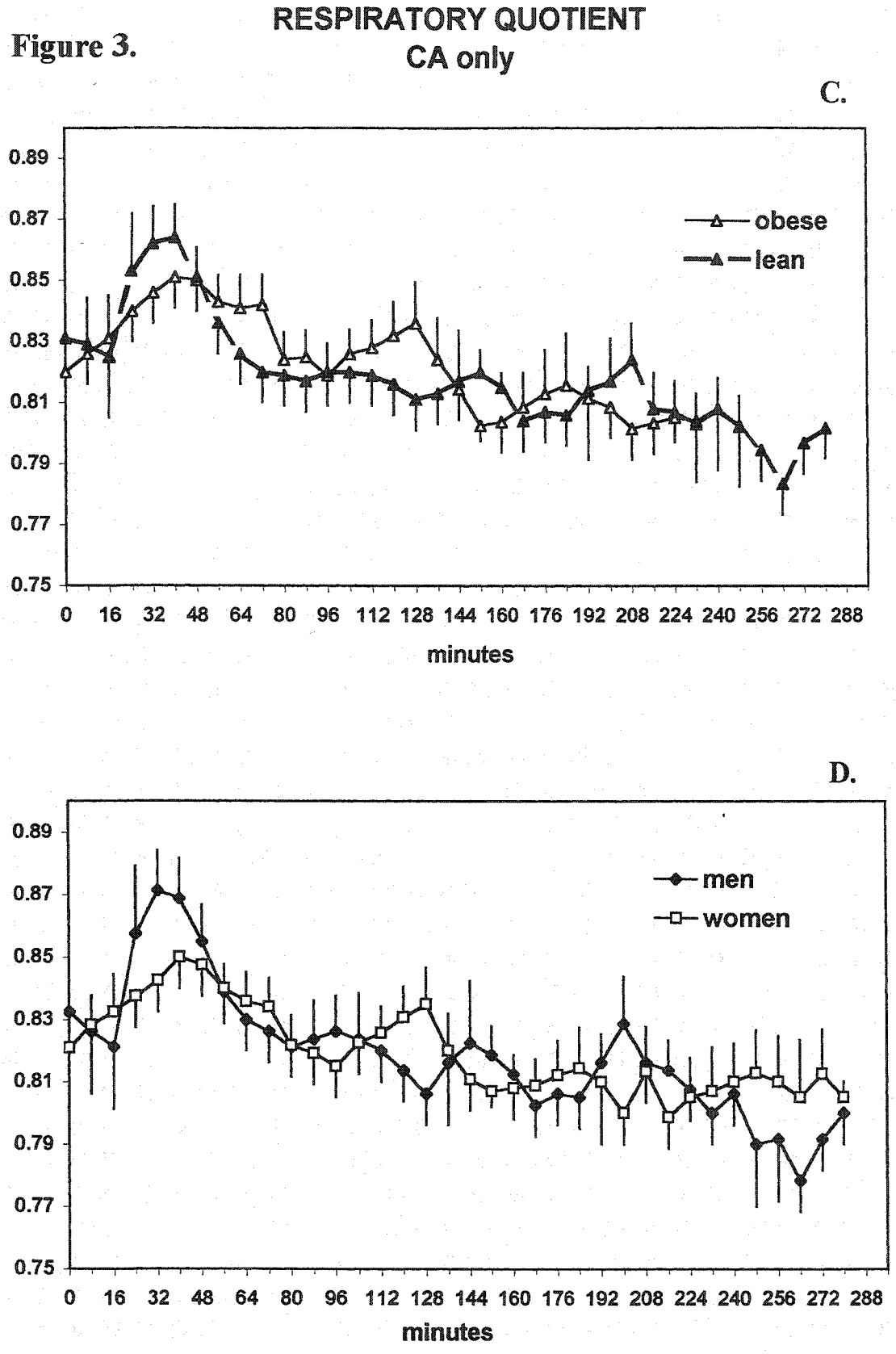
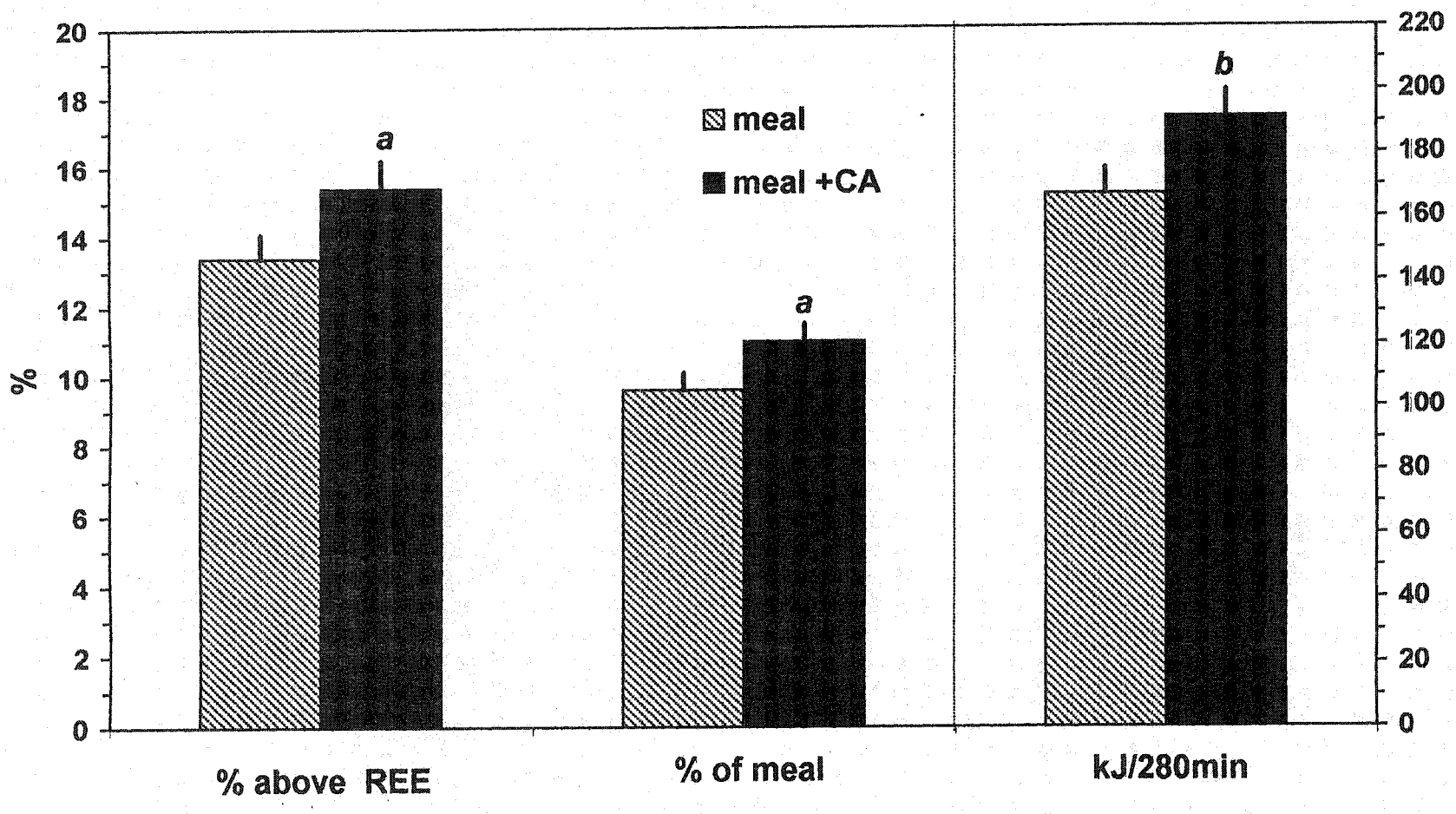

