Obesity and dietary transition and their correlates with fatty acids and desaturases in three distinct populations

Yuan Zhou

School of Dietetics and Human Nutrition

McGill University, Montreal

November 2010

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctorate of Philosophy

ABSTRACT

Adipose tissue has long been considered to be inert fat mass, and only in recent years has its role as an active endocrine organ been recognized. Evidence from animal models suggests that adiposity along with insulin and diet could be an independent regulator of desaturases, which are keys in the biosynthesis of unsaturated fatty acids. Few epidemiological studies, however, have been performed in terms of the impact of adiposity on desaturases. Among the existing studies, there was no examination of whether the regulation would be independent of insulin action and dietary intake. The dietary transition among Canadian Indigenous Peoples and the consequent health transition, including the emergence of obesity, have been well documented; however, it is not known how dietary and metabolic changes might affect the status of highly unsaturated n-3 fatty acids (HUFA n-3) that are implicated to be protective against chronic disease risk.

The thesis work is composed of three studies.

Study 1: The fatty acid composition of fasting plasma from 178 apparently healthy female adolescents from a Montreal gestational diabetes cohort was analyzed. Independent of dietary saturated fatty acids (SFA) intake and the level of insulin resistance, adiposity was demonstrated to be positively predictive of Δ9. The activity of Δ9 was, in turn, positively correlated with fasting plasma triglycerides (TG) and apoliproprotein B (Apo B).

Study 2: The fatty acid composition of erythrocyte membranes from fasting blood was assessed from 168 Cree adults living in a single Cree community in northern Québec who were participating in a diabetes screening program. Inter-generational differences existed in terms of HUFA n-3 status. Adiposity was significantly but inversely associated with Δ5. The latter relationship was also observed among Cree with impaired fasting glucose, among whom insulin resistance was not a significant predictor of Δ5.

Study 3: The fatty acid composition of erythrocyte membranes from fasting blood was determined from 2200 Inuit adults from Nunatsiavut, Nunavut

and Inuvialuit Settlement Region (ISR) participating the International Polar Year Inuit Health Survey. Biochemical measures including of hemoglobin, serum ferritin and C-reactive protein (CRP) from 1528 Inuit adults were examined. Pronounced inter-generational and regional differences across Canadian Arctic regions regarding HUFA n-3 status were observed among Inuit. HUFA n-3 status was inversely related to SFA and TFA status. Additionally, HUFA n-3 status was associated with the presence of iron deficiency (ID) among Inuit; however, only a weak correlation was demonstrated indicating the need for confirmatory studies.

In summary, this thesis work involving both female adolescents and the Cree and Inuit adult population has indicated that adiposity plays a more direct role in regulating fatty acid metabolism than previously realized via the demonstration of strong independent associations of adiposity with Δ5 and Δ9 desaturase activities. In terms of the Canadian indigenous peoples, the adverse effects on n-3 fatty acid metabolism mediated by relatively high prevalence of obesity could exacerbate chronic disease risk along with the diminishing consumption of HUFA n-3 rich traditional foods.

RÉSUMÉ

Le tissu adipeux a longtemps été considéré comme étant une masse graisseuse inerte. Cependant, les recherches des dernières années ont permis de reconnaître son rôle en tant qu'organe endocrinien actif. Les modèles animaux ont démontré que l'adiposité, en plus de l'insuline et de facteurs alimentaires, pourrait être un régulateur de désaturases qui sont essentielles dans la biosynthèse d'acides gras insaturés. Cependant, peu d'études épidémiologiques se sont penchées sur l'impact qu'aurait l'adiposité sur les désaturases. Parmi les études existantes, aucune n'a examiné si ce contrôle pouvait être indépendant de l'action de l'insuline et de celle de l'apport alimentaire. La transition nutritionnelle que vivent présentement les peuples autochtones du Canada de même que les conséquences sur la santé qui leur sont associées, telles que l'apparition de l'obésité, sont bien documentées. Néanmoins, il reste à déterminer comment de tels changements alimentaires et métaboliques peuvent avoir un impact sur le statut en acides gras oméga-3 hautement insaturés (AGHI n-3) qui ont un rôle protecteur face au risque de maladies chroniques.

Cette thèse comprend trois études.

Étude 1 : La composition en acides gras du plasma prélevé à jeun chez 178 adolescentes en apparence en santé faisant partie d'une cohorte montréalaise sur le diabète de grossesse a été analysée. Indépendamment de l'apport alimentaire en AGS et de l'importance de la résistance à l'insuline, l'adiposité a semblé être positivement prédictive de la Δ9. L'activité de la Δ9 était, quant à elle, positivement corrélée avec les statuts en triglycérides (TG) et en apolipoprotéine B (apo B) à jeun.

Étude 2 : La composition en acides gras des membranes érythrocytaires de sang prélevé à jeun chez 168 adultes Cris vivant dans une communauté du nord du Québec et participant à un programme de dépistage du diabète a été analysée. Des différences intergénérationnelles ont été observées quant au statut en AGHI n-3. L'adiposité était significativement mais inversement associée avec la Δ5

chez les Cris. Cette relation a aussi été observée chez les Cris ayant une glycémie anormale à jeun chez qui la résistance à l'insuline n'était pas un prédicteur significatif de Δ5.

Étude 3 : La composition en acides gras des membranes érythrocytaires de sang prélevé à jeun chez 2200 adultes Inuit du Nunatsiavut, du Nunavut et de la région de l'Inuvialuit participant à l'étude «International Polar Year Inuit Health Survey» a été déterminée. Les mesures biochimiques de l'hémoglobine, de la ferritine sérique et de la protéine C réactive chez 1528 adultes Inuit ont été examinées. Des différences intergénérationnelles et régionales importantes ont été observées pour le statut en AGHI n-3 des Inuit à travers les différentes régions arctiques canadiennes. Le statut en AGHI n-3 était inversement relié aux statuts en AGS et en acides gras trans. De plus, le statut en AGHI n-3 était associé à la présence d'une déficience en fer. Cependant, seule une faible corrélation a été observée ce qui indique un besoin pour davantage d'études sur le sujet.

En résumé, cette thèse qui porte sur des adolescentes montréalaises ainsi que sur des adultes Cris et Inuit a démontré que l'adiposité joue un rôle plus direct dans le contrôle du métabolisme des acides gras comparé à ce qui a été observé précédemment via la démonstration d'importantes associations indépendantes entre l'adiposité et les activités des Δ5 et Δ9. En ce qui concerne les populations autochtones canadiennes, l'effet opposé sur le métabolisme des acides gras n-3, influencé par la prévalence relativement élevée de l'obésité, pourrait exacerber le risque de maladies chroniques en même temps que la diminution de la consommation d'aliments traditionnels riches en AGHI n-3.

ADVANCE OF SCHOLARLY KNOWLEDGE

The current thesis work has provided evidence for the first time supporting the regulation of energy homeostasis as mediated by the Δ9-leptin pathway in the human context. The independent relationships of Δ9 with adiposity and certain key metabolic measures in this postulated pathway as demonstrated by the present thesis work were previously only reported in rodent models. Based on this hypothesis, adipose tissue would regulate its own mass by directing hepatic lipids towards either beta-oxidation or storage in adipose tissue. In this latter proposed leptin-modulated pathway, Δ9 would be a critical mediator of hepatic lipids as it would facilitate hepatic synthesis of VLDL in order to transport hepatic lipids to extra-hepatic tissues. The present thesis thus provides evidence that implicates Δ9-leptin pathway in the regulation of energy balance and energy expenditure in humans in addition to the well-established pathway of leptin-modulated energy homeostasis mediated via the central nervous system. Another implication of the above thesis findings is that adipose as an active endocrine organ, could be much more directly involved in regulating fatty acid metabolism than previously considered. The latter concept is further supported by the thesis findings that showed that adiposity is predictive of Δ5 independent of insulin, a well-recognized hormonal regulator of fatty acid metabolism.

The present thesis work provided a novel approach to adjust for dietary saturated fatty acid intake as saturated fat intake has only been previously assessed using dietary questionnaires. The thesis approach utilized the fact that odd-number carbon atom fatty acids are derived solely from dietary saturated fat intake. Thus, tissue levels of the exogenous C15 fatty acid can be used as an indicator of dietary saturated fat intake, which is a more precise alternative approach to estimate dietary saturated fat intake as opposed to questionnaire data.

The present thesis work provided new insights regarding the health transition that Canadian indigenous peoples are experiencing in terms of rising prevalence of obesity and other lifestyle related diseases including T2D. The current thesis work explored whether obesity and dietary transition are associated

with tissue fatty acid profiles, particularly in relation to HUFA n-3 status and Δ5 activity. Based on data from a Cree community, adiposity was inversely related to Δ5 activity. This latter finding, along with reducing HUFA n-3 consumption observed among the Cree youth, suggested that obesity and reduced consumption of n-3 rich traditional foods are leading to decreased HUFA n-3 status among the younger generations of Cree. The implication of the above findings is that adverse dietary and lifestyle transitions occurring among Canadian Cree pose a chronic disease risk.

The current thesis work uniquely explored whether regional differences in HUFA n-3 status existed across Canadian Arctic Inuit residing in a vast territory ranging from the Eastern to the Western Arctic coasts of Canada. The thesis explored regional differences regarding HUFA n-3 status since Inuit populations inhabiting different regions likely experience distinct physical and social environments, which may contribute to pronounced differences in their HUFA n-3 consumption and tissue concentrations. This above hypothesis was supported by the present thesis observations showing inter-generational differences of HUFA n-3 status that varied greatly depending on the arctic region. The latter finding also supported the suggestion that Inuit communities from arctic and sub-arctic regions are not experiencing an equal degree of social-economic-environment transition. The current thesis also explored inter-regional and inter-generational differences in terms of dietary patterns via the use of erythrocyte membrane fatty acid indicators. In addition, the present thesis work examined the presence of inverse associations of HUFA n-3 status with SFA and TFA status among a large scale of indigenous population based on a previous pilot study. The overall implication of the above findings is that nutritious Inuit foods appear to be replaced by lower-quality market foods. Thus, a multiple approach strategy is likely needed to maintain and improve HUFA n-3 status among Canadian Arctic and Sub-arctic indigenous peoples. The current thesis work explored the relationship between HUFA n-3 and iron deficient status for the first time in a relatively large population size showing only a weak positive association, which needs verification in populations with a greater extent of iron deficiency.

CONTRIBUTION OF AUTHORS TO MANUSCRIPTS

Manuscript 1: The association of desaturase 9 and plasma fatty acid composition with insulin resistance-associated factors in female adolescents

Yuan E. Zhou, the candidate, analyzed the data and drafted the manuscript.

Dr. Grace M. Egeland, thesis supervisor of the candidate, designed the study and supervised the data analysis, and contributed to the revision of the manuscript.

Dr. Sara J. Meltzer, a medical doctor and researcher from Division of Endocrinology and Metabolism, Royal Victoria Hospital, Montreal, Canada, designed the study and also contributed to the revision of the manuscript.

Dr. Stan Kubow, thesis supervisor of the candidate, supervised the manuscript drafting.

Ms. Sina Gallo, a Master Candidate from the School of Dietetics and Human Nutrition, McGill University, and Ms. Donna Leggee, a researcher from Center for Indigenous Peoples' Nutrition and Environment, McGill University, carried out the plasma fatty acid analysis.

Manuscript 2: Decreased activity of desaturase 5 in association with obesity and insulin resistance aggravates declining long-chain n-3 fatty acid status in Cree undergoing dietary transition

Yuan E. Zhou, the candidate, analyzed the data and drafted the manuscript.

Dr. Stan Kubow, thesis supervisor of the candidate, supervised the manuscript drafting.

Dr. Grace M. Egeland, thesis supervisor of the candidate, designed the study, supervised the data analysis and contributed to the revision of the manuscript.

Dr. Eric Dewaily, a researcher from Unité de Recherche en Santé Publique, Centre de Recherche du CHUQ, Universite Laval, Canada, designed the study and also contributed to the revision of the manuscript.

Dr. Pierre Julien, a researcher from Unité de Recherche en Santé Publique, Centre de Recherche du CHUQ, Universite Laval, Canada, analyzed the fatty acid composition of erythrocyte membranes, and also contributed to the revision of the manuscript.

Manuscript 3: Highly unsaturated n-3 fatty acids status of Canadian Inuit: International Polar Year Inuit Health Survey, 2007-2008

Yuan E. Zhou, the candidate, analyzed the data and drafted the manuscript.

Dr. Stan Kubow, thesis supervisor of the candidate, supervised the manuscript drafting.

Dr. Grace M. Egeland, thesis supervisor of the candidate, designed the study, supervised the data analysis and manuscript drafting, and contributed to the revision of the manuscript.

Lipid Analytical Laboratories Inc in Guelph, Canada analyzed the fatty acid composition of erythrocyte membranes.

Manuscript 4: Is iron status associated with highly unsaturated fatty acid status among Canadian Arctic Inuit?

Yuan E. Zhou, the candidate, analyzed the data and drafted the manuscript.

Dr. Stan Kubow, thesis supervisor of the candidate, supervised the manuscript drafting.

Dr. Grace M. Egeland, thesis supervisor of the candidate, designed the study, supervised the data analysis and manuscript drafting, and contributed to the revision of the manuscript.

Lipid Analytical Laboratories Inc in Guelph, Canada analyzed the fatty acid composition of erythrocyte membranes.

Jennifer A. Jamieson, a Ph.D. candidate from School of Dietetics and Human Nutrition and the Centre for Indigenous People's Nutrition and Environment, McGill University, carried out the serum ferritin analysis.

AKNOWLEDGEMENTS

I would like to thank all the people who, throughout my time at McGill, contributed towards making my PhD thesis work a wonderful experience.

First of all, I would like to thank my co-supervisor, Dr. Grace M. Egeland, for her incredible support and encouragement throughout my study, and for her giving me the "once in a lifetime" opportunity to work on a PhD program.

I would also like to express my gratitude to my co-supervisor Dr. Stan Kubow who has patiently guided me during my study at McGill, and has spared no efforts to help me and support me on every step towards the completion of my PhD work.

I am thankful to Dr. Sarah J. Meltzer and Dr. Eric Dewaily for their kind help during my PhD project paper drafting, and my appreciation to Dr. Kristine G. Koski for her guidance on my PhD dissertation preparation.

Dr. Katherine Gray-Donald deserves special mention for generously tutoring me and sharing with me her rich research experience in nutrition epidemiology. Ms. Lise Grant also deserves my deep appreciation. Without her great help and assistance during my years of study at McGill, my journey towards a PhD would be even tougher and longer. I am also thankful to the help from Francine Tardif and Elizabeth Ansell during my study at McGill. Catherine Huet very kindly offered French translation for the abstract of my dissertation. Natalia Zienczuk very generously offered her precious time for the final proofreading of my dissertation.

I would like to acknowledge the contribution of all the people who participated in the Montreal Gestational Diabetes Mellitus (GDM) cohort, Health Survey in the Cree community of Mistissini, and International Polar Year Inuit Health Survey.

I would like to thank all the staff who worked in these studies for their dedication, and I am proud to have been one member of the GDM study team, Nituuchischaayihitaau Aschii (Cree) study group, and Inuit Health Survey research team: Donna, Helga, Nelofar, Louise, Renata, Guylaine, Claire, Maria, Yella, Zhilong. I would also like to thank the Cree Nation of Mistissini and the Cree Board of Health and Social Services of James Bay, and Inuvialuit, Nunavut and Nunatsiavut IPY Inuit Health Survey Steering Committees for their permission to conduct the studies.

I am very grateful to my fellow students and my friends at McGill, and cherish a great supporting environment during my study in McGill, particularly during stressful times.

Last but not least, I would like to thank my family and friends. I am truly appreciated for what my parents, Yongning Wang and Liantong Zhou, and my brother Kai Zhou have given to my life. Special thanks to my friends G.P. Schmid for being my mentor throughout this learning process, and W. Hao for offering me encouragement and guidance upon important decisions, and C. Edwards whose friendship made my life during my years at Montreal so much more enjoyable.

In many ways, you have all contributed to my learning and I appreciate and treasure all the talents and gifts that you have had to share.

DEDICATION

This thesis is dedicated with the deepest gratitude to my dear mother, Yongning Wang.