RESPIRATORY QUOTIENT
CA only
C.
0.89
0.87
0.85
0.83
0.81
0.79
0.77
0.75
obese
lean
0 16 32 48 64 80 96 112 128 144 160 176 192 208 224 240 256 272 288
minutes
D.
0.89
0.87
0.85
0.83
0.81
0.79
0.77
0.75
men
women
0 16 32 48 64 80 96 112 128 144 160 176 192 208 224 240 256 272 288
minutes

Figure 4

Thermic Effect of Meal and Meal +CA in the Group



a < p=0.05 vs meal

b = p=0.053 vs meal

Figure 5

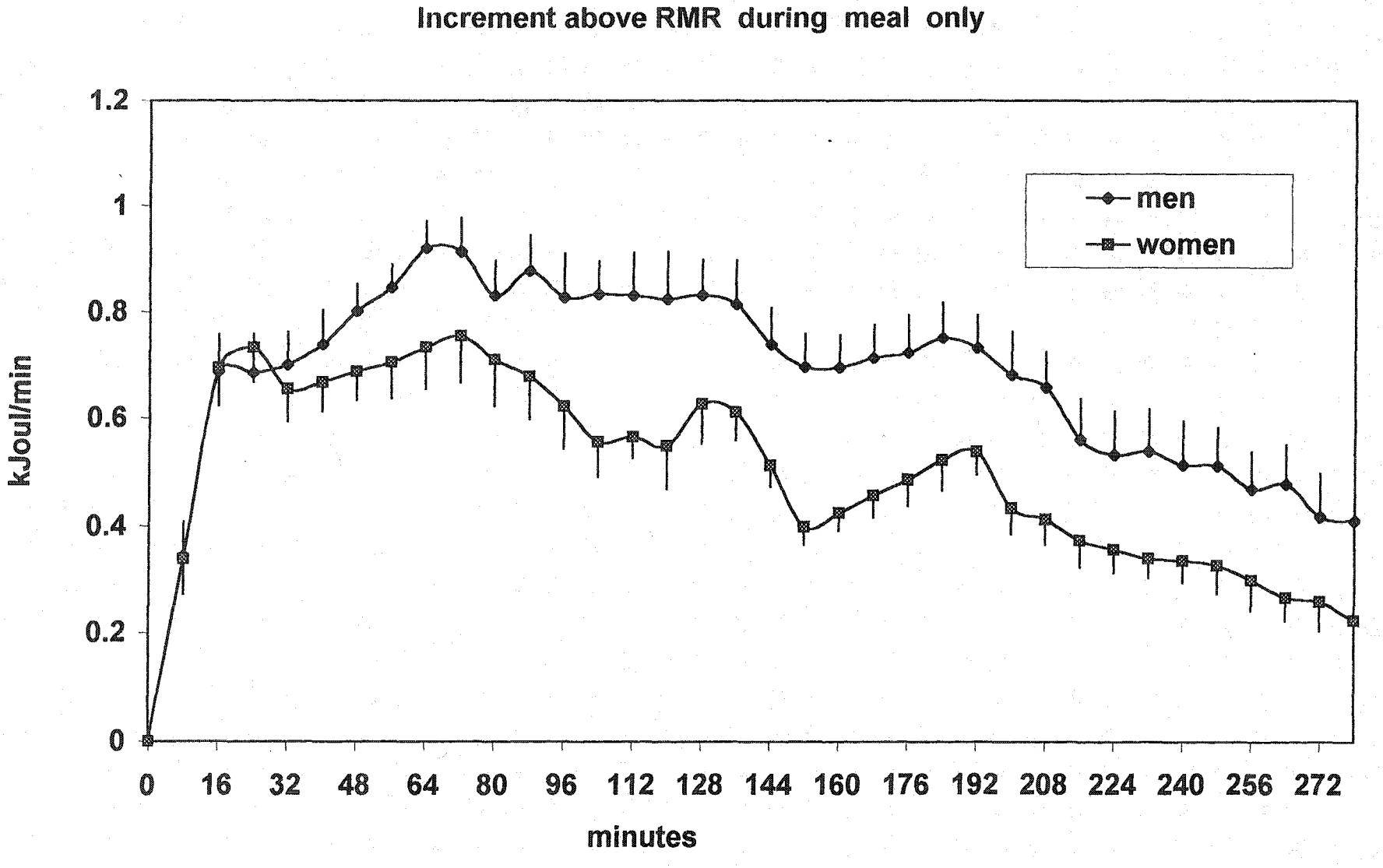

Increment above RMR during meal only
1.2
1
0.8
0.6
0.4
0.2
0
kJoul/min
men
women
0
16
32
48
64
80
96
112
128
144
160
176
192
208
224
240
256
272
minutes