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ALA	Alpha linoleic acid
Apo B	Apolipoprotein B
CINE	Centre for Indigenous Peoples' Nutrition and Environment
CRP	C-reactive protein
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DXA	Dual energy X ray scanning
EPA	Eicosapentaenoic acid
ETA	Eicosatrienoic acid
GDM	Gestational diabetes mellitus
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assessment-insulin resistance
HUFA	Highly unsaturated fatty acids
ID	Iron deficiency
INAC	Indian and Northern Affair Canada
ISR	Inuvialuit Settlement Region
LA	Linoleic acid
LDL	Low-density lipoprotein
MOND	McGill Obstetrics and Neonatal Database
MUFA	Monounsaturated fatty acids
NEFA	Non-esterified fatty acids
NHANES	National Health and Nutrition Examination Survey
PPAR	Peroxisome proliferator-activated receptor
PUFA	Polyunsaturated fatty acids

SCD	Stearoyl CoA Desaturase
SFA	Saturated fatty acids
SREBP-c	Sterol regulatory element binding protein-1c
T2D	Type 2 diabetes mellitus
TFA	*trans* fatty acids
TG	Triglycerides
VLDL	Very low-density lipoprotein
Δ5	Desaturase 5
Δ6	Desaturase 6
Δ9	Desaturase 9

CHAPTER 1. INTRODUCTION

1.1. Background and rationale

Fat metabolism is under the control of both genetic and environmental factors: consumption, assimilation and absorption in the gastrointestinal tract; β-oxidation, synthesis, elongation and desaturation in tissues; redistribution in tissues and incorporation into the bio-membrane. A simplified model is to regard the fatty acid composition in blood circulation, cells and body tissues as reflecting dietary intake and the modulation by regulators in the metabolic pathway of fatty acids.

In the biosynthesis pathway of unsaturated fatty acids, there are three important desaturation reactions (Shils, et al., 2006). Desaturase 9 (Δ9) converts saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA) by introducing a double bond on their Δ9 site. MUFA are the substrates of a diversity of lipids: triglycerides (TG), phospholipids, cholesterol and ceramide, etc. The most important Δ9 product is oleic acid, which is a ubiquitous component of all bio-membranes. Desaturase 6 (Δ6) is the key enzyme whereby the formation of longer and more highly unsaturated fatty acids (HUFA) from two essential fatty acids LA and ALA begins. Desaturase 5 (Δ5) is another rate limiting enzyme in the biosynthesis of HUFA. Δ9, Δ6 and Δ5 can modulate fatty acid biosynthesis and thereby exert an impact on the blood and tissue fatty acid patterns. The long chain HUFA, as the primary products of Δ6 and Δ5, are incorporated into bio-membranes to play important roles in maintaining bio-membrane function and signaling transduction.

The activity of desaturases has usually been estimated by the fatty acid composition of body tissues or blood. Direct measurement of desaturase activity is feasible via *in vitro* studies and animal experiments, but is not generally feasible in the human context. Therefore, desaturase activities are typically calculated as the ratios of parent fatty acids to the daughter fatty acids in epidemiological studies and clinical trials. Fatty acid profiles of plasma, plasma

phospholipids or erythrocyte membranes are the most frequent approaches used in population-based research due to their sampling convenience relative to other body tissues such as adipose tissue (Baylin, et al., 2005). Plasma or serum fatty acid profiles can be obtained using straightforward laboratory analysis procedures. Erythrocytes have a life span of about 120 days and thus fatty acid profiles of erythrocyte membranes are largely unaffected by recent dietary fat influx. As erythrocytes cannot synthesize fatty acids, the fatty acid composition of plasma, serum and erythrocytes reflects consumption of exogenously derived dietary fatty acids, such as long chain n-3 fatty acids, trans fatty acids (TFA) and odd-numbered carbon fatty acids (Willett, 1998), with the caveat that these profiles are also modulated by endogenous biosynthesis.

Dietary regulation of the activity of desaturases has been examined in previous studies (Nakamura & Nara, 2004b). In terms of nutritional regulators, polyunsaturated fatty acids (PUFA) have been intensively studied and the influence of other dietary components such as carbohydrates and cholesterol has also been addressed (Ntambi, 1992). PUFA are the main dietary components that regulate all three desaturases. Known for their inhibition of lipogenesis, PUFA suppress the expression of enzymes needed in this pathway including Δ9, Δ6 and Δ5 via regulation of corresponding gene transcription factors. In contrast to PUFA intake, high intake of carbohydrates has been well demonstrated to exert lipogenic effects (Miyazaki, et al., 2001)(Mangravite, et al., 2007). As the primary option for fuel energy, carbohydrate exerts fat sparing effects - as excessively consumed carbohydrate is stored as triglyceride (TG) via *de novo* synthesis.

Hormonal regulation of desaturase activities, particularly via insulin, has been studied intensively (Brenner, 2003)(Vessby, et al., 2002). As an anabolic hormone, insulin regulates the absorption, transportation, utilization and endogenous metabolism of all the three macronutrients. The activities of desaturase Δ9, Δ6 and Δ5 have all been closely related to insulin action as positive associations were reported between insulin resistance indices and the activities of Δ9 and Δ6, while a negative association was reported between Δ5 and insulin

resistance (Brenner, 2003)(Vessby, et al., 2002). In that regard, the above associations could account partly for the disturbed lipid metabolism that has been acknowledged as a fundamental component of the insulin resistance syndrome. Dyslipidemia is detectable even before the appearance of fasting or postprandial hyperglycemia, suggesting that the disturbed lipid metabolism occurs long before type 2 diabetes (T2D) mellitus fully develops (Lewis, et al., 2002).

Evidence has recently emerged from experimental studies suggesting that there may be other key physiological regulators of desaturase such as adiposity. The regulation of Δ9 activity by the level of adiposity has been robustly established in rodent models via leptin as a key regulatory agent (Cohen, et al., 2002). Leptin is an adipose tissue derived hormone whose diverse physiological functions include regulating energy intake and energy regulation. In mouse models, the *SCD-1* gene has been ranked at the top of the list of genes uniquely repressed by leptin - as shown in mouse models with the gene mutation or knockout of the leptin (Cohen, et al., 2002)(Cohen & Friedman, 2004). The metabolic consequences of disturbed function of Δ9 were clearly demonstrated in *SCD-1* knockout mice including abnormalities in biosynthesis of hepatic TG and cholesterol esters, indicating that Δ9 could greatly influence lipid metabolism (Miyazaki, et al., 2000). Available information regarding the effects of adiposity on Δ6 and Δ5, however, has been limited. In that regard, decreased activity of Δ6 and Δ5 has been reported in hepatic microsomes from obese rats versus lean rats (Blond, et al., 1989). Similar observations were reported regarding differences in desaturase activity as assessed by the fatty acid ratios measured in hepatic lipids in the comparison of obese versus lean rats (Blond, et al., 1989)(Cunnane, et al., 1985).

Conclusions from epidemiological studies support the relationship between adiposity and desaturase activity indicated from *in vitro* and *in vivo* animal experiments. Strong positive associations were demonstrated between the level of adiposity and Δ9 activity among elderly people (Warensjö, et al., 2005), middle-aged populations (Warensjö, et al., 2006) and children (Okada, et al.,

2005). In concert with *in vitro* experiments and animal studies, epidemiology studies have demonstrated higher levels of Δ6 and lower levels of Δ5 with increased adiposity among Pima Indians (Pan, et al., 1995) and Caucasians (Warensjö, et al., 2005)(Warensjö, et al., 2006)(Warensjö, et al., 2007).

Previous human studies, however, have had important limitations that have limited interpretation of the above relationships. Firstly, it has not been clear whether the associations of desaturase activities with the level of adiposity were independent of the degree of insulin resistance. Since insulin resistance level is a well-recognized important modulatory factor on desaturase activities, the potential confounding effects of insulin resistance should be taken into careful consideration. Secondly, none of the previous human studies considered the impact of dietary fatty acid intake on the activity of the desaturases. For example, the level of dietary saturated fat intake could greatly affect the fatty acid ratios (palmitoleic acid/palmitic acid and oleic acid/stearic acid) indices of Δ9 activity, which could bias the observed associations with Δ9.

In contrast with the generally well-elaborated interplay between the metabolic pathways of fatty acids and macronutrient intakes, there has been an evident lack of information regarding the relationship between iron intake and fatty acid metabolism. In previous animal studies, rats fed iron deficient diets showed decreased activities of Δ9 (Rao, et al., 1980)(Rao, et al., 1983)(Hirosue & Hosogai, 1993), Δ6 and/or Δ5 (Hirosue & Hosogai, 1993)(Tichelaar, et al., 1997). To our knowledge, only two human studies to date have reported associations between iron status and the activity of desaturases (Smuts, et al., 1994)(Krajcovicova-Kudlackova, et al., 2004). The human studies have implied a negative association between desaturase activity and iron status, however study design limitations, namely small sample size and lack of adjustment for possible confounding effects, have limited their conclusions.

Obesity has been well recognized to increase the risk of a variety of lifestyle related adverse health consequences such as insulin resistance,

hypertension, dyslipidemia, overt T2D, cardiovascular disease and certain cancers leading to a reduced life expectancy. The mechanisms involved during the development of obesity that could increase the risk of chronic diseases are still unclear. Recent evidence has implicated adipose tissue as a highly active endocrine organ that releases not only non-esterified fatty acids (NEFA), but also a large family of cytokines, adipokines and hormones (Ronti, et al., 2006). Thus, it is increasingly evident that there may be multiple obesity-mediated pathways that contribute to metabolic abnormalities associated with obesity. Evidence from *in vitro* experiments and animal studies regarding the impact of adiposity on the activity of desaturases has suggested that adiposity could play an important role in the regulation of fatty acid metabolism. In particular, increased adiposity could disturb the function of desaturases, leading to abnormal bio-membrane structure and function that could have an impact on disease risk. As the fatty acid products of Δ9, Δ6 and Δ5 have distinct physiological roles, it is conceivable that their regulation involves different dietary modulators.

A public health concern associated with obesity, which is increasingly prevalent in the general Canadian population, is gestational diabetes mellitus (GDM). Women with previous GDM usually have a higher level of adiposity prior to, during and after pregnancy, which partly explains their higher incidence of T2D in the postpartum period. Their offspring are also suggested to be susceptible to T2D and their female offspring are at elevated risk of developing GDM. The reasons for the elevated disease risk among the offspring are unclear but could be due to intrauterine exposure to an abnormal metabolic milieu, shared life style with their mothers, or a combination of the above. During adolescence, however, it is possible that the offspring of GDM mothers may not yet experience substantial metabolic consequences from the GDM pregnancy. This latter explanation could account partly for the non-significant differences observed between female offspring of GDM mothers and non-GDM mothers regarding fatty acid composition of fasting plasma and Δ9 activity.

Unfortunately, obesity has also become a serious public health problem among Canadian indigenous people in the last few decades (Young & Sevenhuysen, 1989)(Young, et al., 2007). The health transition, including increased prevalence of obesity, started since early contact with Western culture. For Inuit, dietary transition and other acculturation changes were noticeable following WWII when Canadian indigenous people moved from nomadic camps to permanent settlements given the requirement for government to provide better health care and education. The social, economic, and environmental changes had an impact on every aspect of daily living of Inuit, including causing altered dietary patterns and reduced physical activity. Dietary transition away from traditional foods (animals or plants harvested locally) rich in a variety of nutrients to market foods (shipped from the south) that were high in refined carbohydrates, fat and saturated fat has been well documented. The younger generations are thus suggested to be more vulnerable to dietary transition, whereby their traditional diets are being replaced by low quality market foods (Kuhnlein, et al., 2004). Only fragmented information is available regarding dietary and lifestyle patterns of Canadian native peoples in terms of the individual regions that are scattered over the vast Canadian Arctic territory. The native populations, as a group, are distinct from the general population living in southern Canada in terms of lifestyle. The Canadian Arctic native populations, however, are not likely homogenous given the widely different natural and social environments and individual community histories. Therefore, tissue fatty acid status, particularly HUFA n-3, might be different among indigenous peoples depending on region and life stage as affected by dietary transition. It is conceivable that dietary transition together with an increased prevalence of obesity among native populations could work in tandem to exacerbate health consequences, including disturbed n-3 fatty acid status.

1.2. Objectives

The thesis objectives were:

1. To determine the influence of adiposity on endogenous fatty acid metabolism through the examination of ratios of specific plasma or erythrocyte membrane fatty acids from: (1) female adolescents in the Montreal gestational diabetes cohort study; (2) Cree adults from the Cree health screening program "Nituuchischaayihitaau Aschii: A Multi-Community Environment-and-Health Longitudinal Study in Iiyiyiu Aschii"; and (3) Inuit adults from the International Polar Year Inuit Health Survey.
2. To evaluate the impact of the level of insulin resistance and/or dietary fat intake on the relationship between adiposity and endogenous fatty acid synthesis assessed in the Montreal gestational diabetes adolescent cohort and adults in the Cree health screening program.
3. To explore in Inuit adults the associations of iron status with the endogenous HUFA metabolism as evaluated via estimates of Δ5 activity
4. To explore the impact of dietary transition on fatty acid status among Cree and Inuit.
5. To compare regional differences regarding HUFA fatty acid status across Canadian Arctic Inuit regions.

1.3. Hypothesis

The overall null hypothesis was that there would be no independent significant associations of measures of desaturase activities for Δ9, Δ6 and Δ5 with adiposity and iron status. Further, an additional null hypothesis was that there would be no age or regional differences in fatty acid status among Cree and Inuit.

CHAPTER 2. LITERATURE REVIEW

Fatty acid desaturases introduce a double bond into the carbon chain of fatty acids. The three desaturases present in human tissues are Δ9, Δ6 and Δ5. Direct measurement of their activities is difficult in human context, which is instead estimated by the ratios of product fatty acids to their precursor fatty acids, The activities of desaturases influence a variety of physiological functions. One fundamental role of desaturases is homeostatic maintainance of unsaturation of tissue fatty acids. Fatty acids form the backbone of bio-membranes that are made of a bilayer of phospholipids. The unsaturation level of fatty acids in bio-membranes is critical in terms of maintaining the physical properties of biological membranes including membrane fluidity and membrane physiological functions. MUFA, the products of Δ9, are favored substrates of phospholipids, cholesteryl ester and TG stored in adipose tissue (Nakamura & Nara, 2004b). HUFA, the products of Δ6 and Δ5, are essential for various cellular functions, such as eicosanoid signaling (Funk, 2001), ion channel modulation (Harris, et al., 2008), and regulation of gene expression (Nakamura, et al., 2004a). The overlapping function of the three desaturases involves: (a) maintaining a homeostatic level of unsaturation in membrane phospholipids; and (b) generating specific fatty acid products that have distinct physiological roles. It is believed that the latter aspect may account for the opposing associations with insulin resistance observed with the different types of desaturases.

2.1. Desaturases characteristic features

All human desaturases are present in endoplasmic reticulum and work on fatty acyl-CoAs as substrates (Nakamura & Nara, 2004b). The activity of desaturases requires a variety of enzymes, coenzymes and co-factors to work in concert with each other including NADH, coenzyme A, oxygen and the electron transport system (ferredoxin-NADPH reductase and ferredoxin, cytochrome

reductase, cytochrome b). The fatty acid desaturase structure contains nonheme iron, which is utilized in the oxidation-reduction during desaturation, which produces a double bond at a fixed position from the carboxyl end of fatty acids.

2.1.1 Desaturase 9 (Δ9)

Mammalian Δ9, which was first purified from rat liver (Strittmatter, et al., 1974) is also referred to as stearoyl-CoA desaturase 1 (*SCD-1*) in animal models. There are four isoforms identified in mice, *SCD-1*, *-2*, *-3* and *-4*, which are suggested to exhibit tissue-specific expression (Nakamura & Nara, 2004b). *SCD-1*, which is actively expressed in adipose tissue and liver in mice, can be pronouncedly induced by high carbohydrate diets (Ntambi & Miyazaki, 2003) and is highly homologous to human Δ9. *SCD-1* has three regions of conserved histidine-box motifs that act at the catalytic center of desaturases (Shanklin, et al., 1994) and are the potential ligands of iron atoms.

Desaturase 9 (Δ9) introduces a *cis*-double bond at the 9 and 10 carbon atoms from the carboxyl end of fatty acids. Δ9 converts two important SFA, palmitic acid and stearic acid, into their corresponding MUFA, palmitoleic acid and oleic acid. Palmitic acid is the natural endproduct of the fatty acid synthesis pathway starting from acetyl CoA. Once synthesized, palmitic acid can undergo further metabolism via: (a) conversion into longer chain SFA via elongation; (b) conversion to palmitoleic acid via desaturation; or (c) transport into mitochondria for beta oxidation. Oleic acid is the most abundant fatty acid in human tissue pool as it comprises almost one-half of all stored fatty acids (Nakamura, et al., 2004a). In addition, oleic acid is important for cellular physiological functioning via its role in the maintenance of membrane fluidity. Oleic acid, however, does not participate in trans-membrane signaling.

2.1.2. Desaturase 6 (Δ6) and desaturase 5 (Δ5)

As compared to Δ9, the Δ6 and Δ5 desaturases share surprisingly high similarity in terms of their structures and encoding genes. Both Δ6 and Δ5 desaturases structurally consist of the cytochrome b_5 domains, the highly

conserved heme-binding motif and histidine rich regions (Cho, et al., 1999). The homologous sequence of genes for Δ6 was cloned from humans (Cho, et al., 1999), mice (Cho, et al., 1999) and rats (Aki, et al., 1999)(Cho, et al., 1999). Genes of Δ5 were also cloned from humans (Cho, et al., 1999) and rats (Zolfaghari, et al., 2001) showing 92% similarity in their coding sequence between the two species (Zolfaghari, et al., 2001).

Both Δ6 and Δ5 are involved in the rate-limiting steps of the HUFA endogenous biosynthesis pathway (Shils, et al., 2006). In contrast to SFA and MUFA that can be completely derived from bio-synthesis, PUFA LA and ALA cannot be synthesized in animals because they do not have the enzymes that can introduce double bonds beyond Δ9 in the fatty acid structure. Hence, the latter two fatty acids are essential in the diet. In animal tissues, LA and ALA can be converted into longer and more unsaturated fatty acids. In the bio-synthesis pathway of HUFA, Δ6 utilizes LA and ALA as substrates to catalyze the first desaturation step. The Δ5 desaturase catalyzes the synthesis of AA and EPA, which have critical physiological roles in terms of acting as precursors for eicosanoids, trans-membrane signaling and maintainance of membrane structure and properties.

2.2. Regulation

2.2.1. Insulin

Among hormonal regulators, insulin is the only anabolic hormone and is the sole activator of all three desaturases (Brenner, 2003). Depressed liver microsomal Δ9, Δ6 and Δ5 have been observed from rats with alloxan or streptozotocin-induced diabetes, which was overcome by injection of low doses of insulin (Mercuri, et al., 1966) (Mercuri, et al., 1967) (Eck, et al., 1979) (Mimouni & Poisson, 1992). In contrast, rats given troglitazone to improve insulin sensitivity did not show significant improvement of ratio of AA to C20:3 n-6 (homo gamma linoleic acid) in muscle phospholipids. The latter

ratio, however, is a less accurate estimate of liver Δ5 activity than direct measures of liver microsomal activity since the muscle phospholipid fatty acid composition is the outcome of several other metabolic pathways including inter-organ fatty acid transport and muscle tissue acylation enzyme activity (Clore, et al., 2000).

Similarly, human research literature has consistently implicated insulin-mediated activation of desaturase activities (Vessby, et al., 2002). For example, disorders related to insulin resistance such as type 2 diabetes, obesity and coronary heart disease share similar aberrant tissue fatty acid profiles of elevated levels of palmitic acid, palmitoleic acid and homo gamma linoleic acid (Vessby, et al., 2002). Such fatty acid profiles are indicative of increased activities of Δ9 and reduced Δ5 in association with insulin resistance. On the other hand, patients with type 1 diabetes showed a reduced ratio of AA to LA in serum lipids, suggesting reduced activities of Δ6 and Δ5 (van Doormaal, et al., 1984) (Bassi, et al., 1996). After insulin treatment, an increase of AA and total PUFA was observed, implying improved activity of these desaturases (van Doormaal, et al., 1984) (Bassi, et al., 1996). Using daughter fatty acids to parent fatty acids ratios as the estimates of the activity of desaturases, Borkman et al. were the first to examine in humans the associations of insulin sensitivity with the fatty acid composition of skeletal muscle phospholipids showing positive associations between insulin sensitivity and Δ5 (Borkman, et al., 1993). Subsequent observational studies conducted among adult Pima Indian and elderly Swedish men showed that palmitoleic acid was positively and independently associated with insulin resistance level, indicating elevated Δ9 activity (Pan, et al., 1995) (Vessby, et al., 1994).

2.2.2. Adiposity

In previous studies, the role of adiposity in regulating the activities of desaturases has received relatively little research attention as compared to the impact of insulin action on desaturase activities. In particular, the regulatory

function of adiposity on desaturase activities has been unclear as opposed to the well-characterized effects of insulin on Δ6 and Δ5 activities.

2.2.2.1. Δ9

Direct evidence regarding the role of adiposity and related hormones on regulation of human Δ9 is scarce as understanding of these latter relationships has mainly derived from animal models. To date, four isoforms of *SCD* have been identified in mice whereas only a single human gene of Δ9 has been identified. The human Δ9 gene is highly homologous to *SCD-1*, which is one of the four isoforms of mice *SCD* (Miyazaki, et al., 2000). In mouse models, *SCD-1* is highly expressed in liver where it is involved in the production of VLDL. *SCD-1* is also suggested to play a critical role in leptin-mediated energy homeostasis (Ntambi & Miyazaki, 2004).

In mouse models, the *SCD-1* gene is ranked on the top of the list of genes uniquely repressed by leptin (Cohen, et al., 2003). Observations from epidemiological studies support such a relationship as the activity of Δ9 of 59 obese children was demonstrated to be higher than age- and sex-matched healthy controls (Okada, et al., 2005). The activity of Δ9 was positively and significantly associated with leptin but not with insulin or HOMA-IR among this study population (Okada, et al., 2005). Similarly, positive associations between Δ9 and waist circumference or BMI were observed in three Swedish health surveys among adults (Warensjö, et al., 2005) (Warensjö, et al., 2006) (Warensjö, et al., 2009). Although such findings are promising, the observed associations of desaturation ratios with insulin indices and obesity markers were not adjusted by dietary intake data. The latter confounder could weaken greatly the generalizability of the above epidemiological findings.

2.2.2.2. Δ5 and Δ6

Evidence from *in vitro* studies and rodent models suggested a direct link between adiposity and Δ5 activity. In that regard, obese Zucker rats (*fa*/*fa*), an animal model characterized of a faulty leptin receptor in the brain,

demonstrated reduced affinity of hepatic Δ5 to its substrates as compared to lean Zucker rats (FA/-), which was not explainable by differences in insulinemia (Blond, et al., 1989). Similar findings have been reported in genetically diabetic mice (*db*/*db*), a model used for the study of T2D and obesity in humans, in comparison with their lean littermates (*db*/-) (Cunnane, et al., 1985). The higher hepatic Δ6 activity in obese (*ob*/*ob*) mice versus lean (*ob*/-) mice was attributed to the hypothyroid status of the obese (*ob*/*ob*) mice whereas insulinemia could not explain the differences in hepatic Δ6 activity Hughes & York, 1985). There is a limited amount of information from human trials that has also indicated adiposity could impact upon Δ5 activity and tissue HUFA profiles independent of insulin action. For example, some clinical studies have demonstrated strong negative correlations between adiposity measures and Δ5 activity in Pima Indian (Pan, et al., 1995), obese Hungarian children (Decsi, et al., 1996) and Caucasians (Warensjö, et al., 2006) (Warensjö, et al., 2009). Apart from the Pima Indian study, however, the other two studies did not adjust for the level of insulin resistance, which could bias the observed association between adiposity and Δ5.

2.2.2.3. Possible mechanisms for the associations of adiposity with the activity of desaturases

The regulation of adiposity on the activity of Δ9, Δ6 and Δ5 may not occur through the same mechanisms. It is now generally acknowledged that leptin, an adipose tissue derived hormone, is one of the main contributors to the independent association between Δ9 and adiposity level. It is still unclear, however, whether the associations of adiposity with Δ6 and Δ5 could be independent of other adiposity related factors, especially the level of insulin resistance. Available information about leptin and its regulatory function on *SCD-1* or Δ9 is summarized in the following sections.

2.2.2.3.1. Leptin

Working both centrally and peripherally, leptin has diverse functions, including regulating fatty acid metabolism and cardiovascular function. Since

the regulation of leptin on *SCD-1* was identified several years ago in rodent models, this pathway has been intensively studied (Cohen, et al., 2002). The rodent studies have indicated that leptin is suggested to down-regulated *SCD-1*. In the leptin gene knock-out rodent model, activity of hepatic *SCD-1* increased dramatically together with similar increases in the *SCD-1* products Oleic acid and palmitoleic acid in hepatic lipids. Correspondingly, blood TG levels in circulation were elevated. In humans, absolute leptin deficiencies are very rarely clinically observed. Leptin deficiency in humans leads to increased plasma concentrations of Oleic acid and palmitoleic acid. The latter observations, however, contrast with findings seen with leptin-resistance in humans, which occurs in most circumstances of obesity. In the majority of obese humans, leptin levels are positively correlated with estimates of Δ9 as assessed by the plasma ratios of oleic acid to stearic acid and palmitoleic acid to palmitic acid. Consequently, leptin levels show a positive association with circulating TG as activated Δ9 leads to increased synthesis of TG-rich VLDL. The positive relationship between plasma leptin levels and the estimates of Δ9 in obese humans coincides with observations in rodent models of leptin resistance.

2.2.2.3.2. Leptin and adiposity

Leptin levels in circulation are mainly determined by adipose tissue volume and/or subcutaneous adipose tissue, as shown in *in vitro* studies (Masuzaki, et al., 1995) (Montague, et al., 1997) (Li, et al., 2007) and rodent models (Guo, et al., 2004) (Zhang, et al., 2002) (Shillabeer, et al., 1998) (Casabiell, et al., 1998). From the above studies, leptin has been shown to be primarily synthesized in adipocytes (i.e., ~95%) and is released in response to changes of body fat composition. Results from the population-based studies are largely consistent with the above observations. Subcutaneous adipose tissue, total body fat mass or body fat% were shown to be the main predictors of circulating leptin among different populations (Doucet, et al., 2000) (Tai, et al.,

2000) (Roemmich, et al., 1998) (Doucet, et al., 2000) (Ostlund, et al., 1996) (Dua, et al., 1996) (Fisker, et al., 1997).

2.2.2.3.3. Leptin resistance

The concept of "leptin resistance" refers to the reduced sensitivity to leptin action resulting in an adequate physiological response to leptin despite elevated leptin levels (Martin, et al., 2008) (Considine, et al., 1996). In the latter circumstance, the metabolic action of leptin is blocked, including effects on satiety, weight control and fatty acid metabolism, whereas the other aspects of leptin function are maintained. Rodent models of common human obesity such as *db*/*db* mice, *fa*/*fa* mice, VMH (ventromedial hypothalamus)-lesioned mice have shown a positive correlation between body fat mass and serum leptin levels and tissue leptin expression (Frederich, et al., 1995a)(Frederich, et al., 1995b)(Maffei, et al., 1995). Similarly, most obese human subjects showed increased leptin levels in circulation (Hassink, et al., 1996) (Maffei, et al., 1995) with increased expression of leptin also observed among obese subjects (Hamilton, et al., 1995). Hyperleptinemia among obese animals or human subjects, however, has not demonstrated the expected leptin-mediated effects such as curbing of appetite and maintenance of body weight. Also, evidence from weight loss clinical trials has reinforced the leptin resistance theory. Large doses of leptin given to both lean and obese subjects showed no consistent effects on weight loss or body fat composition (Heymsfield, et al., 1999)(Zelissen, et al., 2005). Thus, it seems that for the majority of obese subjects, endogenous nor exogenous leptin does not provide sufficient signaling to maintain healthy body weight.

At present, it is not clear why leptin resistance occurs although there has been conjecture that leptin access into tissues including the brain may be impaired (Martin, et al., 2008) or that negative regulators are present in the leptin signaling pathway (Yang & Barouch, 2007).

2.2.2.3.4. Implication of the regulation of adiposity on Δ9

The consideration of the regulation of Δ9 activity by leptin provides a novel dimension regarding the effects of leptin on energy metabolism. This latter pathway might work in concert with the well-documented pathway whereby leptin influences energy metabolism via the central nervous system. In the latter pathway, leptin affects the hypothalamus to modulate appetite and energy expenditure via neuropeptide Y signaling pathway. The latter pathway appears to inhibit orexigenic effects and activate anorexigenic peptides in the hypothalamus to maintain body weight (Shils, et al., 2006). Based on evidence from animal models, Δ9 activity has been indicated to reflect cellular energy status by partitioning energy (fatty acids) towards oxidation or storage (Cohen & Friedman, 2004). Thus, decreased Δ9 activity would reflect an accumulation of palmitic acid and stearic acid in hepatocytes that, in turn, repress the bio-synthesis of fatty acids through a negative feedback. As a result, malonyl-CoA, the intermediate at the start of fatty acid biosynthesis, is reduced. Malonyl-CoA is the main inhibitor to beta-oxidation in mitochondria by inhibiting activity of carnitine parmitoyltransferase 1 (CPT-I), which is responsible for the transportation of fatty acids into mitochondria. Decreased concentrations of malonyl-CoA will thus diminish inhibitory effects on CPT-1 resulting in increased beta oxidation. In this manner, via inhibition of Δ9 activity, leptin can re-direct hepatic fatty acid metabolism from VLDL assembling towards fatty acid oxidation. Thus, transportation of lipids from liver to adipose tissue would be reduced thereby avoiding excessive energy storage and fatty acid accumulation in adipose tissue and prevent the development of "lipotoxicity" in insulin sensitive tissues.

2.2.3. Dietary PUFA

Dietary PUFA intake exerts a dominant influence on fatty acid profiles in bio-membranes, tissue pools and blood circulation, due to the low efficiency of HUFA endogenous synthetic pathway (Plourde & Cunnane, 2007). Dietary levels of PUFA intake is thus closely related to the activity of PUFA-regulated metabolic pathways.

2.2.3.1. PUFA regulation

A few early studies have demonstrated that PUFA is the main dietary component that regulates all the three desaturases (Nakamura & Nara, 2004b). *In vitro* experiments have shown that PUFA inhibit expression of all three desaturases (Ramanadham, et al., 2002). In rodent models, PUFA supplementation was shown to down-regulate expression of hepatic *SCD-1* (Ntambi, 1992) (Waters & Ntambi, 1996). Also, rats fed with high PUFA diets showed significantly reduced levels of hepatic mRNA for Δ6 and Δ5 as compared to rats fed MUFA rich diets (Cho, et al., 1999).

2.2.3.2. Mechanism

The effects of PUFA on the activity of desaturases have been suggested to be exerted through the regulation of gene expression related to lipogenesis (Sampath & Ntambi, 2004). Two transcription factors, sterol regulatory element binding protein-1c (SREBP-1c) and peroxisome proliferator activated receptor-α (PPAR-α), have key roles in the PUFA-mediated regulation of desaturases .

SREBPs are the master lipogenic gene regulators by stimulating the complete lipogenic pathway with its related enzymes (Horton, et al., 2002)(Matsuzaka, et al., 2002). Among isoforms of SREBP, SREBP-1c preferentially activates genes encoding enzymes involved with fatty acid and TG synthesis (Brown & Goldstein, 1997)(Horton, et al., 2002). The expression of SREBP-1c is high in hepatocytes, adipose tissue and other tissues in humans and mice (Horton, et al., 2002)(Shimomura, et al., 1997). The active form of SREBP-1c can be reduced by PUFA, thus mediating the suppression of PUFA on target genes, such as those regulating Δ9 (Tabor, et al., 1999)(Waters, et al., 1997) and Δ6 (Nara, et al., 2002). There may be several mechanisms involved. Firstly, PUFA can reduce SREBP-1c mRNA levels by inhibiting its transcription. Secondly, PUFA can inhibit the proteolytic processing of SREBP-1c (Hannah, et al., 2001). Thirdly, dietary PUFA may inhibit SREBP-1c at post-transcriptional level by reducing SREBP-1c mRNA stability then accelerating its degradation (Xu, et al., 1999). Additionally, activation of SREBP-1c by the

liver X Receptor (LXR) is inhibited by PUFA due to their competition with the ligand of LXR/RXR, which is the activator of the SREBP-1c promoter (Ou, et al., 2001)(Yoshikawa, et al., 2002).

HUFA such as AA and EPA are suggested to be more potent than their PUFA precursors to inhibit SREBP-1c.The PUFA LA is a stronger inhibitor than the MUFA Oleic acid, whereas SFA generally do not show effects on SREBP-1c (Xu, et al., 1999) (Hannah, et al., 2001) (Yoshikawa, et al., 2002). By feeding diets rich in PUFA, hepatic SREBP-1c protein is reduced 50-85% (Xu, et al., 1999). When PUFA intake accounts for as little as 5% of the total calories, lipogenic gene expression is reduced by 50%, while when the PUFA content reaches 20% of total energy intake level, the inhibition of PUFA on lipogenic gene expression reaches its maximum (Xu, et al., 1999).

As compared to SREBP-1c, the peroxisome proliferator-activated receptor α **(**PPAR-α) induces a different set of genes related to fatty acid oxidation and ketogenesis in mitochondria, peroxisomes and microsomes (Gulick, et al., 1994). It is generally recognized that long chain PUFA activate fatty acid oxidation by acting as ligands of PPAR-α and inducing transcription of target genes (Nakamura, et al., 2004a).