Figure 6

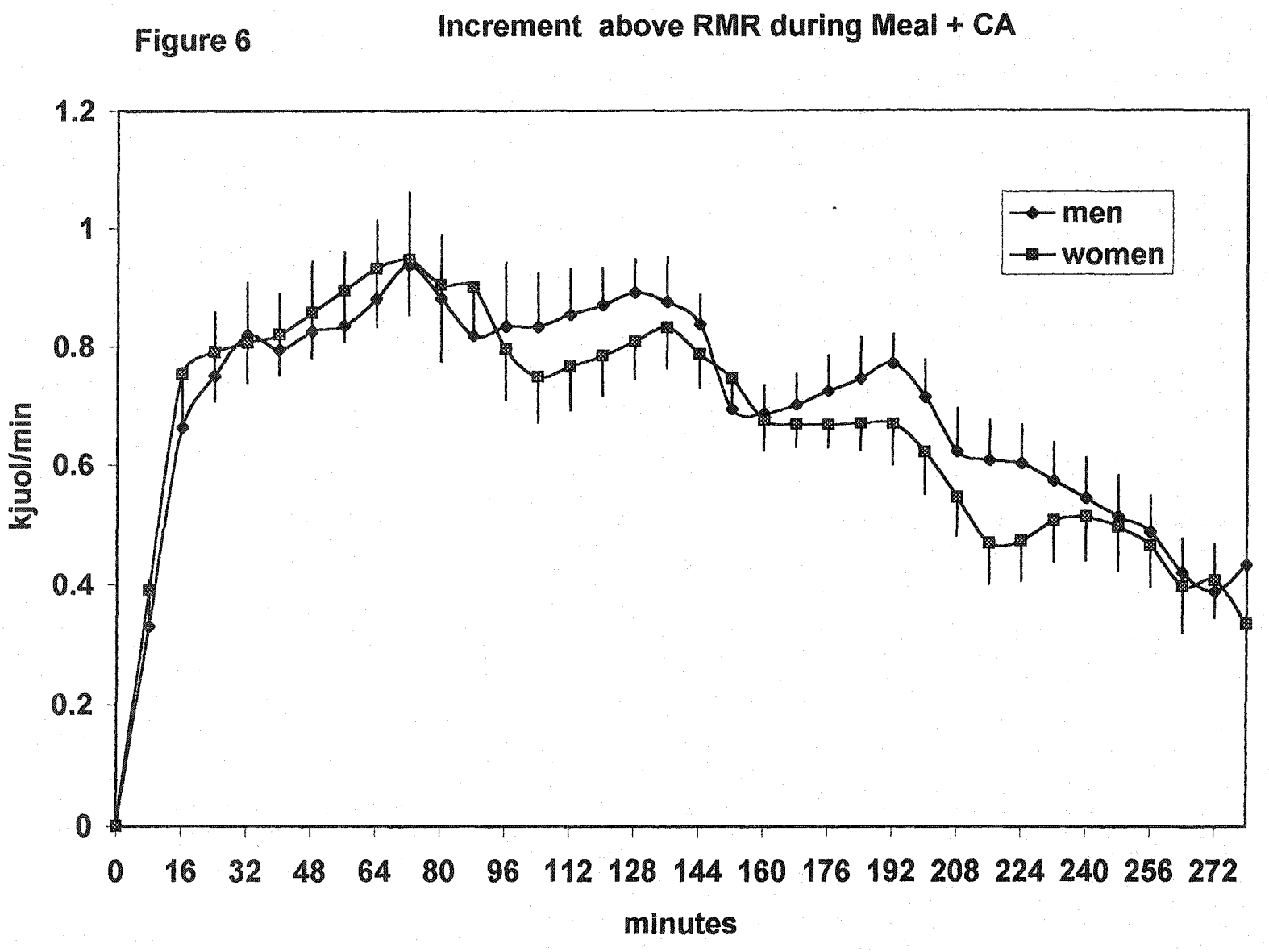

Increment above RMR during Meal + CA
men
women
kjuol/min
1.2
1
0.8
0.6
0.4
0.2
0
0 16 32 48 64 80 96 112 128 144 160 176 192 208 224 240 256 272
minutes