2.2.4. Iron

Despite the fact that the iron deficiency is a global public health problem and is the most prevalent nutrient deficiency in developed countries, there is little research attention regarding whether iron deficiency could influence the metabolism of other nutrients including fatty acids.

2.2.4.1. Animal studies

Two rat studies have been performed to examine whether moderate iron-deficiency induced by diet could influence the activity of desaturases (Cunnane & McAdoo, 1987) (Stangl & Kirchgessner, 1998). Iron deficient male Sprague-Dawley rats were fed an iron intake of 12 mg/kg as compared to control animals with an iron intake at 27 mg/kg diet (Cunnane & McAdoo,

1987). Another iron fed group received 237 mg/kg that is comparable to human consumption when individuals take iron supplementation at a maximum level (200-250 mg/kg) (Cunnane & McAdoo, 1987). After 12 weeks of feeding, the iron-deficiency rats demonstrated low hemoglobin, low hematocrit and mean erythrocyte cell volume although plasma iron levels were not reduced (Cunnane & McAdoo, 1987). Despite similar food intakes and body weights, the iron deficient rats showed a significantly higher ratio of LA to AA in plasma and erythrocyte lipids, indicating a reduced conversion of short chain PUFA to long chain PUFA (Cunnane & McAdoo, 1987).

Other studies using more severely iron-deprived rat models induced by diet have also demonstrated the impaired activity of desaturases as shown in Table 2.1.

2.2.4.2. Human studies

In an adult human study (Krajcovicova-Kudlackova, et al., 2004), subjects with low serum iron levels (men: < 12 μmol/L; women: <10 μmol/L) demonstrated reduced plasma ratio gamma linoleic acid/LA, indicating lower activity of Δ6 relative to normal controls.

2.2.4.3. Possible explanations

An association between iron and fatty acid metabolism is premature based on the limited information from previous studies and given that the underlying possible mechanism(s) have not yet been clearly defined. There could be at least two possible mechanistic explanations. It is conceivable that as iron is part of the structure of desaturases, low iron status could affect the function of iron containing enzymes such as desaturases. On the other hand, maintaining the activity of the desaturases requires the normal function of all their other components as well: NADH, stearyl coenzyme A, oxygen, lipids, cytochrome b5 reductase and cytochrome b5, which also might be adversely influenced by low iron status and thereby inhibit the function of the desaturase enzyme system.

2.2.5. Summary of desaturase regulation

Information from literature, mostly from animal models, demonstrates that insulin, adiposity, and possibly other metabolic factors regulate endogenous fatty acid synthesis. The activity of desaturases is closely related to insulin, the only activator of all three desaturases. An elevated insulin resistance level is related to activated Δ9 and depressed activity of Δ5. Additionally, animal studies indicate that adiposity exerts an independent influence on Δ9. Although there also seems to be a direct impact of adiposity on Δ6 and Δ5, the latter relationships are still uncertain. The results from a limited number of studies have implied that low iron status could be associated with inhibited fatty acid biosynthesis.

The PUFA products of Δ6 and Δ5, especially HUFA, are concentrated on bio-membranes where they play critical role in maintaining their physical properties and in the regulation of membrane signaling transduction. Additionally, HUFA are the only precursors of eicosanoids, which are involved in physiological responses such as inflammation, immunity and thrombosis. In contrast, the MUFA products of Δ9 are the substrates of a wide diversity of lipids including TG, phospholipids and cholesterol. Although SFA and MUFA are also the components of bio-membranes, they do not participate in cell signaling. Abundant levels of MUFA along with SFA are stored in adipose tissue.

Desaturases are regulated by an intricate network of hormones, adipokines and transcriptional factors. There is still, however, a great deal of uncertainty regarding understanding the factors operating: ultimately mechanistic *in vitro* and animal studies as well as epidemiological studies are needed to examine possible mechanisms of regulation in humans that are indicated from *in vitro* and animal models.

2.3. Fatty acid composition of plasma and erythrocyte membranes as biomarkers of dietary fatty acid intake

The fatty acid composition of whole blood and blood lipid fractions has been used commonly in observational studies as an indicator of dietary fat intake due to sampling convenience and the assumption that dietary intake habits of subjects do not change dramatically over a short period of time (Willett, 1998). This approach is particularly useful for the assessment of fatty acids largely of dietary origin such as long chain n-3 and n-6, trans fatty acids (TFA) and odd numbered carbon fatty acids. The Nurses' Health study showed that certain plasma and erythrocyte fatty acids were both significantly and positively correlated with their intake levels as assessed by food-frequency questionnaires (Sun, et al., 2007). Among these latter fatty acids, erythrocyte and plasma concentrations of DHA showed the strongest correlation with its intake (Sun, et al., 2007). Other strong correlations were also seen for erythrocyte and plasma concentrations of EPA, LA, total trans fatty acids and trans oleic acid (Sun, et al., 2007). As compared to plasma and serum fatty acid composition, the fatty acid composition of erythrocyte membranes has generally demonstrated a stronger correlation with intake levels, indicating that these are better biomarkers for long-term intake (Sun, et al., 2007). Erythrocyte fatty acids are also less sensitive to recent dietary intake as they have a slower turnover rate of fatty acid composition. Since erythrocytes do not synthesize fatty acids and erythrocytes survive for approximately 120 days, the erythrocyte membrane fatty acid composition is relatively stable in comparison to plasma lipid profiles that are affected by alterations in short-term dietary fat intake. On the other hand, plasma fatty acid composition is more convenient to measure. A comparison of fatty acid intake as estimated from food frequency questionnaire among 200 Costa Rican people with plasma, adipose tissue, and whole blood fatty acid composition showed that adipose fatty acid composition is the best indicator of long term fatty acid intake whereas plasma fatty acids are the most suitable bio-maker of short-term intake (Baylin, et al., 2005). In a study of repeated 24-h recalls collected during a 3-yr period among 276 male subjects and 257 female subjects, plasma EPA and DHA

levels correlated significantly with their respective percentages of energy intake (Astorg, et al., 2008). An earlier dietary study of male and female adults of the same population showed that main dietary sources of EPA and DHA were fish and seafood, while the main source of DPA was meat, poultry and eggs (Astorg, et al., 2004). Moreover, this latter study demonstrated that plasma levels of EPA and DHA but not DPA correlated significantly with fish consumption, especially fatty fish (Astorg, et al., 2008). The study, however, did not find significant associations of dietary intake of LA and ALA with the plasma levels of their respective long chain derivatives (Astorg, et al., 2008).

The association of dietary intake of long chain n-3 fatty acids with long chain n-3 erythrocyte membrane fatty acid levels has also been assessed in dietary intervention studies. A EPA and DHA enriched diet containing 200 g fish given once per day (providing approximately 150 mg EPA and 410 mg DHA) and five days per week for 6 weeks elevated significantly levels of EPA and DHA in erythrocyte membranes as compared to a fish-free diet (Brown, et al., 1990). Dietary EPA strongly correlated ($r=0.908$) with erythrocyte membrane levels of EPA in the intervention group at the end of the trial. A study of middle-aged Japanese males and females observed significant correlations of long chain n-3 fatty acid intake measured in 7-d weighted diet records with plasma levels of EPA ($r\geq0.66$) and DHA ($r\geq0.59$) in both genders (Kuriki, et al., 2003). Subjects were given a daily salmon fish oil diet over 4 weeks, which provided a daily intake 20-29 g of n-3 fatty acids that had a 55% content composed of EPA and DHA (Harris, et al., 1983). These individuals showed significantly elevated plasma levels relative to control subjects fed diets without EPA and DHA (Harris, et al., 1983). In a clinical trial, free-living male and female subjects who were given n-3 enriched food providing HUFA n-3 fatty acid intake (around 200 mg/day) showed a 43% increase in erythrocyte HUFA n-3 content from baseline levels over a 3-month intervention period (Murphy, et al., 2007).

2.4. Canadian Arctic Inuit and Canadian Cree

Inuit are a group of indigenous peoples residing in the circumpolar area of Canada, Greenland, Russia and the United States (US), who share a similar culture and speak an Inuit/Eskimo or Aleut language. The Cree, on the other hand, is one of the largest groups of First Nations, who primarily live in the sub-Arctic regions across Canada with some also residing in the US. The Cree language is closely related to Algonquian languages.

Since the end of World War II, Canadian Arctic Inuit and James Bay Cree have experienced rapid social transition similar to other native populations living in circumpolar area and other regions of Canada. With the end of their nomadic traditional life style, the Inuit and Cree moved into permanent settlements to adapt to a more modernized lifestyle that has included dietary changes. A dietary transition away from traditional foods (the animals or plants harvested locally) to market foods (store foods shipped from the south, also referred to country foods) has been previously documented.

2.4.1. Contribution of traditional foods to nutrition

2.4.1.1. Traditional high protein and n-3 PUFA diets versus market food-based diets

The traditional foods of Canadian indigenous peoples are relatively low in SFA, but high in protein and PUFA, particularly n-3 PUFA, in comparison to market foods. Data regarding the food composition of Inuit diet, however, are limited. A Greenland study carried out compositional analysis of daily meal samples from Inuit households from the mid-1970's, which were obtained at the end of the winter season when seal hunting was dominant (Bang, Dyerberg, & Sinclair, 1980). Food composition analysis showed that the total dietary fatty acid content comprised of 22.8% SFA in contrast to the estimated 52.7% in Danish diets during the same period of time.

Historically, native Arctic indigenous peoples had depended almost completely on high protein and fat-based traditional foods that contained mostly

animal meat. Before their contact with Europeans, there were a relatively few carbohydrate sources, including berries, green plants, roots and seaweed which were collected locally and preserved for later use (Anderson, 1939) (Porsild, 1953). Animal meat such as animal liver also provided small amount of carbohydrate in the form of glycogen, which was estimated to be around 10 gram per day (Draper, 1977). In addition, rumen content of herbivorous animals such as caribou and muskoxen was also a source of carbohydrate among Inuit, as rumen content carried the bulk of plants eaten by these animals (Porsild, 1953). In a Greenland study, rumen content of muskoxen was tested for the quantitative analysis of plant species and types composed of the diet of muskoxen throughout the year (Thing, et al., 1987).

The dietary pattern changed at the beginning of the 20th century when Hudson's Bay stores were established in Arctic areas. Tea/coffee, sugar and flour were among the food items that were introduced into the daily diet of indigenous peoples. A dietary study conducted in late 1990s showed that sugar was the most consumed market food among 18 Inuit communities in Northwest Territories, Nunavut and Labrador (Kuhnlein, et al., 2004). Similar observation has been noted consistently in several studies of Inuit dietary surveys among Inuit (Kuhnlein, et al., 1996b)(Wein, et al., 1998). A survey among both children and adults in the Baffin community of Qikiqtarjuaq showed that their average daily intake of soft drinks and table sugar contributed to higher proportions of energy than the intake of more nutritious market foods such as meat, vegetables and milk for both genders (Kuhnlein, et al., 1996b). Similarly, an observational study in the Sanikiluaq community in the southeastern area of Hudson Bay showed that sugar was one of the top market foods consumed by locals throughout the year including hunting season when traditional food intake is typically higher (Wein, et al., 1998).

Similar findings were also reported in dietary studies among other Canadian native populations living in Arctic and sub-Arctic regions. Receveur et al. (1997) examined dietary data of adult Dene/Métis in 16 communities of the

Northwest Territories. On days when only market foods were consumed, intake of fat, saturated fat, sucrose as well as sodium was higher (Receveur, et al., 1997). The authors concluded that dietary transition away from traditional foods towards market foods was characterized by increased intake of absolute energy and increased intake of fat and SFA as well as carbohydrates, especially sucrose (Receveur, et al., 1997). An earlier study in two Dene/Métis communities of Northwest Territories showed that table sugar and soft drinks were ranked as the most consumed market foods, far ahead of dairy products, meat and vegetables (Morrison, et al., 1995). In the latter study, it was estimated that about 15% energy for children came from sucrose (Morrison, et al., 1995). Moreover, more than half of children did not report any fruit, vegetable or dairy products in their 24-h recall, whereas 82% children and 66% teens reported soft drink and crystal powdered drinks in their recalls. The authors concluded that market foods consumed by the study participants were cheap, highly processed and considerably high in refined sugar and fat, but generally had low nutrient-density.

High content of refined sugar in market foods has also been seen in studies among indigenous peoples living in the circumpolar area of other countries. A Greenland historical record showed that the traditional adult Inuit diet in 1855 contained about 377 g protein, 59 g carbohydrate and 162 g fat whereby carbohydrate contributed to approximately only 7% of daily energy intake. The average proportion of daily energy from carbohydrate rose to about 38% in 1970, although it was still considerably lower than the 48% of energy from carbohydrate observed in the typical Danish diet (Bang, et al., 1980). A study in Alaska suggested that table sugar was the 2nd most consumed market food wheras sugar ranked as the 4th most common in the US National Health and Nutrition Examination Survey (Nobmann, et al., 1992).

2.4.1.2. Nutrient density versus energy density

Traditional foods have a higher nutrient-density than market foods. Traditional foods have been considered to be important sources of nutrients such as protein, iron, zinc, copper, selenium and vitamin A (Kuhnlein, et al., 2008).

Dietary data collected from children and adults in a Baffin community (Kuhnlein, et al., 1996b) showed that a greater amount of energy, fat, carbohydrate, calcium and sodium came from market foods, while traditional foods contributed more protein, iron, zinc, and vitamin A. Also, the PUFA content was high in Inuit foods from food items such as whale muktuk, whale blubber, seal meat and seal blubber (Kuhnlein, et al., 1996b). A dietary study among 164 Baffin Inuit children and adolescents showed that Inuit traditional foods were a source for the majority of nutrients, particularly for protein, iron and zinc, despite the relatively low consumption of Inuit foods (Berti, et al., 1999). For instance, 16% of energy was provided from traditional foods according to 24-h recalls among the children as compared to 84% of energy from market foods; however, more than 50% of dietary iron and zinc came from the traditional diet (Berti, et al., 1999). In another study, on the days when traditional foods were consumed among adult Dene/Métis in the Canadian Arctic, the intake of iron, zinc and potassium was significantly higher, while the consumption of sodium, fat, saturated fat and sucrose was significantly lower than on days when no traditional foods were consumed (Receveur, et al., 1997). In a dietary survey of Inuit adults in Sanikiluaq, Nunavut, Inuit foods provided, on average, 47% of daily energy but contributed to 65-92% of daily protein, iron, zinc, phosphorous, thiamin, riboflavin and niacin (Wein, et al., 1998). A dietary study among adult Inuit women in Nunavik showed that while most energy came from market foods, 40% of the nutrients came from traditional foods (Blanchet, et al., 2000).

Given their higher consumption among young generations, however, market foods play an important role in the maintenance of nutritional status of the young population. Market foods have been suggested to be important sources of energy, fat, carbohydrate, calcium and sodium (Kuhnlein, et al., 1996b). Some market foods were considered to be important contributors to nutrients such as calcium as observed in dietary surveys among indigenous populations residing in Canadian Arctic and Sub-arctic regions (Kuhnlein, et al., 1996b) (Kuhnlein, et al., 2004). Higher intake of market food versus traditional food has also been seen among Inuit children and adolescents. A dietary study among Inuit children and

adolescents in a Baffin community demonstrated that the study subjects largely relied on market foods for calcium and vitamin A (Berti PR, 1999). Unfortunately, nutritious market foods such as meat, low-fat dairy items, vegetables and whole grains have not been consumed on a regular basis, according to dietary survey data collected in the Canadian Arctic (Kuhnlein, et al., 2004). Surveys among Dene/Métis children showed that more than half of the energy they consumed came from food items with poor nutritious quality providing more than 40% of energy from sugar or fat whereas traditional foods only contributed to an average 4.3% to 4.7% of energy in this population (Nakano, et al., 2005).

Details about the food composition of Canadian Indigenous People's traditional foods and market foods are summarized in Table 2.2. Information about foods frequently consumed by Canadian Indigenous People is noted in Table 2.3. Information about the nutrition contribution by Canadian Indigenous People's traditional foods are summarized in Table 2.4.

2.4.2. Fatty acid profiles of blood and tissues of Inuit and Cree

For indigenous people living in the northern Canada, their high blood concentrations of n-3 and low blood concentrations of n-6 as compared to populations living in the southern Canadian areas has been consistently documented. On the other hand, it is conceivable that the blood and tissue fatty acid profiles could be different for indigenous people from different regions due to the variability among the Inuit groups regarding the relative dietary importance of fish, marine mammals and land mammals that have different fatty acid composition.