Figure 7

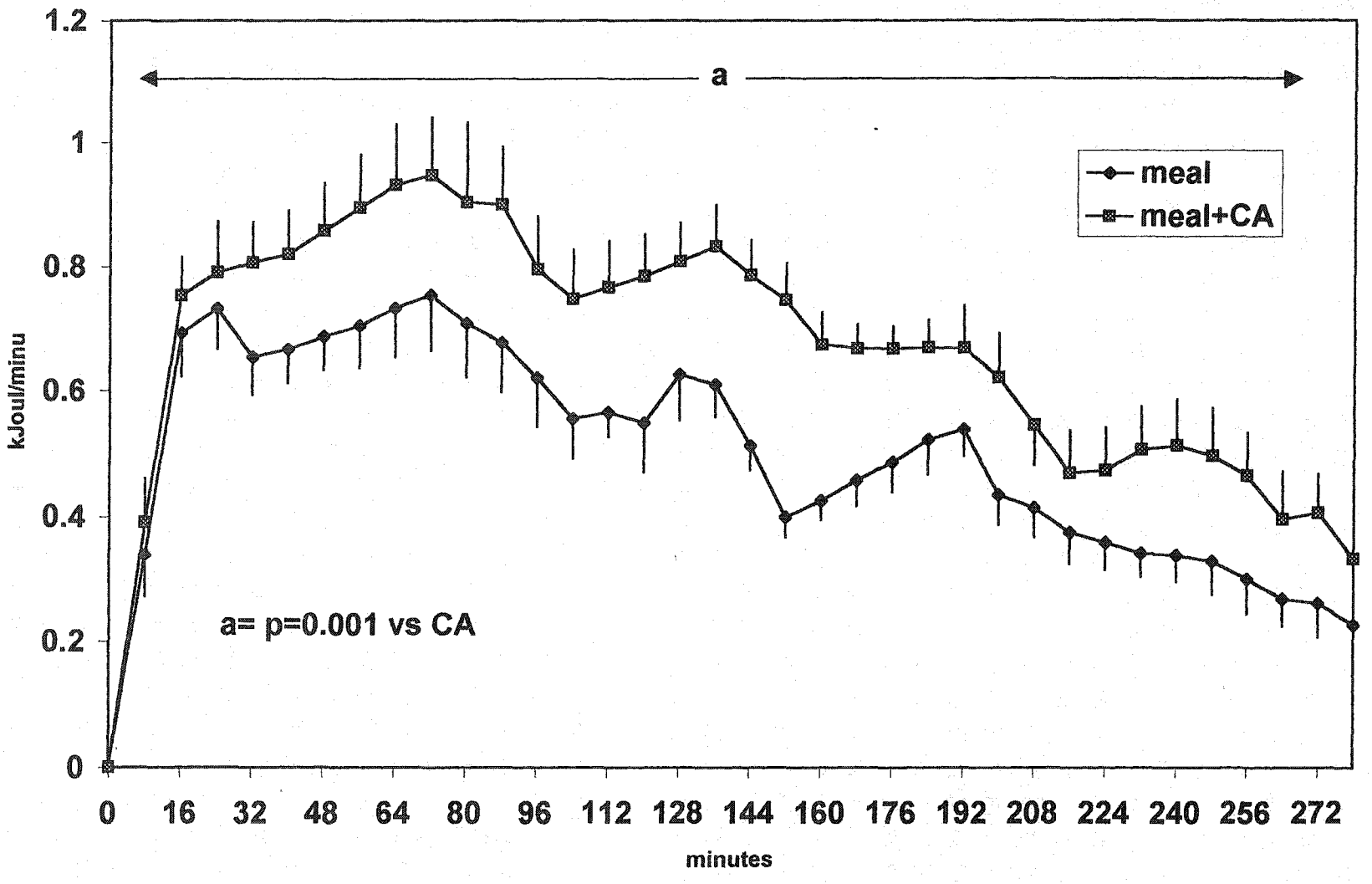

Increment above RMR in WOMEN
a
meal
meal+CA
a= p=0.001 vs CA
kJoul/minu
minutes
1.2
1
0.8
0.6
0.4
0.2
0
0 16 32 48 64 80 96 112 128 144 160 176 192 208 224 240 256 272

Figure 8

INCREMENT above RMR in MEN

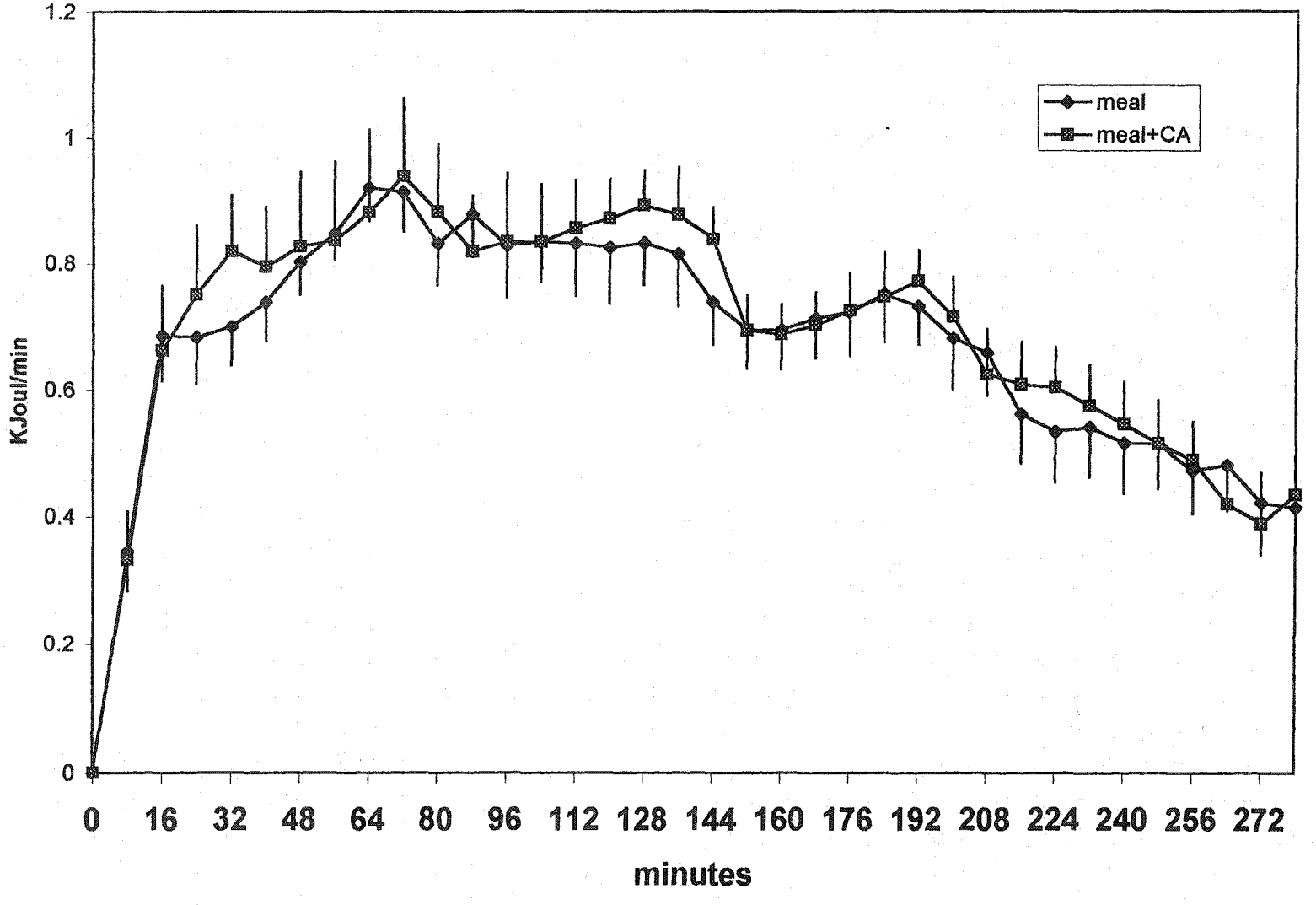
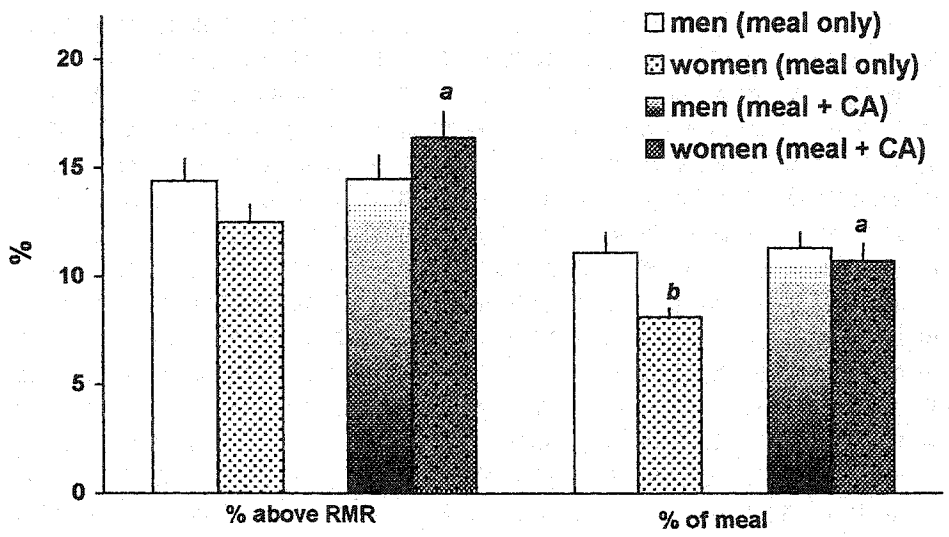
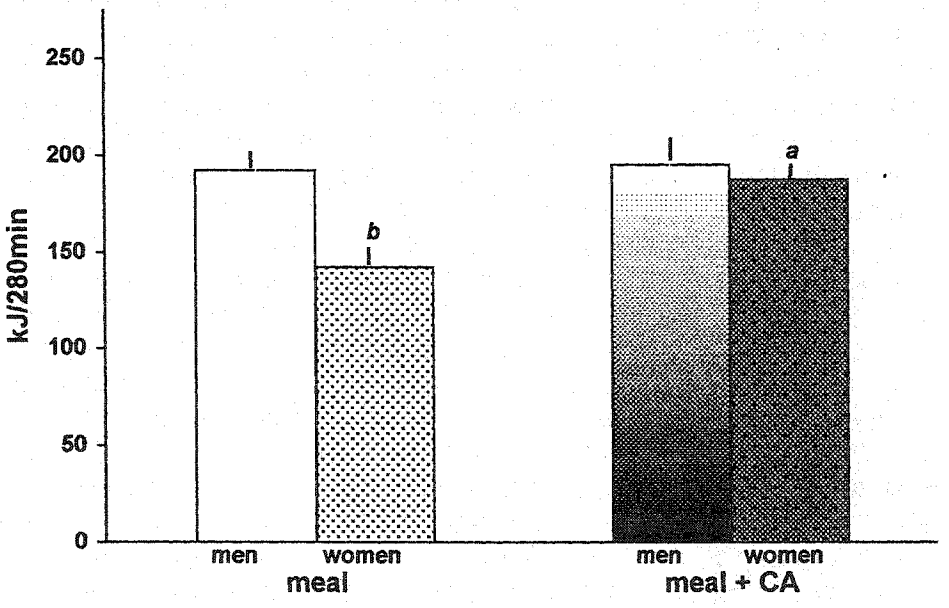