An earlier cross-sectional health survey among the central Canadian Arctic Inuit showed that Inuit had higher plasma concentration of EPA, but lower plasma concentrations of AA and total n-6 than Caucasians residing in the same region who consumed lower amounts of fish or other traditional foods (Young, et al., 1999). The plasma phospholipid concentration of EPA and DHA in Nunavik Inuit, James Bay Cree and Quebecers reflected clearly their distinctive habitual

dietary patterns (Dewailly, et al., 2003). The Nunavik Inuit who had high consumption of fish and marine mammals showed the highest plasma phospholipid concentrations of n-3 fatty acids among the three populations. The James Bay Cree who had a greater intake of freshwater fish and terrestrial mammals rather than sea mammals demonstrated intermediate n-3 fatty acid profiles relative to Nunavik Inuit and Quebecers. It was estimated that the daily fish intake of Nunavik Inuit, Cree and the Quebecers provided 2115, 700 ~ 900 and ~170 mg of the sum of EPA and DHA, respectively. Fish consumption among the three groups was thus closely related to their plasma phospholipid HUFA n-3 concentrations.

Similar reports have been published showing a differential n-3 fatty acid status among indigenous peoples living in Greenland and Alaska. The dietary n-3 content has been suggested to be a strong indicator of traditional food consumption among Greenland Inuit as the correlation coefficient between the n-3 content in diets and local traditional food content in diets has been as high as 0.7 (Deutch, et al., 2007). As Greenland Inuit diet still contained significant amounts of traditional food, their diet had markedly lower levels of LA and AA, leading to substantially lesser amounts of LA and AA in their plasma lipid fractions (Dyerberg, et al., 1975). For example, the C18:2 levels in plasma lipid fractions were only 1/3 to 1/2 of those observed from Danes and Inuit living in Demark (Dyerberg, et al., 1975). Another study that compared the plasma fatty acid composition among Greenland Inuit from three communities showed that Inuit who self-reported higher consumption of Inuit meals particularly sea mammals and fish as compared to Danish meals had plasma n-3 fatty acids levels (Deutch, et al., 2004). Furthermore, the Inuit plasma n-6 fatty acids levels including LA showed moderate inverse correlations with their reported traditional food consumption (Deutch, et al., 2004). In Alaska, Inuit who still depend on fishing and hunting marine mammals their livelihood showed plasma levels of n-3 PUFA, EPA and DHA that were 4.3, 13 and 6.8 times respectively higher than non-native controls living in a nearby region (Parkinson, et al., 1994). In the latter study, coastal Inuit had relatively elevated levels of EPA and DHA than river Inuit,

which was consistent with the higher consumption of marine fish and marine mammals among the coastal Inuit (Parkinson, et al., 1994). In the latter study, higher LA levels were noted among river Inuit, which may have been due to their greater dependence on non-native foods. Another study collected dietary intake data and measured red blood cell fatty acids for Yup'ik Inuit living in Alaska native communities (Bersamin, et al., 2008). Increased traditional food consumption, estimated as the proportion of energy from traditional foods, was associated with an increase in erythrocyte EPA and DHA levels and decreased erythrocyte content of LA and AA (Bersamin, et al., 2008).

Details about the fatty acid composition of blood and blood lipid fractions among Canadian indigenous people and reference populations are summarized in Table 2.5.

2.4.3. Distinctive health status of indigenous people residing in arctic and sub-arctic area

The epidemiologic pattern of diseases in Canadian Arctic indigenous populations has been generally different from that observed in non-native Canadians residing in southern Canadian regions. Young, et al. (1993) examined mortality, morbidity and risk factors of cardiovascular disease between 1950-1989 for residents in the Northwest Territories. Despite the prevalence of obesity and smoking, the age-adjusted mortality rate for ischemic heart disease was lower among this population than the mostly Caucasian Manitoba population (Young, et al., 1993). Also, the age-standardized mortality rate for all circulatory diseases was lower among the Canadian Arctic indigenous population than the general Canadian population (Young, et al., 1993). Furthermore, the Inuit of Kivalliq, Nunavut had relatively low plasma levels of cholesterol and TG as well as high plasma levels of HDL, particularly among the older adults (Young, et al., 1993). A recent survey among residents at and beyond 20 years old from Yukon, Northwest Territories and Nunavut showed that these populations had a lower self-reported crude prevalence of hypertension, diabetes, heart disease and stroke than southern Canadian respondents (Deering, et al., 2009). Despite higher levels

of obesity and smoking among these northern residents, the age- and sex- adjusted prevalence of all chronic diseases among northern residents was significantly lower than that among southern residents. In other words, the higher adiposity level of northern residents did not appear to result in observably higher obesity related disease to the same degree as observed in their southern Canadian counterparts (Deering, et al., 2009).

Similar results have also been seen among indigenous peoples living in Alaska and Greenland. In Greenland, despite the fact that local Inuit had a higher proportion of smokers and a higher prevalence of hypertension as compared with Danes, mortality from ischemic heart disease was 352 per 100,000 among Greenland Inuit as opposing to 434 per 100,000 among Danes (Bjerregaard, et al., 1998). This discrepancy could be partly explained by a lower n-3/n-6 ratio in the Danish diet as well as lower plasma HDL levels among Danes (Bjerregaard, et al., 1998). Based on electrocardiographic evidence, a comparison between historical and current records showed that although ischemic heart disease existed before dietary westernization, its prevalence was much lower than presently seen in Greenland Inuit (Kjaergaard, et al., 2009). A review on cardiovascular mortality and risk factors in Alaska natives (mostly Inuit) showed that mortality from ischemic heart disease in this population was generally lower than U.S. Caucasians (Schumacher, et al., 2003).

Comparisons among Arctic and sub-Arctic native populations from Canada, Alaska and Greenland have supported the above conclusions regarding distinctive epidemic disease patterns between indigenous peoples and Caucasians. A cross-sectional health screening that compared the blood pressure among Greenland Inuit, Nunavik Inuit, Kivalliq Inuit and Alaska Inuit suggested that despite their relatively high adiposity compared to European populations, blood pressure levels among the Inuit were relatively low (Bjerregaard, et al., 2003). A study that compared the age standardized diabetes prevalence among indigenous populations in the circumpolar Arctic and sub-Arctic areas, including Russia, Alaska and Canada (Young, et al., 1992) showed that the prevalence of diabetes

of 3.6/1000 among Inuit living in Northwest Territories was substantially less than the US all-race prevalence of 23.5/1000 (Young, et al., 1993). When comparing age-standardized diabetes prevalence between these indigenous populations, Canadian Inuit and other Canadian indigenous populations had lower prevalence of diagnosed diabetes than indigenous people residing in Alaska (3.6/1000 for Canadian northwest territories Inuit *vs.* 7.9/1000 for Alaska Inuit)(Young, et al., 1992). The longer westernization history of Alaskan Inuit may partly explain their higher prevalence of diabetes. As compared to Canadian Arctic, Alaska had experienced earlier contact with Europeans who came for whaling. Thus, local Inuit participated in a wage engagement, contacted with trade goods and experienced dietary transition at an earlier stage.

2.4.4. Influential factors for traditional foods consumption

For indigenous people, food selection and consumption are influenced by a variety of factors (Kuhnlein & Receveur, 1996a). These factors vary from: (a) natural environment such as climate change, pollution and local food species; (b) political environment such as policies related to food availability; (c) cultural preferences; (d) availability of market foods via food distribution networks; (e) affordability such as income and food price; and (f) media influences such TV food commercials. The above factors may work either individually or synergistically to result in the ongoing dietary transition among Inuit populations. Currently, depending on food availability, Inuit and Cree can consume either a mixture of market foods and country foods or market foods alone. Based on data dietary data collected in 44 communities from Yukon Territory, Northwest Territory, Nunavut, and Labrador in the mid to late 1990s (Kuhnlein, et al., 2004), energy derived from traditional foods was around 17-28% with an average level at 22% (Kuhnlein, et al., 2008). The influential factors commonly suggested to be related to traditional foods consumption are discussed below.

2.4.4.1. Age

The inter-generational differences regarding dietary intake patterns among native populations living in the Canadian Arctic and Sub-arctic areas has been

clearly described in previous studies. There could be multiple reasons for this phenomenon including young people spending more time in schools and more engaged in wage-earning employment. Therefore, youth have less time for hunting as compared to older individuals in the same communities and so have less exposure to cultural activities and less chance to gain related knowledge (Kuhnlein, et al., 1996a). Moreover, as traditional food holds an important status in Inuit culture, it is the etiquette among Inuit to save traditional foods for consumption by the elderly (Wein, et al., 1998). Although the causes of the inter-generational differences for traditional food consumption can vary, the consequences for nutritional outcomes are similar. A dietary study in a representative community in Baffin showed that young adults tended to have higher intake of market foods, especially those high in refined sugar (Kuhnlein, et al., 1996b). Young adults at 20-40 years of age had considerably lower average daily energy intake from traditional foods than elderly adults (Kuhnlein, et al., 1996b). Similar results were seen in the studies conducted in other Arctic areas. A study among adults in 16 Dene/Métis communities of Northwest Territories showed that with decreasing age, the intake of carbohydrate significantly increased, particularly sucrose. Total energy intake was not significantly different across age groups whereas total carbohydrate and sucrose consumption was higher in the younger groups (Receveur, et al., 1997). In the latter study, higher saturated fatty acid intake was seen in the younger groups, but the difference of total PUFA consumption across age groups was non-significant. A study that collected 24-hour dietary recall data from adult Inuit in Sanikiluaq, Nunavut over two seasons (Feb/Mar, Oct/Nov) showed that traditional foods only contributed 33% of energy for younger men compared with 56% of energy for older men (Wein, et al., 1998). Likewise, traditional foods only contributed 25% energy for younger women in contrast to 63% for older women (Wein, et al., 1998). When dietary data from 226 Nunavik Inuit females were examined, older females had higher intakes of traditional foods than younger females (Blanchet, et al., 2000). Similar observations have been noted among other indigenous populations residing in Arctic and sub-Arctic regions. For example, a dietary study of Sahtu

Dene/Métis showed that market food consumption was substantially higher among children, teens and adults at 20-40 years of age (Morrison, et al., 1995).

Data interpretation for the above studies, however, is problematic because of the cross-sectional nature of most studies carried out among indigenous populations residing in Arctic and sub-Arctic regions. It is thus not clear that the observed low consumption of country foods among young Inuit would change as this population ages or that the dietary patterns established in childhood continue into adulthood (Dewailly, et al., 2001).

2.4.4.2. Location

Few studies have addressed whether or how geographic factors are related to the consumption levels of traditional or market foods. The consumption of traditional foods is often restrained by locally available wildlife species (Kuhnlein & Receveur, 1996a). A dietary study that examined data from adults in 44 communities Dene/Métis, Yukon First Nations and Inuit communities showed large differences regarding the average energy derived from traditional food with ranges of 6-40% between communities, depending on closeness to urban areas and transportation (Kuhnlein, et al., 2004). Similarly, a dietary survey among adults in 16 northwestern Dene/Métis and Yukon communities observed that the proportion of energy from traditional foods increased among people from communities at higher latitudes (Receveur, et al., 1997). A study conducted in Northwest Territories reported that Dene/Métis and Yukon children living in the more northern communities consumed higher amount of traditional foods and consequently more intake of protein, iron, copper, vitamin B6 and manganese, and less intake of energy, saturated fat and sodium (Nakano, et al., 2005).

2.4.4.3. Seasonality

Because harvesting country foods is constrained by hunting season the consumption of country foods fluctuates during the year. A dietary study in a representative Baffin community showed that the percentage of total energy from market foods peaked in January with about 77% and 70% observed for women

and men, respectively. The lowest intake of market foods as a percent of energy took place around September with 59% for women and 53% for men, when the maximum consumption of traditional foods was observed (Kuhnlein, et al., 1996b). In the same study, energy contributed by traditional foods ranged from 41.1% in September to 23% in November (Kuhnlein, et al., 1996b). Examining 24-h recall and food frequency questionnaire collected in 18 communities of the Northwestern Territories showed that consumption of traditional foods in the summer was higher than in the winter (Batal, et al., 2005). In contrast, another dietary study in 16 communities of Northwest Territories reported that neither intake of market foods nor intake of land animals and birds varied significantly during the year, except for fish (Receveur, et al., 1997).

2.4.4.4. Cultural preference

Country foods, which are also called "Inuit foods", are at the heart of Inuit culture as those foods give Inuit a sense of identity. Traditional foods are still rated as the favorite foods among both Inuit adults and children. A study conducted in a Nunavut community showed that both adults and children still rated traditional foods such as caribou, char, beluga and geese as "well-liked" as these foods reminded locals of their tradition, and were associated with their home and childhood (Wein, et al., 1996). On the other hand, children generally gave store-bought foods higher preference ratings than adults (Wein, et al., 1996).

2.4.4.5. Cost and other considerations

Hunting, trapping and fishing activities have become more expensive in recent years. Inuit and Cree are facing increasing expense for practicing their traditional activities, which include necessary equipment facilities such as arsenal for hunting animals, transportation costs between home residence and hunting camps, and the preservation costs for the traditional food items obtained.

When nutritious traditional foods are not available, market foods are problematic since store foods in the north are more expensive than in the southern regions due to the costs of transport of food to remote and isolated northern

communities. Lengthy time of transportation can also pose problems such as perishability and lower food quality (Wein, et al., 1998). In order to improve the affordability among northern residents for nutritious store foods, Indian and Northern Affairs Canada (INAC) has carried out the Food Mail Program in collaboration with Health Canada and Post Canada (Hill, 1998). Under this program, retailers in northern eligible regions can order eligible foods, which are mostly nutritious perishable foods, from assigned southern cities without paying extra fees for transportation as INAC subsidizes the fee difference. Even with the help from this latter program, the price gap between northern communities and southern cities still exists, which is shown by food price surveys conducted regularly by INAC (Indian and Northern Affairs Canada, 2009). Compounding the food availability problem, wage-employment opportunity is limited in the Arctic and sub-Arctic area. Limited numbers of wholesalers in northern communities may result in the similarity of foods in the stores while reducing the variability in the types and amounts of market foods available (Kuhnlein, et al., 1996b). Other challenges include lack of knowledge of identifying the nutrient value of market foods (Wein, et al., 1998).

2.4.4.6. Summary

Food consumption among native Canadian residing in the arctic and sub-arctic area is influenced by multiple factors. Traditional foods are the heart of their culture and are still favorably preferred. But this attitude does not seem to translate into high consumption of traditional foods, particularly among the youth. Availability, accessibility and affordability are the main barriers for native Canadians to increase or maintain their traditional food consumption levels.

2.4.5. Health consequence of nutrition transition

The American epidemiologist Abdel R. Omran proposed the concept "epidemiologic transition" to interpret the change of disease pattern in human history, the age of pestilence and famine, the age of receding pandemics and the age of degenerative and man-made diseases (Young, 1988). This theory has been applied to countries such as England and the United States, Japan and Eastern

Europe, as well as developing countries. This theoretical model is suggested to explain the increase in chronic disease occurrence among indigenous peoples in North America experience, including the Canadian Arctic Inuit (Young, 1988). Typical infectious diseases such as measles, smallpox, scarlet fever and influenza emerged by the early and mid-18th century. These latter diseases soon became rampant among Inuit communities. Vaccination among Canadian Arctic Inuit implemented by the Hudson's Bay Company and later by the Canadian government from the early part of the 19th century greatly reduced the threat of infectious diseases. In the recent decades since the end of World War II, chronic diseases such as heart disease, stroke, diabetes and certain type of cancers increasingly emerged among Inuit and Cree after massive government interventions with the intention to improve social welfare, medical care and economic development of the Inuit (Young, 1988). Review on mortality data during 1987-1996 from Kivalliq residents whose majority population were Inuit demonstrated that the total mortality rate of local Inuit was 1.8 times of the rest of the Canadian population (Macaulay, et al., 2004). Leading causes of death included cancers, circulatory disease, respiratory disease, and injury (Macaulay, et al., 2004).

The evolving disease profiles of Inuit and Cree population in recent decades have indicated important implications from their changing food consumption patterns. Obesity risk has increased, which is likely due to decreased traditional food consumption combined with the substitution by market foods that are high in energy, refined carbohydrates, fat and saturated fat. In addition to the risk of obesity as public health problem among Inuit and Cree, other health problems are emerging as public health concerns, particularly for life style related degenerative diseases.