Figure 9. THERMIC EFFECT of MEAL

A.



B.



a = P<0.01 vs meal, b = P<0.01 vs men

group

Figure 10.

RESPIRATORY QUOTIENT

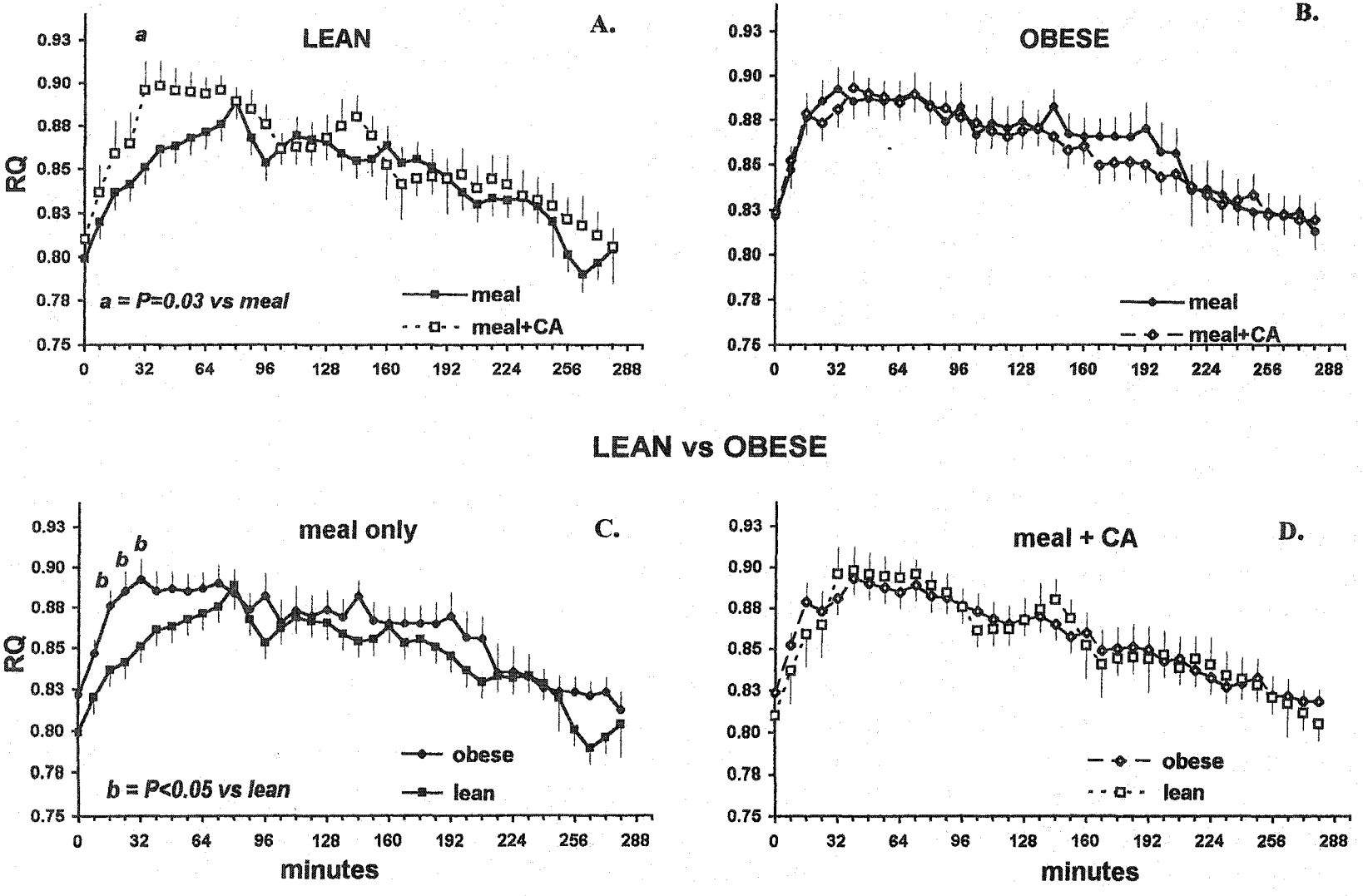


Figure 11.

PLASMA GLUCOSE during TEF

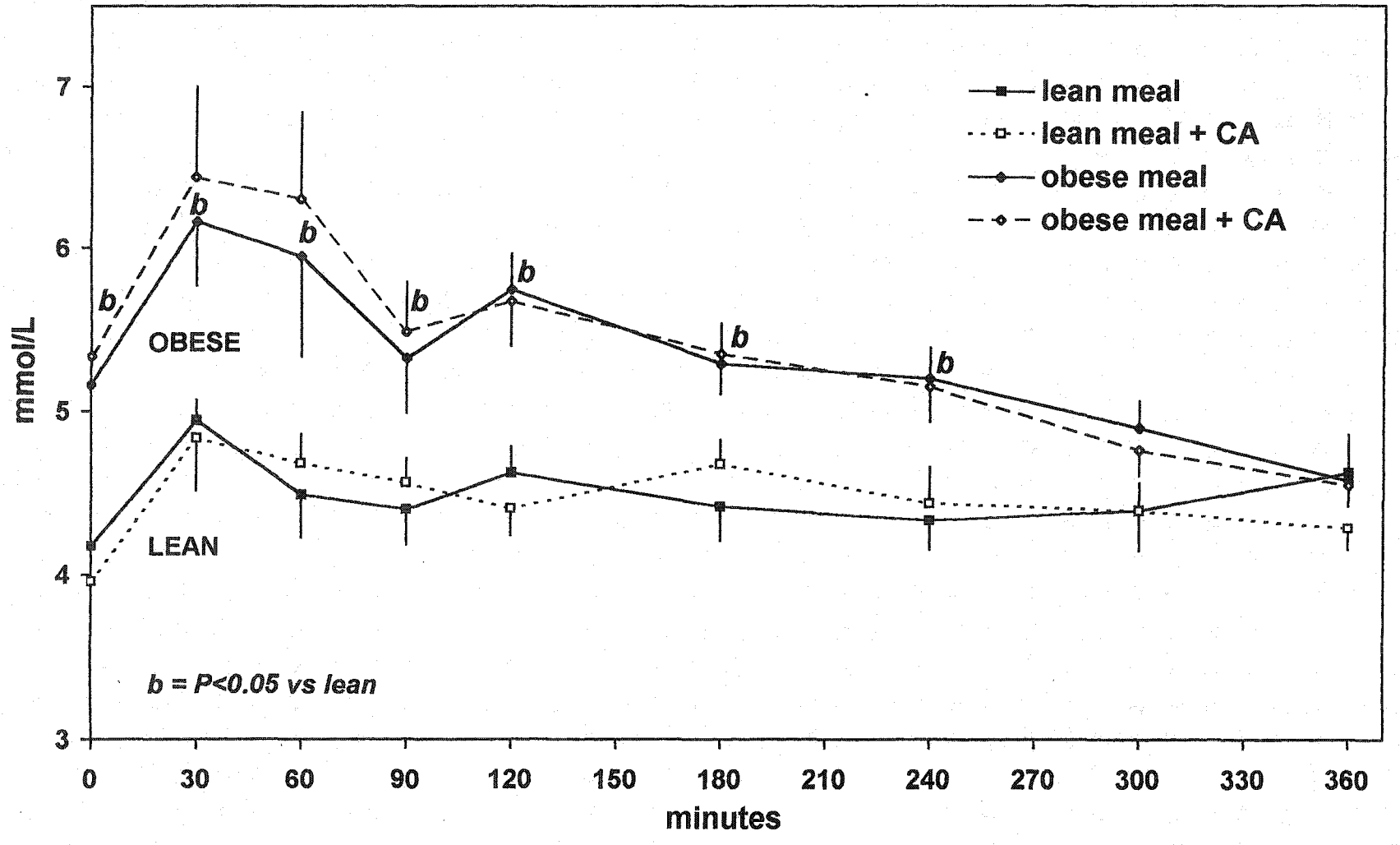


TABLE 1: Nutrient Composition of Mixed Meal

Energy (Kcal)	418
Protein (g)	58
Carbohydrate (g)	96
Fat (g)	44
Moisture (g)	3.976
Total Dietary Fibre (g)	0.0222
Cholesterol (g)	3.162
Saturated Fat (g)	3.13
Mono-unsaturated fat (g)	3.256
Poly-unsaturated fat (g)	0.378
Total omega-3 EFAs (g)	2.88
Total omega-6 EFAs (g)	2.88
Linoleic acid (g)	2.88
Potassium (g)	0.402
Sodium (g)	0.001
Calcium (g)	0.238
Phosphorus (g)	0.374
Vitamin A(IU)	216
Vitamin D(IU)	28
Vitamin E (IU)	0.352
Vitamin C (g)	0.194
Thiamine (g)	0.0072
Riboflavin (g)	0.0216
Niacin (g)	0.0124
Vitamin B6 (g)	0.00 0
Vitamin B12 (g)	0.1106
Folate (g)	0.04
Biotin (g)	0.004
Pantothenate (g)	0.0194
Iron (g)	0.552
Iodine (g)	0.0
Magnesium (g)	0.076
Copper (g)	0.0632
Zinc (g)	1.944
Manganese (g)	0.21

From Bariatrix International Inc., Montreal Quebec

TABLE 2 BASELINE ANTHROPOMETRIC DATA, BODY COMPOSITION & METABOLIC VARIABLES

	Lean	Obese	P-value
n	9 F 2 M 7	13 F 9 M 4	*<0.05*
Age (y)	21.7 ±0.8	40.9± 2.8	*<0.05*
Weight (kg)	64.2 ± 3.1	90.7 ± 3.1	*<0 .05*
BMI(Kg/m^2)	22.6 ± 0.6	34.8 ± 1.0	*< 0.05*
Waist (cm)	76.8± 2.4	104.8 ± 2.5	*< 0.05*
Hip	92.4 ± 1.0	119.8 ± 1.6	*< 0.05*
W:H	0.84 ± 0.03	0.88 ± 0.02	NS
RMR (kJ/d)	6284 ± 251	6657 ± 356	NS
RMR (kcal/d)	1502 ± 60	1591± 85	NS
Fat Free Mass (kg)	51.9 ± 3.0	52.6± 2.5	NS
Fat mass (kg)	12.3±1.0	38.1 ± 2.4	*<0.05*
% body fat	19.3 ± 1.6	41.9 ± 2.0	*<0.05*
Fasting plasma glucose (mmol/L)	4.7± 0.1	5.3± 0.3	*<0.05*
SH (Hormone) (0.4 - 4.4) mU/L	na	1.94 mU/L	
Pulse (beats/m)	62 ± 4	67 ± 4	NS
Blood pressure (mm Hg)			
Systolic	112 ± 4	124 ± 3	*<0.05*
Diastolic	77 ± 3	76 ± 3	NS

1. Study groups were compared using unpaired t –test
2. Difference in proportion between groups was determined by Chi Square

TABLE 3: PREDICTIVE EQUATIONS

Harris & Benedict (Harris & Benedict 1919) (F) REE* = 655+ (9.5 × weight) + (1.9 height) - (4.7 × age) (M) REE = 66 + (13.8 × weight)+ 5.0 × height) - (6.8 × age)
OWEN (Owen et al 1987) (F) REE= 795+ (7.18 × weight) (M) REE=879+ (10.2 × weight
MIFFLIN (Mifflin et al 1990) (F) REE= (9.99 × weight) + (6.25 × height) - (4.92 × age) –161 (M) REE= (9.99 × weight)+ (6.25 × height) - (4.92 × age) +5
WHO (WHO 1984) (F) REE= (8.7 × weight)+ 829{30-60y} (M) REE= (11.6 × weight)+ 879{30-60y}
JAMES (James WPT 1984) (F) REE = (8.17 × weight)+ 845 (M) REE = (11.6 × weight) +873
BERNSTEIN (Bernstein et al 1983) (F) REE = (7.48 × weight) - (0.42×height)-(3.0×age)+844 (M) REE = (11.0× weight) +(10.2×height)-(5.8×age)-1032

*REE = Resting energy expenditure in kilocalories

TABLE 4 a : MEASURED AND PREDICTED RESTING METABOLIC RATE (kcal/day)

	Obese		Lean	
Variable	Men	Women	Men	Women
Measured RMR Pre meal	1886±123	1460±78	1545± 68	1349 ±46
Measured RMR Pre Meal +CA	1884±116	1453± 81	1577 ±73	1343± 27
HB	2051± 32	1576± 47*	1681± 58*	1407 ±4
OWEN	1869 ±18	1426± 29	1558± 36	1197 ±7
MIFFLIN	1894± 30	1476± 57	1619± 49	1331± 12
WHO	2005± 21	1594± 36*	1651± 40*	1316± 9
JAMES	1999± 21	1563± 33	1645± 40*	1303± 8
BERNSTEIN	1605± 43	1300± 33*	1299± 63*	1134± 2

* $p < 0.05$ versus measured RMR

TABLE 4 b: Percent difference between measured RMR and predicted RMR

	Measured	HB	Owen	Mifflin	WHO	James	Bernstein
Obese Men							
Pre Meal	1886±123	10.2± 6.2	0.4±7.0	1.5±5.8	7.7±7.5	7.4±7.5	-14.0±4.3*
Pre Meal+CA	1884±116	9.9±5.6	0.4±6.3	1.5±5.3	7.6±6.8	7.3±6.8	-14.1±3.9*
Lean Men							
Pre Meal	1545±68	9.1±2.3*	1.5±2.8	5.3±2.5	7.5±2.8*	7.1±2.8*	-15.9±2.2*
Pre Meal+CA	1577±73	7.0±2.2*	- 0.5±2.8	3.2±2.5	5.4±2.8	5.0±2.8	-17.6±2.1*
Obese Women							
Pre Meal	1460±78	9.1±2.9*	-0.8±3.5	1.8±1.9	10.7±3.8*	8.7±3.8	-9.8±2.8*
Pre Meal+CA	1453±81	9.8±3.1*	-0.3±3.7	2.4±2.2	11.4±4.0*	9.3±4.0*	-9.3±3.0*
Women							
Pre Meal	1349±46	4.4±3.3	-11.1±3.6	-1.2±2.5	-2.3±4.0	-3.3±3.9	-15.9±3.0
Pre Meal+CA	1343±27	4.8±2.4	-10.8±1.3	- 0.8±2.9	-2.0±1.3	-3.0±1.3	-15.6±1.5

* = $p < 0.05$ versus 0% difference from measured RMR

Table 5a **Pearson correlation coefficients between RMR and age; anthropometric and body composition variables**

Variables	All		In 11 women		In 11 men	
	r	p	R	p	r	p
Age (yrs)	-0.232	0.298	-0.089	0.795	0.326	0.328
Weight (kg)	0.625	0.002	0.745	0.008	0.807	0.003
Height (cm)	0.638	0.001	0.484	0.131	0.563	0.071
Waist circumference (cm)	0.466	0.029	0.593	0.054	0.667	0.025
Hip circumference (cm)	0.177	0.431	0.315	0.345	0.686	0.020
Waist-hip ratio	0.602	0.003	0.702	0.016	0.366	0.268
Fat free mass (kg)	0.818	<0.001	0.807	0.003	0.770	0.006
Fat mass (kg)	0.225	0.313	0.653	0.029	0.600	0.051
% body fat	-0.003	0.989	0.438	0.178	0.484	0.132
BMI (kg/m^2)	0.231	0.301	0.457	0.157	0.691	0.019
Fasting plasma glucose (mmol/L)	0.317	0.215	0.814	0.014	0.708	0.033
Gender	0.452	0.034	na	na	na	na