2.4.5.1. Obesity

A study that pooled data collected from Inuit residing in circumpolar areas in Canada, Greenland of Demark, and Alaska of the United States during 1990-2001 showed that mean BMI levels in all the study regions were beyond 25,

which is the cutoff for overweight (Young, et al., 2007). Results from a study done in Dene/Métis, Yukon First Nations, and Inuit communities showed that obesity (BMI≥30) occurred in 23% of Inuit women in the age group of 20-40 years, in 38% of Inuit women in the age group of 41-60 year and in 44% of Inuit women at 60 years old and above (Kuhnlein, et al., 2004). The obesity prevalence was 16%, 21% and 23%, respectively, in the corresponding age groups of Inuit men (Kuhnlein, et al., 2004). A survey of 704 adult Cree and Ojibwa people inhabiting 6 remote communities in northwestern Ontario and northeastern Manitoba suggested that obese participants had higher carbohydrate consumption independent of total caloric intake (Young & Sevenhuysen, 1989). As compared to consumption of refined carbohydrate, consumption of fat showed a much weaker association with BMI (Young & Sevenhuysen, 1989). The consumption of diets high in energy and refined sugar is coincident with other lifestyle risk factors such as dramatically reduced physical activities. Additionally, Inuit may possibly be genetically predisposed to the development of obesity. A high prevalence of obesity has also been noted among Inuit children populations in arctic regions. A study among 178 Cree children of eastern James Bay aged 9-12 yr showed that 52.2% of children had central adiposity according to NHANE III reference, and among children with central adiposity, waist circumference was positively correlated with sweetened beverage intake (Downs, et al., 2008). Another health screen among 719 Ojibwa-Cree children and adolescents aged 4-19 years demonstrated that 64% girls and 60% boys exceeded the 85th percentile growth chart (Young, et al., 2000).

2.4.5.2. Circulatory diseases

A study reviewed health record data during the period of 1987-1996 in Kivalliq of Nunavut concluded that mortality rate of circulatory disease was the second leading causes of death among Kivalliq residents who were mostly composed of Inuit. Other causes of mortality such as unintentional injuries, suicides, respiratory disease and lung cancer are suggested to be areas of concern (Macaulay, et al., 2004). A review of cardiovascular mortality rates and risk factors in Alaska natives (mostly Inuit) showed that mortality from ischemic heart

disease among Alaska native used to be lower than that of U.S. whites (Schumacher, et al., 2003). Differences in the mortality of ischemic heart disease, however, had started to disappear over the past twenty years (Schumacher, et al., 2003). Moreover, recent mortality rates from all types of heart diseases were similar between Alaska natives and US whites (Schumacher, et al., 2003). On the other hand, the historically reported low incidence of ischemic heart disease in Inuit populations has been questioned as some have suggested that more thorough examination of mortality statistics is needed (Bjerregaard, et al., 2003).

2.4.5.3. Hyperlipidemia

In a cross-sectional health screening among Inuit residing in Nunavik, Québec, n-3 fatty acids showed a positive association with HDL-cholesterol and an inverse association with TG concentrations (Dewailly, et al., 2001). A health survey among non-native Québec residents, Cree people living in northern Québec and Inuit of Nunavik residing in northernmost area in Québec showed that the highest quartiles of plasma EPA and DHA levels were positively associated with HDL (Dewailly, et al., 2003). Traditional food intake measured as percentage of energy was related positively to HDL-cholesterol concentrations and inversely correlated with TG concentrations (Bersamin, et al., 2008).

2.4.5.4. Cancer

The examination on mortality data during 1987-1996 among Kivalliq residents, who were mostly Inuit, reported that leading causes of death among locals included cancers (Macaulay, et al., 2004). The dominant type of malignant disease patterns shifted from Epstein-Barr virus-associated carcinomas of the nasopharynx and salivary glands which were low in the Caucasian populations, to the lifestyle associated tumors, particularly those in the lung, colon and breast, increased dramatically with changing life style pattern including smoking and diet (Friborg & Melbye, 2008).

2.4.5.5. Diabetes

Using clinic diagnosis records, a heath survey among all eastern James Bay Cree in the mid of 1980s showed that crude prevalence of T2D was 2.7% while the age-standardized rate of T2D was 6.6% among people 20 years and over (Brassard, et al., 1993). Hospital records showed that gestational diabetes was prevalent among Cree women in the eastern James Bay region, Québec with a prevalence of 12.8% between 1995 and 1996 as compared to 3%-5% in the general North American population during the same period of time (Rodrigues, et al., 1999). Also, the prevalence of gestational diabetes among Cree of eastern James Bay in the southern communities (inland communities) was twice as high as that in the northern communities (coastal communities) (Rodrigues, et al., 1999). A health survey among Cree of western James Bay showed 13.1% (95 %CI: 11.5%-14.6%) age-standardized prevalence of T2D compared to the estimated 5% in the Canadian population (Maberley, et al., 2000). In 630 non-diabetic adult Ojibwa Cree aged 20-64 years old in northern Manitoba and Ontario, who were followed-up for 4-5 years, the observed T2D incidence density was 8.0/1000 person-years (95% CI: 5.8-10.3)(Young & Harris, 1994). A review on 1305 singleton births among Ojibwa-Cree women living in Ontario during 1990-1993 showed the overall prevalence of diabetes in pregnancy in this population was 11.6% and a gestational diabetes prevalence of 8.4% (Harris, et al., 1997).

2.4.5.6. Metabolic syndrome

A limited number of studies have compared Canadian Arctic Inuit, Cree residing in Manitoba, Ontario and Québec, with the regional control population of non-native Caucasians in terms of the metabolic syndrome components (Lavallee & Bourgault, 2000) (Young, et al., 2002) (Liu, et al., 2006). Inuit and Cree are shown to be generally more obese; however, Inuit showed a better metabolic profile in terms of lipidemia, blood pressure, blood glucose levels than Cree and non-native Caucasians, while Cree had the highest reported prevalence of T2D (Lavallee & Bourgault, 2000) (Young, et al., 2002). According to NCEP/ATP III

criteria, the age-standardized prevalence of metabolic syndrome ranged from 16.0% in Inuit from Kivalliq, to 24.3% in non-native Caucasian in Manitoba and to 37.5% in Cree from northern Ontario and Manitoba (Liu, et al., 2006). Genetic predisposition may be related to the above differences, but the distinctive life styles of the three ethnic groups are also conjectured to play an influential role (Hegele, 1999). In a health screening study among 297 Western Cree in Alberta, 52.3% of adults and 40.5% of children and adolescents had the metabolic syndrome according to NCEP/ATP III criteria (Kaler, et al., 2006).

2.4.6. Summary

Recent studies have shown major differences in dietary intake of traditional foods in different Arctic and sub-Arctic indigenous populations as well as inter-generational differences, which indicates that dietary acculturation is still occurring rather than irrevocably completed (Szathmary, et al.,1987). These circumstances are an opportunity for public health professionals to improve indigenous people's health in high Arctic areas via intervention/prevention measures. Results from different dietary studies need to be viewed with caution since dietary survey methods are not standardized across studies (Whiting & Mackenzie, 1998). All studies to date are cross-sectional design, which still can characterize the dietary transition among indigenous people living in the north via the comparison of different age groups and locations. Longitudinal studies would more accurately capture the evolution of indigenous people's lifestyle including dietary patterns, but these types of studies are difficult to perform in arctic regions.

Table 2.1. Iron deficiency on the activity of desaturases in rodent models

Reference	Animal, duration and dosage	Results
1 (Hirosue & Hosogai, 1993)	20 Female Wistar rats, 8 wks • Iron-deficient group: 5 ppm • Control group: 40 ppm	Rats on iron-deficient diet: • Reduced ratio AA/LA • Reduced palmitoleic acid% In hepatic lipids
2 (Rao, et al., 1980)	Young male Sprague-Dawley rats, 8 wks • Iron-deficient group: fat free diet low in iron • Control: fat free diet with iron supplemented	Rats on low iron diet: • Decreased significantly ratio palmitoleic acid/palmitic acid, oleic acid/stearic acid, and AA/LA in tissue lipids (plasma, erythrocytes and intestinal mucosa) • No significant change regarding hemoglobin content of blood and packed red cell volume

Reference	Animal, duration and dosage	Results
3 (Rao, et al., 1983)	Male Sprague-Dawley rats,10 wks • Experimental group 1: low iron diet without fat • Control group 1: low iron diet without fat but with iron supplement • Experimental group 2: low iron diet containing 14% hydrogenated coconut oil • Control group 2: low iron diet containing 14% hydrogenated coconut oil with iron supplement • Experimental group 3: low iron diet containing 14% corn oil • Control group 3: low iron diet containing 14% corn oil with iron supplement	Rats on all 3 iron deficient diets: • Decreased significantly ratio of Δ9 in liver microsomes Rats in experimental group 2 and 3 • Reduced ratio of Δ9 before the reduction of blood hemoglobin and hematocrit • Lower ratio of Δ9 than that of rats in experimental group 1
4 (Johnson, et al., 1989)	Male Long-Evans rats, 41 days • Iron deficiency group: 5.6 µg iron per gram diet • Control group: 41.7 µg iron per gram diet	Rats on iron-deficiency diet: • Increased LA in liver and serum, and decreased AA in serum

Reference	Animal, duration and dosage	Results
5 (Sherman, et al., 1982)	15 maternal rats During gestation and a 18-day lactation • Iron deficient group: diet containing 5 ppm iron • Control group: diet containing 320 ppm iron	On the 18th day of lactation, pups of maternal rats from iron deficient group: Apparently reduced ratio AA/LA in both fasting serum and liver
6 (Tichelaar, et al., 1997)	132 male Wistar rats 4 weeks • Iron deficiency group: diet with only trace level iron • Control group: same with iron supplements (ferric citrate 6 g/kg body weight)	Rats on iron deficient diet: Apparently reduced ratio AA/ homo gamma linoleic acid in plasma

Table 2.2. Food composition analysis on Canadian Arctic and Sub-arctic Indigenous People's traditional foods and market foods

Study	Design	Results
Greenland, Denmark (Bang, et al., 1980)	To compare food composition of Eskimos' diet and Danes' diet in 1976; 178 samples of daily food during 3-7 consecutive days from 50 Eskimo couples were collected	• The ratio of PUFA to SFA in Eskimo food was 0.84, compared to 0.24 in typical Danish food • The n-3 was the predominant PUFA in Eskimo foods, while the n-6 was the predominant PUFA in Danish foods
Sahtu Dene/Métis, Canada (Appavoo, et al., 1991)	Fresh and prepared country foods were collected from local hunters and fisherpersons in the summer and winter in 1990s	• The total fat content of whitefish, inconnu, cisco, loche, trout, caribou, moose and rabbit was low (<5% wet weight); dried and smoked flesh of above foods had much higher total fat (>18% wet weight) • Land animals contained higher SFA than fish did, and PUFA were predominantly presented as the n-6 • The Dene foods were lower in total fat and higher in the PUFA:SFA ratio

Study	Design	Results
Yukon and Northwest Territories (Inuvialuit, Nunavut and Labrador), Canada (Kuhnlein, et al., 2002)	24-h dietary recall once every two months from 1875 Inuit and 802 First Nations during 1987-1988	• Land animals contained more SFA than samples from sea mammals or fish • Land animals had higher n-6 family, in which LA and AA are primary fatty acids • The n-3 was considerably higher than n-6 in sea mammals as well as in fish
Greenland (Dyerberg, et al., 1975)	To compare calories from the Inuit diet and average Danish foods:	• Carbohydrate (37% Inuit foods *vs* 49% Dane foods) • Protein (26% Inuit foods *vs* 11% Dane foods) • Fat (37% Inuit foods *vs* 40% Dane foods) • SFA (34% Inuit foods *vs* 53% Dane foods) • LA and AA (4.8% Inuit *vs* 10.0% Dane)
North Greenland (Deutch, et al., 2006)	To compare the 177 Inuit meals collected in 1976 with 90 Inuit meals collected in 2004	• % of local country foods decreased in the 2004 meals • Intake of n-3 fat less than half of its level in 1976

Table 2.3. Frequently consumed food items among Canadian Arctic and Sub-arctic Indigenous People

Study	Design	Results
Sahtu Dene/Métis, Canada (Appavoo, et al., 1991)	Fresh and prepared country foods were collected from local hunters and fisherpersons in the summer and winter in 1990s	• The most frequently consumed market foods included sugar, white bread, and bannock • The regularly consumed market foods with high fat are lard, butter, frankfurters, and French fries
Yukon and Northwest Territories (Inuvialuit, Nunavut and Labrador), Canada (Kuhnlein, et al., 2002)	Food items reported ≥ 5 times in 24-h dietary recall that was collected once every two months from 1875 Inuit and 802 First Nations during 1987-1988	• The most frequently used traditional foods in Yukon are caribou meat, moose meat, rabbit, the flesh of grayling, and several species of salmon and trout • The most frequently consumed traditional foods in Inuit are sea mammals including ringed seal, bearded seal, narwhal, beluga and walrus
East and West Greenland (Deutch, et al., 2004)	Information of food frequency questionnaires was obtained from 250 men and women	• Seal, whale, polar bear, fish and game were examples of important local country foods

Study	Design	Results
Greenland (Deutch, et al., 2007)	Dietary survey with food frequency questionnaires was conducted among 500 Greenland men and women (18-50 yr) Plasma fatty acid profile was analyzed	• Seal was the most commonly consumed traditional food, followed by fish • Market meat and bread were similarly and commonly consumed in all the survey districts
5 Dene/Métis and Yukon communities (Nakano, et al., 2005)	222 local children of 10-12 yr old were interviewed in the winter and fall of 2000-2001	• Traditional foods consumed were mostly animal meat
11 communities in Alaska (Nobmann, et al., 1992)	24-h recall during 5 seasons over an 18-month period among 351 Alaska native adults aged at 20-63 yr during 1987-1988	• Sugar was ranked as the 2nd most frequently consumed foods among Alaska native, while it was the 4th in the NHANES II • Vegetables intake among Alaska native was much lower than NHANES data • Intake of energy and protein decreased in the past 30 years, while the intake of fat remained unchanged

Table 2.4. Nutritional contribution from traditional foods and market foods consumed by Canadian Arctic and Sub-arctic Indigenous People

Study	Design	Results
Baffin, Canada (Berti, et al., 1999)	24-h recall among 164 Inuit children and adolescents once every two months from July 1987 to May 1988.	• Market foods contributed an average of 84% of dietary energy, whereas only 16% came from traditional foods • Total and saturated fat intakes met current recommendations, while sucrose intakes were higher than recommended • Over 50% of dietary iron and zinc was from traditional food • Most age and gender categories had a low prevalence of inadequate intakes of iron, zinc and protein
The Great Whale and Nottaway-Broadback-Rupert area, Québec, Canada (Blanchet, et al., 2000)	Food frequency questionnaires were conducted among 226 Inuit women (≥18 yr) in 1990; food composition analysis	• Most energy came from market foods • Country foods contributed to 40% intake of nutrients such as protein, vitamin D, iron, phosphorous and Zn • Country foods contributed more nutrient intake in the older age group (≥40 yr), while market foods were the greater sources of nutrient intake in the younger group

Study	Design	Results
Yukon and Northwest Territories (Inuvialuit, Nunavut and Labrador), Canada (Kuhnlein, et al., 2002)	24-h dietary recall once every two months from 1875 Inuit and 802 First Nations during 1987-1988	• Land animals contained more SFA than samples from sea mammals or fish • Land animals had higher n-6 family, in which LA and AA are primary fatty acids • The n-3 was considerably higher than n-6 in sea mammals as well as in fish
East and West Greenland (Deutch, et al., 2004)	Information of food frequency questionnaires was obtained from 250 men and women	• Local country foods contributed to 25-30% of the total energy • DPA, which is high in seal and polar bear blubber, showed strong association with diet
Greenland (Deutch, et al., 2007)	Dietary survey with food frequency questionnaires was conducted among 500 Greenland residents (18-50 yr). Plasma fatty acid profile was analyzed	• Traditional foods provided an average of 20-30% of total energy intake • Significant correlations existed between intake of traditional foods and plasma levels of n-3 fatty acids

Study	Design	Results
3 large cultural areas of the Canadian Arctic (Kuhnlein, et al., 2004)	Dietary survey with 24-h recall for market foods and food-frequency questionnaires were conducted in 10 communities of the Yukon First Nations, 16 Dene/Métis communities, and 18 Inuit communities from Northwest Territories, Nunavut and Labrador	• Only 10-36% of energy was derived from traditional foods • In all the 3 survey areas, adults beyond 40 years old consumed more traditional foods than the younger • During the days when only market foods were consumed, the intake of fat, carbohydrate and sugar was high
A Baffin community, Canada (Kuhnlein, et al., 1996b)	Dietary survey with 24-h recall and food frequency questionnaires was conducted once every two months from 1987 to 1988 among 366 local residents	• Market foods contributed greater amounts of energy, fat, carbohydrate, calcium and sodium • Traditional foods contributed to energy ranged from 41.1% in September to 23% in November with yearly average at 34% • Young children and teenagers had less intake of energy from traditional foods

Study	Design	Results
16 Dene/Métis communities of Northwest Territories (Receveur, et al., 1997)	Dietary survey with 24-h recall and food frequency questionnaires, Socio-cultural questionnaire. There were 385 interviews conducted in 9 communities during Mar-April, 1994 and 677 interviews conducted in 16 communities during October-November, 1994.	• Intakes of iron, zinc and potassium were higher when traditional foods were consumed (P<.05) • Sodium, fat, saturated fat and sucrose were higher when market foods only were consumed (P<.05) • The shift away from traditional diet to diet composed of only market foods was characterized by a higher proportion of energy from carbohydrate (particularly sucrose), fat and saturated fat (P<.01) and higher absolute energy intake (P<.05)
Yukon and Northwest Territories communities (Kuhnlein & Receveur, 2007)	24-h dietary recall and food frequency interviews 164 students attending the 4^{th} to 6^{th} grader	• The traditional foods contributed to 6-40% of daily energy among adults, and 0.4-15% of energy among children • Even a single portion of traditional foods intake would increase the intake level of a variety of nutrients, such as protein, vitamin E, iron, zinc, etc (P< .05)

Study	Design	Results
18 Inuit communities in Inuvialuit, Kitikmeot, Baffin, Kivalliq, Labrador (Egeland, et al., 2004)	24-h dietary recall, 7-day food record, and 3-month traditional food frequency interviews in summer (June-August) and winter (December-Feburary) 715 men and 909 women participated in the survey conducted in 1998-1999	• Traditional food items including animal liver were less frequently consumed among individuals at 15-40 yr old than elderly Inuit. A higher proportion of persons among the 15-40 yr old showed sub-optimal intake level (<%EAR) of Vitamin A.
5 Dene/Métis and Yukon communities of the Northwest Territories (Nakano, et al., 2005)	222 local children of 10-12 yr old were interviewed in the winter and fall of 2000-2001	• Traditional foods contributed to only an average of 4.3%-4.7% of energy • Among market foods, those with less nutrient density such as fat, sweet and mixed dished contributed to more than half of the energy • Children in the more northern communities consumed more traditional foods (P<.05), protein, iron, copper, vitamin B6 and manganese, but less energy, fat, saturated fat, sodium

Study	Design	Results
11 communities in Alaska (Nobmann, et al., 1992)	24-h recall during 5 seasons over a 18-month period among 351 Alaska native adults aged at 20-63 yr during 1987-1988	• Saturated fat intake mainly came from Eskimo ice cream (shortening mixed with berries), beef and butter

Table 2.5. Fatty acid composition of blood and blood lipid fractions between Canadian Arctic & Sub-arctic Indigenous People and reference population

Study	Design	Results
Kivalliq, Canada (Young, et al., 1999)	To measure plasma phospholipids fatty acid composition in 505 adults aged at 18-74 yr, including 379 Inuit, 95 Caucasians and 31 others, collected in a health survey during 1990-1991	• Ratio of Inuit/non-Inuit: 0.46 (95% CI: 0.40-0.53) for AA 0.65 (95% CI: 0.58-0.73) for n-6 1.37 (95% CI: 1.02-1.85) for EPA
Québec (Dewailly, et al., 2003)	1460 Québec residents, 917 James Bay Cree, 426 Nunavik Inuit, aged at 18-74 yr, with blood samples collected between 1990 and 1992	• Combined concentration of plasma EPA and DHA: 8.0% among Inuit, 3.9% among Cree, and 1.8% among Québec residents
Greenland (Dyerberg, et al., 1975)	To compare fatty acid composition of blood lipid fractions among 130 Greenland Inuit, 32 Danish Inuit and 31 Danes whose blood samples were collected in the fall of 1970	• For fatty acid composition of plasma phospholipids among Greenland Inuit, Danish Inuit and Danes were: 6.6%, 22.6% and 21.0% respectively for LA 0.8%, 1.3% and 8.0% respectively for AA 7.0%, 0.7% and 0.2% respectively for EPA

Study	Design	Results
Greenland (Cote, et al., 2004)	To measure plasma fatty acid composition of 153 Greenland women in year 2000 (age, 55.3±4.4 yr)	• Total n-3 fatty acids was 13.8% (95% CI: 13.1-14.6%) • The relative concentration of EPA was 7.2% and DHA was 4.7% • The ratio of n-3/n-6 was 0.67
Greenland (Deutch, et al., 2004)	250 Inuit from 3 communities with blood samples collected in year 2000	• The plasma n-3/n-6 ratio, LA (%), AA (%), EPA(%) and DHA(%) among the 3 communities: 0.70, 14.25±4.09, 4.68±0.98, 5.48±3.44, 5.89±1.35 0.37, 19.22±3.86, 5.79±1.10, 2.64±2.01, 5.05±1.68 0.45, 19.16±3.39, 4.81±0.92, 3.40±2.22, 5.91±1.58
Greenland (Deutch, et al., 2007)	500 Inuit randomly selected in each of the 6 Greenland districts, whose blood samples were collected during 1999-2005	• Plasma n-3/n-6 was 0.48±0.34 among men, 0.39±0.21 among women • Plasma n-3 was 10.54±4.71% among men, 9.69±3.61% among women

Study	Design	Results
Greenland, Demark and Guelph, Canada (Stark, et al., 2002)	A clinical trial about fish oil supplementation on 15 Greenland Inuit women and 16 non-native Canadian women aged at 45-65 yr	• Baseline fatty acid composition (%) of serum phospholipids in Greenland Inuit: 13.97±0.86 for LA, 5.24±0.25 for AA, 4.90±0.54 for EPA, 7.89±0.39 for DHA, 0.73±0.08 for n-3/n-6 • The baseline fatty acids profile (%) of serum phospholipids in Canadian women: 18.40±0.49 for LA, 10.03±0.28 for AA, 1.30±0.18 for EPA, 4.23±0.28 for DHA, 0.21±0.02 for n-3/n-6
Alaska (Parkinson, et al., 1994)	To compare fatty acids concentration of plasma and platelet collected in September and October, 1984 from 80 Alaska Inuit and 26 non-indigenous people.	• Alaska Yupik showed that their total plasma n-3 fatty acids, EPA and DHA were 4.4, 13 and 6.8 times higher than those of non-indigenous group • EPA/eicsoatetraenoic acid was higher in coastal-villages than in river-village participants.

Study	Design	Results
Alaska (Bersamin, et al., 2008)	To compare reported dietary intake among 530 Yup'ik with their relative concentration of fatty acids in red blood cell membranes	• Red blood cell membranes (self reported traditional food intake at the 1st quintile *vs* at the 5th quintile) EPA (1.75±0.13% *vs* 3.68±0.15%) C22:6 n-6 (5.72±0.14% *vs* 7.19±0.16%) LA (13.09±0.19% *vs* 10.72±0.23%) AA (9.35±0.15% *vs* 8.38±0.18%)

Bridge 1

In animal models, a growing body of evidence has suggested that adipose tissue is an independent regulator of Δ9 via the adipose tissue derived hormone leptin, which was shown to suppress the transcription of Δ9. The MUFA products of Δ9, particularly oleic acid, are related to the assembly of VLDL that transports hepatic fat for storage into extra-hepatic tissues such as adipose tissue. Evidence for such a hypothesized pathway, however, had not been closely examined in the human context. Although human observational studies have demonstrated positive associations between adiposity measures and Δ9, it has not been clear whether the observed association was independent of insulin, which is known for its influence on fatty acid metabolism. Although estimation of the activity of Δ9 by tissue ratios of daughter fatty acids to parent fatty acids is a practical approach in humans, such estimates can be easily biased by dietary intake because tissue fatty acids represent both *de novo* lipogenesis and dietary intake. Thus, to overcome limitations of previous human studies it is necessary to adjust for insulin action and dietary intake in the examination of the associations of Δ9 with adiposity. Female adolescents from a Montreal gestation cohort provided a healthy and young study population to study the above associations, particularly since this cohort would be less likely to be biased by chronic diseases that impact fatty acid metabolism.

Chapter 3. MANUSCRIPT 1

The association of desaturase 9 and plasma fatty acid composition with insulin resistance associated factors in female adolescents

Yuan E. Zhou[a], Grace M. Egeland[a], Sara J. Meltzer[b], Stan Kubow[a]

Author affiliation:

[a] School of Dietetics and Human Nutrition, McGill University, H9X 3V9 Montreal, Canada

[b] Division of Endocrinology and Metabolism, Royal Victoria Hospital, H3A 1A1, Montreal

This manuscript has been published in *Metabolism*

Zhou YE, Egeland GM, Meltzer SJ, Kubow S. The association of desaturase 9 and plasma fatty acid composition with insulin resistance-associated factors in female adolescents. Metabolism. 2009; 58(2): 158-66

This study was supported by Canadian Institute of Health Research (CIHR) grant

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3.1. Abstract

Desaturase 9, which converts SFA into MUFA, is an important component in leptin mediated energy homeostasis in rodent models. Few human studies, however, have been performed regarding the clinical relevance of Δ9, particularly whether Δ9 is involved in the relationship between blood fatty acid profiles and insulin resistance associated factors. The aim of the present study was to examine fatty acid data from 178 apparently healthy female adolescents whether: (a) Δ9 has independent associations with adiposity, insulin resistance level and fasting plasma PUFA; (b) Δ9 is a predictor of fasting blood lipid profile; (c) the associations between fasting plasma fatty acid component and insulin resistance level are independent of abdominal obesity level. Δ9-16 (surrogate of Δ9 as calculated by plasma ratio palmitoleic acid/palmitic acid) correlated with waist girth ($r = .160$, $P < .05$), HOMA-IR ($r = .201$, $P < .01$), plasma PUFA [e.g., AA ($r = -.269$, $P < .001$), DHA ($r = -.274$, $P < .001$)]. After adjustment for dietary SFA, Δ9-16 had stronger correlation with waist ($r = .227$, $P < .01$) and significant correlation with PUFA, whereas it had a non-significant correlation with HOMA-IR. The same pattern was observed with Δ9-18 (surrogate of Δ9 as calculated by plasma ratio oleic acid/stearic acid). After adjustment for dietary SFA, waist and HOMA-IR, Δ9-16 and Δ9-18 were still positive predictors of triglyceride (TG) (both $P < .001$) and Apo B (Δ9-18, $P < .001$; Δ9-16, $P = .052$). After adjustment for waist, HOMA-IR only remained a positive determinant of medium chain SFA [myristic acid, $P < .001$; palmitic acid, $P < .05$], but it emerged to be inversely related to AA ($P < 0.1$). The positive and independent associations of medium chain SFA with insulin resistance level suggest their vital roles in diabetes pathogenesis, while certain PUFA such as AA appears to be protective. The observed associations of Δ9 with adiposity and plasma lipid profile in these apparently healthy female adolescents support the concept derived from rodent models that Δ9 activity is independently reflective of higher BMI and higher circulatory TG levels.

3.2. Introduction

Disturbed lipid metabolism is an important component of the insulin resistance syndrome (McGarry, 2002). Dyslipidemia is detectable even before the appearance of fasting or postprandial hyperglycemia, suggesting the altered lipid metabolism occurs long before T2D fully develops (Lewis, et al., 2002).

The role of the fatty acids in the modulation of insulin resistance is supported by animal models (Kusunoki, et al., 2006). In addition, there is now evidence that biosynthesis of fatty acids is also related to insulin resistance and adiposity. Fatty acid profile of plasma, serum and erythrocytes reflects both dietary intake and modulation by endogenous synthesis. The activity of Δ9 has been positively associated with insulin action (Vessby, et al., 2002). In cross-sectional studies, serum medium chain saturated fatty acids were positive determinants of insulin resistance measures in both males and females over a wide range of glucose tolerance (Folsom, et al., 1996)(Lovejoy, et al., 2001). In longitudinal studies, where a middle aged male population was followed for 10 years and another elderly male population was followed for 4 years, serum saturated fatty acids were predictors of the development of impaired fasting glycaemia and T2D (Laaksonen, et al., 2002)(Vessby, et al, 1994). To date, however, previous human studies have not examined whether insulin action and associated factors predict fatty acid composition in blood.

In addition to the insulin-inducing action on Δ9 well demonstrated in various rodent models (Vessby, et al., 2002), mouse models with gene mutation or knock-out have also demonstrated that Δ9 is closely under the regulation of leptin (Cohen & Friedman, 2004)(Cohen, et al., 2002), indicating the role of adiposity is not negligible. Moreover, dietary PUFA supplement inhibited the gene expression of murine liver Stearoyl-CoA desaturase 1 (*SCD*-1) which is equivalent to Δ9 in human (Ntambi, 1992), and this repression effect was independent of insulin (Waters & Ntambi, 1996). High carbohydrate intake induced the elevated level of palmitoleic acid and Oleic acid in both mice (Miyazaki, et al., 2001) and human (Mangravite, et al., 2007). The aforementioned evidence suggests that besides

insulin, other regulatory factors also exist, which modify the activity of desaturases to influence the plasma fatty acid composition. Furthermore, evidence from mouse studies have demonstrated that *SCD*-1 knock out mouse showed impaired biosynthesis of hepatic cholesterol esters and triglycerides (TG) (Miyazaki, et al., 2000), suggesting that Δ9 could exert an important influence on lipid metabolism. Despite abundant animal studies examining indicating an important role of Δ9 affecting insulin, energy and lipid metabolism, human studies are limited. A positive association between adiposity and Δ9 was reported in several recent studies on elderly people (Warensjö, et al., 2005), middle age population (Warensjö, et al., 2006) and children (Okada, et al., 2005). A key limitation of the latter studies was that the observed association was not adjusted by dietary saturated fatty acids (SFA) intake that could affect the ratios palmitoleic acid /palmitic acid (Δ9-16) and oleic acid /stearic acid (Δ9-18), the surrogate measures of Δ9 used in these studies. Moreover, no previous human studies have examined for associations of Δ9 with either dietary polyunsaturated fatty acids (PUFA) intake or plasma PUFA.

In the present study, we investigated the cross sectional associations of fasting plasma fatty acid profile and Δ9 with insulin resistance indices, blood lipids, adiposity and dietary SFA intake in apparently healthy teenage girls. Young adolescents were chosen to potentially provide new insights regarding the early mechanisms that underlie the development of metabolic disorders in later life and as adolescents are relatively free of confounders compared to the previously studied elderly population. The tested hypotheses were that in apparently healthy adolescent females: (1) a positive association of plasma medium SFA and HOMA-IR exists independent of abdominal adiposity; (2) Δ9 and abdominal adiposity are positively associated independently of insulin resistance and dietary SFA intake; and (3) Δ9 is positively associated with blood lipids independent of the insulin resistance, abdominal adiposity and dietary SFA intake.

3.3. Subjects and methods

3.3.1. Subjects

The study population consists of adolescent girls (n=189) from the Montreal mother-daughter gestational diabetes mellitus (GDM) case-control cohort (Egeland & Meltzer, 2008) (Appendix A: Egeland GM and Meltzer SJ, submitted). Mother daughter pairs affected or unaffected by GDM that resulted in singleton term (37-42 weeks) deliveries at the Royal Victoria Hospital (Montreal, Canada) in 1989-1991 were recruited for a 15-year follow-up evaluation. Pregnancies with pre-existing or pregnancy-related medical complications were excluded to ensure no confounding influence of other diseases. Eligible mother-daughter pairs were identified from patient records and the McGill Obstetrics and Neonatal Database (MOND). Controls were frequently matched by 4 maternal age groups and 4 socioeconomic strata based on postal code census income at the time of delivery. Among the case control cohort of 189 mother daughter pairs, fatty acid data were available for 179 adolescent subjects. One of these 179 subjects was excluded due to her diabetic status, thus, there were 178 adolescents' fatty acid data used for data analysis. The study was approved by the ethical review board of the McGill University Health Center and informed consent was obtained from all participants.

3.3.2. Anthropometric and biochemical measures

Subjects came to the Royal Victoria Hospital in the morning after an overnight fast. Height was measured without shoes to nearest centimeter using a stadiometer. Waist girth was assessed at the end of exhalation by using a standard tape measuring the level midway between the costal margin and the iliac crest when the subjects were standing. Circumference was measured to the nearest 0.1 cm. For each subject, the measurements were repeated three times, the average of values were presented and used in analyses. Weight and the percentage of body fat (BF) were evaluated by bioelectrical impedance (Tanita TBF-310, Tanita Corporation, Tokyo, Japan). Body mass index (BMI) was calculated as weight (kg) / height^2 (m). Fasting plasma was used for the fatty acid analysis and fasting

serum for biochemical tests on TG, Apo B, LDL-cholesterol, HDL-cholesterol, hemoglobin A1c.