Table 5b **Stepwise multiple regression coefficients (RC) for resting metabolic rate (RMR) (Kcal/day)**

All subjects			
Variables	RC	Beta	P
Intercept	$141.6_{\pm}196.5$		0.480
Fat free mass (kg)	$20.0_{\pm}4.0$	0.678	<0.001
Weight (kg)	$4.6_{\pm}2.1$	0.298	0.040
Adjusted $R^2 = 0.708$			

Excluded independent factors: gender, weight, height, waist circumference, waist-hip ratio and fat free mass

Table 5c

11 women			
Variables	RC	Beta	P
Intercept	$-241.9_{\pm}411.9$		0.571
Fat free mass (kg)	$35.9_{\pm}8.7$	0.807	0.003
Adjusted $R^2 = 0.613$			

Excluded independent factors: weight, waist-hip ratio, fat free mass, fat mass and fasting plasma glucose

Table 5d

11 men			
Variables	RC	Beta	P
Intercept	$719.9_{\pm}236.8$		0.014
Weight (kg)	$12.2_{\pm}3.0$	0.807	0.003
Adjusted $R^2 = 0.612$			

Excluded independent factors: weight, waist circumference, hip circumference, fat free mass, BMI and fasting plasma glucose

TABLE 6: Stepwise multiple regression analyses in 22 subjects.

A. dependent variable: % above RMR			
Variables	RC	Beta	P
Intercept	$22.2_{\pm}2.7$		<0.001
Weight	$-0.11_{\pm}0.03$	-0.593	0.004
Adjusted $R^2 = 0.319$			

Excluded independent factors: BMI, waist circumference, hip circumference, fat mass, % body fat, RMR, gender and fasting plasma glucose

B. dependent variable: % of the energy content of meal			
Variables	RC	Beta	P
Intercept	$8.1_{\pm}0.5$		<0.001
Gender	$3.0_{\pm}0.7$	0.679	0.001
Adjusted $R^2 = 0.433$			

Excluded independent factors: height, BMI, hip circumference, fat free mass, fat mass and % body fat.

C. dependent variable: kJ/280 minutes			
Variables	RC	Beta	P
Intercept	$142.2_{\pm}9.1$		<0.001
Gender	$49.9_{\pm}12.8$	0.657	0.001
Adjusted $R^2 = 0.403$			

Excluded independent factors: height, hip circumference, fat mass and % body fat.

TABLE 7: THERMIC RESPONSE TO MIXED MEAL

	RMR kcal/d	% above RMR	% of meal	kcal / 280 minutes
Men meal only	1669 ± 78	14.4 ± 1	11.1 ± 0.6	192.1 ± 11.5
Women meal only	1439 ± 65	12.5 ± 0.8 #	8.1 ± 0.3#	3142.2 ± 5.6#
Men meal + CA	1689 ± 75	14 .5 ± 1.2	11.3 ± 0.9	195.4 ± 14.7
Women meal + CA	1433 ± 67	16.4 ± 1.1*	10.7 ± 0.5*	187.5 ± 9.0*

* $p < 0.05$ versus without CA
# $p < 0.05$ versus men

TABLE 8: Hemodynamic Effects of Citrus Aurantium

	Systolic Blood pressure (mmHG)		Diastolic Blood pressure (mmHG)		Mean Arterial pressure (mmHG)		Heart Rate beats/minute	
	Meal	Meal + CA	Meal	Meal+CA	Meal	Meal+CA	Meal	Meal+CA
Obese								
Baseline	119±3*	124±3	76±2	76±3	90±2	92±3	66±4	67±4
1 hour	116±4	121±3	74±4	74±3	89±3	90±5	67±3	68±5
2 hour	120±3	120±3	76±3	79±3	92±2	93±3	70±4	69±5
3 hour	121±4	113±4	78±3	79±2	93±3	89±3	65±7	65±3
4 hour	116±4	121±3	76±3	74±3	89±3	90±5	70±4	68±5
5 hour	120±6	120±3	76±3	79±3	92±2	93±3	70±4	69±5

* All measurements are mean ± SEM

	Systolic Blood pressure (mmHG)		Diastolic Blood pressure (mmHG)		Mean Arterial pressure (mmHG)		Heart Rate beats/minute	
	Meal	Meal + CA	Meal	Meal+CA	Meal	Meal+CA	Meal	Meal+CA
Lean								
Baseline	122±5	111±4	83±4	77±3	97±4	88±3	65±5	61±4
1 hour	121±4	113±4	78±3	79±2	93±3	89±3	66±4	64±3
2 hour	120±6	115±6	83±3	81±3	98±3	92±3	63±3	62±3
3 hour	116±4	121±3	74±4	74±3	89±3	89±9	67±3	68±5
4 hour	121±4	113±4	78±3	79±2	93±3	89±3	65±8	64±3
5 hour	120±6	115±6	83±3	81±3	98±3	92±3	63±3	62±3

TABLE 9: Urinary catecholamines (nmol/h)

	Overnight Fasting		Post CA	
	Obese (1M, 8F)	Lean (6M, 3F)	Obese	Lean
Norepinephrine	7.6 ± 1.3*	6.1 ± 1.3	7.3 ± 0.6	6.5 ± 1.1
Epinephrine	0.6 ± 0.2	1.7 ± 0.5[a]	1.6 ± 0.4[b]	4.1 ± 0.6[ab]
Dopamine	76.5 ± 5.1	57.0 ± 5.8[a]	78.2 ± 5.1	80.4 ± 8.0[b]
	Men (n=7)	Women (n=11)	Men	Women
Norepinephrine	7.9 ± 1.7	6.2 ± 1.9	6.9 ± 1.3	6.9 ± 0.6
Epinephrine	1.9 ± 0.6	0.7 ± 0.2[a]	4.7 ± 0.5[b]	1.6± 0.3[ab]
Dopamine	58.8 ± 8.1	71.8 ± 4.8[a]	87.6 ± 9.2[b]	74.0 ± 4.5

* data reported as mean ± SEM

a $p < 0.05$ versus obese or men

b $p < 0.05$ versus fasting in same group