Glucose was analyzed on the Beckman-Coulter LX20 analyzer (Beckman Instruments, Fullerton, CA) using the glucose oxidase technique, insulin by an immunometric assay with chemiluminescent detection on a DPC Immulyte Immunoanalyzer (Diagnostic Products Corporation, Los Angeles, CA, USA) and hemoglobin A1c was measured using a Roche Cobas Mira analyzer (Roche, Laval, QC, Canada) with the reagent Roche Unimate 3 hemoglobin A1c. Lipid profiles were performed using the Beckman-Coulter LX20 analyzer. ApoB was measured using an immunonephelometric technique on a Beckman-Coulter Image nephelometer (Beckman Instruments, Fullerton, CA)

3.3.3. Fatty acid analysis

A one-step extraction and transesterification method was applied instead of a traditional fatty acid analysis procedure which requires multiple steps (Lepage & Roy, 1986)(Masood, et al., 2005). Briefly speaking, 50 μL plasma samples were added into 10 mL screw cap tubes containing 1.9 mL run solution which consisted of 1.7 mL BHT-methanol, 100 μL acetyl chloride and 100 μL internal standard. After waterbath heating at 100°C for 1 h, 1.0 mL hexane was added to each tube. The tubes were then vortexed for 30 sec and the upper organic phase was collected. The extract was evaporated under nitrogen, re-dissolved in hexane, and transferred to GC vial inserts for the test on a 100 m × 0.25 mm I.D. × 0.25 μm Varian CP-select CB for FAME fused-silica capillary column in a Varian 3400 CX gas chromatograph equipped with a flame ionization (Varian Inc., Pala Alto, CA). The temperature program was as follows: initial, 80ºC with a 1 min hold; ramp: 30ºC/min to 180ºC, 1ºC/min to 196ºC, 20ºC to 230ºC with a 15 min hold and 30ºC/min to 270ºC with a 8 min hold. The detector temperature was set at 275ºC and injector temperature at 250ºC. The carrier gas was helium set at a flow rate of 2 mL/min. The signal:noise ratio was set at 3 allowing a method detection limit of 8.6×10^{-4} mg/mL. The fatty acids were identified by comparing each peak's retention time with those of methyl ester standards (GLC-6923

containing 31 FAMEs plus added lauric acid and C18:3 FAMEs, Nu-Check Prep Inc.). Seronorm^{TM} lipid (SERO AS, Billingstad, Norway) containing 23 fatty acids of animal origin was used as an additional external control. Data was collected in a Varian Star 3400 CX gas chromatograph (Varian, Montreal, Canada) and Saturn W/S Ver 5.4.1 (Integration) software (Varian Inc., Pala Alto, CA). The relative amount of each fatty acid was quantified by integrating the area under the peak and dividing the results by the total area for all fatty acids. Due to the fact that the C18:1 trans isomers appeared as a series of overlapping peaks which the Saturn program was unable to separate to quantify, thus, the C18:1 trans isomers (t6, t9 and t11) were identified and integrated as one broad peak (C18:1t total).

Inter-assay coefficients of variation (CV) percentages were assessed by analyzing duplicate standard references during each run of fatty acid analysis. The overall CV percentages of the most abundant saturated fatty acids and monounsaturated fatty acids in plasma were less than or around 10%: myristic acid5.51%, palmitic acid 9.26%, stearic acid 10.40%, palmitoleic acid 8.92%, oleic acid 10.70%. Inter-assay CV percentages of primary polyunsaturated fatty acids are: LA 10.27%, AA 18.00%, EPA 12.19%, DHA 18.53%.

3.3.4. Statistical analysis

Data are presented as mean (SD) if normally distributed, and median (interquartile range) if not normally distributed. Fatty acid in plasma was expressed as a percentage of total fatty acids in plasma. SFA, MUFA, omega 3 fatty acids (n-3 PUFA), omega 6 fatty acids (n-6 PUFA), and trans fatty acids (TFA) were calculated by summing the concentrations of individual fatty acids if they were detectable.

The use of the palmitoleic acid/palmitic acid and oleic acid/stearic acid ratios as crude surrogate measures of the activity of Δ9 is well established in human studies since direct measure of enzyme activity is not normally feasible. As expected, in the current study palmitoleic acid/palmitic acid and oleic

acid/stearic acid are related to the fasting plasma MUFA profile since the Spearman correlation coefficients between the surrogate markers of Δ9 and MUFA were highly positively correlated (data not shown). A major limitation to the use of palmitoleic acid/palmitic acid and oleic acid/stearic acid ratios as indices of metabolic activity of Δ9 is the inherent bias of medium chain SFA intake. To circumvent this latter confounding influence, endogenous medium chain SFA was adjusted according to levels of pentadecanoic acid. This latter odd number carbon atom fatty acid is not synthesized by humans and is obtained from dairy fat and ruminant meat products (Smedman, et al., 1999). In the linear regression models, pentadecanoic acid was a predictor for the predicted variables of palmitoleic acid/palmitic acid and oleic acid/stearic acid ratios. One may regard the residuals of these ratio as that which can not be explained by dietary intake, i.e., the remaining endogenous product. Similar statistical approach is widely applied in other epidemiological studies (Baylin, et al., 2005) to assess the importance of other predictors after adjustment for one major predictor. Though it is only able to partly remove the effect of dietary intake due to a variety of dietary sources of palmitic acid and C16:1, and the variability of fatty acid composition in foods, this attempt allows for a closer estimate to the true endogenous levels of palmitic acid and C16:1. Both Δ9-16 and Δ9-18 were calculated. Although oleic acid is important in terms of maintenance of normal physiological functioning such as membrane fluidity and lipoprotein metabolism, its abundant existence in food makes it more difficult to estimate its endogenous levels. In contrast, Δ9-16 represents potentially a more accurate measure of Δ9, since palmitoleic acid is mainly derived from endogenous pathways.

Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR), calculated as follows: HOMA-IR = [Fasting insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5]. HOMA-IR was used since this measure is suggested to be applicable for a broad range of possible co-effects of insulin resistance and beta cell function and across ethnic groups (Wallace, et al., 2004). Interactions of mother's gestational diabetes status on the

associations of Δ9 with anthropometric measures and glucose homeostasis indices were tested with general linear models. Because there were no observed interactions, the results of associations of Δ9 and fasting plasma fatty acid profile with measures of interest are presented for all daughters pooled into one group. Spearman correlations were conducted for the correlations of Δ9 with adiposity measures, HOMA-IR and PUFA. Student's t test was used to examine the significance of Spearman correlation coefficients before and after adjustment (Hittner & May, 1998)(Kutner, et al., 2005). Multivariable linear regression was used to evaluate whether the associations of the two unadjusted Δ9 ratios and the two Δ9 ratios adjusted for pentadecanoic acid with TG and lipoprotein would remain when adiposity level and HOMA-IR were entered as covariates. Because of their skewed distribution, HOMA-IR, fasting insulin and TG were log transformed before correlation and regression analysis. All P values were obtained from 2-sided tests. Data were analyzed with the SAS software (version 9.1; SAS Institute, Cary, NC).

3.4. Results

3.4.1. Subjects' characteristics

The mean age of the female adolescents was 15.3 (SD = 0.7) years, and their mean BMI was 22.9 (SD= 4.4) (Kg/m^2), mean waist was 77.9 (SD=10.5) cm. Majority of participants were Caucasian (77.8%). Other anthropometric and biologic characteristics are presented elsewhere (Egeland & Meltzer, 2008). All the subjects had at least one menses to be eligible to participate into the study.

Desaturase 9 and fatty acid profiles of fasting plasma of the two groups were unrelated to mothers' previous GDM status (Table 3.1.). Significant differences were only found for palmitic acid and C18:1t ($P < .05$); however, the differences were less than 1%, and of no clinical significance.

3.4.2. Fatty acid profile of fasting plasma, glucose homeostasis measures and adiposity

After adjustment for waist girth, fasting insulin and HOMA-IR were significantly and positively associated with median chain saturated fatty acids: myristic acid, pentadecanoic acid and palmitic acid (Table 3.2.). Conversely, the significant positive association of fasting insulin and HOMA-IR with palmitoleic acid disappeared after adjustment for waist girth. For n-3 PUFA, fasting glucose was a negative predictor of EPA ($P < .01$), and total n-3 fatty acids ($P < .01$).

For n-6 PUFA, fasting insulin and HOMA-IR inversely predicted total n-6 fatty acids and AA (Table 3.2.) after adjustment for waist girth. Fasting insulin and HOMA-IR were inversely predictive of LA, however, after adjustment for waist girth, the associations were not significant ($P > .05$). In contrast, the associations of fasting insulin and HOMA-IR with AA were only significant (for fasting insulin, $P < 0.05$; for HOMA-IR, $.05 \leq P \leq .10$) after adjustment for waist girth.

3.4.3. Δ9, dietary saturated fat intake, adiposity, insulin resistance level and PUFA, lipids and lipoprotein in fasting blood

Both unadjusted Δ9-16 and Δ9-16 adjusted for pentadecanoic acid were positively correlated with adiposity measures, but adjusted Δ9-16 was more strongly correlated with BMI, %body fat and waist girth than unadjusted Δ9-16 ($P < .05$). On the other hand, the significant ($P < .01$) correlation between Δ9-16 and HOMA-IR disappeared after adjustment for dietary SFA intake (Table 3.3.). The Δ9-18 showed a similar pattern of changes of the correlations with adiposity measures and HOMA-IR with and without adjustments (Table 3.3.). Both Δ9-16 and Δ9-18 were strongly negatively correlated ($P < .001$) with the primary plasma PUFA both before and after adjustment for pentadecanoic acid, particularly for LA, but not for the relationship between Δ9-16 and EPA.

The medium chain saturated fatty acid, pentadecanoic acid was highly correlated with other medium chain fatty acids such as palmitic acid ($r = .501$, $P <$

.001) and C16:1 (r =.424, P < .001), which can be explained by the common food source from which they originate. For example, as shown in Table 3.4., before adjustment, in the regression model with Δ9 as a predictor of TG, the $\beta_{\Delta 9\text{-}16}$ was 4.222 and the $\beta_{\Delta 9\text{-}18}$ was 0.254. As Δ9-16 and Δ9-18 represent activity of the same desaturase enzyme, their predictive values should be similar. As expected, after adjustment for pentadecanoic acid, the beta coefficients for Δ9-16 and Δ9-18 were similar (0.066 versus 0.096, respectively). Similar patterns were also observed in the regression model for the other plasma lipids (Table 3.4.). Also as was expected, β-coefficients of pentadecanoic acid for fasting insulin and HOMA-IR were weaker relative to other saturated fatty acids (Table 3.2.), given that pentadecanoic acid is an unlikely factor in the causal pathway between Δ9 and either insulin resistance or adiposity. In addition, the associations of pentadecanoic acid with adiposity measures and glucose homeostasis index were in consistent with those of other median chain SFA. As expected, no significant correlations were observed between pentadecanoic acid and adiposity measures [e.g., BMI (r = -.081, P = .283), waist girth (r = -.070, P = .355), body fat% (r = -.045, P=0.554)]. Also, weak correlations of pentadecanoic acid with fasting insulin [r = 0.214, P=0.005] and HOMA-IR [r = 0.200, P=0.009] were observed and there was no significant correlation between pentadecanoic acid and fasting glucose [r = -0.032, P=0.670].

Adjustment for saturated fat intake did not change the significance or magnitude of the associations of Δ9-16 and Δ9-18 with plasma lipoprotein profiles (Table 3.4.). Both adjusted and unadjusted Δ9-16 and Δ9-18 were strong predictors of TG (P < .001), independent of waist girth and HOMA-IR. Likewise, both adjusted and unadjusted Δ9-18 was a pronounced predictor of Apo B and HDL (P< .001).

3.5. Discussion

The present study is the first study conducted in a human context to examine and demonstrate important relationships between Δ9 and certain key metabolic indices, which previously have only been noted in rodent models (Cohen & Friedman, 2004)(Ntambi & Miyazaki, 2004). In that regard, a positive relationship was shown between Δ9 and adiposity and a negative association of Δ9 to plasma PUFA was observed, which occurred independently of dietary saturated fat intake. The present work also demonstrated novel associations between Δ9 and blood lipid profiles, which remained significant after the adjustment of adiposity, dietary saturated fat intake and insulin resistance level. The present findings thus showing important metabolic associations with Δ9 indicate that this enzyme activity could be also clinically relevant in the adolescent female population.

As hypothesized, positive associations were shown between Δ9 and all adiposity measures, including waist girth, BMI and body fat%. The above associations became even stronger after adjustment of dietary saturated fat intake, suggesting that these associations were not spurious due to confounding effects of saturated fat intake. In contrast to the strong positive association between adiposity and Δ9, the weak positive associations between Δ9 and HOMA-IR became non-significant after the adjustment for pentadecanoic acid, suggesting that the latter association was largely mediated by saturated fat intake.

It is conceivable that the present findings might reflect a regulatory role of adipose tissue derived hormones such as leptin in suppressing Δ9. In rodent models, the gene of stearoyl CoA desaturase-1 (*SCD*-1), one of the four Δ9 gene isoforms predominantly existing in mice liver and highly homologous to human Δ9, was ranked on the top of the list of genes uniquely repressed by leptin (Cohen & Friedman, 2004). Mice lacking *SCD*-1 gene expression showed the lipodystrophy syndrome and were hyper-metabolic (Cohen, et al., 2002). Insulin is considered to be capable to induce *SCD*-1 through sterol regulatory element-

binding protein (SREBP)-1c mediated pathway (Heinemann & Ozols, 2003). The effect of leptin on *SCD*-1 in mice was apparently independent of insulin and SREBP-1c as leptin inhibited the transcription, translation and activity of hepatic *SCD*-1 regardless of blood insulin levels and expression levels of SREBP-1c (Biddinger, et al., 2006). This latter study indicates that insulin is subordinate to leptin in terms of regulating *SCD*-1, which is supported by the data of the present work which shows a strong relationship of Δ9 with adiposity relative to a non-significant association of Δ9 with HOMA-IR.

To our knowledge, the present study is the first study done in a human context to report negative associations of Δ9-16 and Δ9-18 with plasma PUFA, especially LA, AA and DHA. PUFA have been suggested to be involved in inhibiting expression of genes related to lipogenesis (Sampath & Ntambi, 2004). Therefore, fasting plasma PUFA could be indicative of down-regulation of the expression of genes related to Δ9. Since LA is an essential fatty acids and DHA is largely exogenously derived, these fatty acids reflect dietary intake levels and could indicate the capability of PUFA to downregulate Δ9-16 and Δ9-18. In support of this concept, previous mouse studies have shown that PUFA supplementation downregulates expression of hepatic *SCD*-1 (Ntambi, 1992). Similarly, diabetic mice supplemented with either (3% w/v) linolenic acid or (1% w/v) arachidonic acid exhibited repression of insulin-induced hepatic *SCD*-1 mRNA by 97% and 99%, respectively (Waters & Ntambi, 1996). In the present study, the association of PUFA with Δ9-16 and Δ9-18 remained significant after adjustment with HOMA-IR, also suggesting an insulin-independent effect of PUFA on Δ9. The repression of PUFA on lipogenesis may not only be hepatic specific, as inhibition of expression and activity of rat lipogenic enzymes in adipose tissue by corn oil has also been reported in adipose tissue, although the effects were less pronounced than those observed in the liver (Foufelle, et al., 1992).

The strong and positive associations of Δ9-16 and Δ9-18 with plasma TG and Apo B that were independent of adiposity, insulin resistance level and dietary

saturated fat are strongly supported by previous rodent studies showing that Δ9 is critical in the synthesis of hepatic cholesterol ester and TG, which plays a key role in the hepatic VLDL synthesis (Cohen & Friedman, 2004)(Ntambi & Miyazaki, 2004). Moreover, the transcript level of Δ9 in human adipose tissue has been reported to be positively associated with plasma TG (Mangravite, et al., 2007). The role of Δ9 in hepatic lipid synthesis is due to the role of Oleic acid, the major product of this enzyme, as the preferred substrate of acyl-CoA:cholesterol acyltransferase (ACAT) and diacylglycerol acyltransferase (DGAT), responsible for the synthesis of cholesterol ester and TG, respectively (Cohen & Friedman, 2004)(Ntambi & Miyazaki, 2004) . In that regard, *SCD*-1 knockout mice or mice lacking of *SCD*-1 expression showed deficient hepatic concentrations of TG and cholesterol and reduced hepatic production of VLDL (Cohen, et al., 2003)(Miyazaki, et al., 2000)(Miyazaki, et al., 2001). Conversely, in hyperlipidemic mice, a four-fold increase in hepatic *SCD* activity was accompanied by a two-fold increase in plasma TG (Attie, et al., 2002). Additionally, the expression and activity of Δ9 in primary human skeletal myocytes exhibited a strong negative correlation with fatty acid oxidation, and a strong positive correlation with intramuscular TG synthesis (Hulver, et al., 2005). Similarly, a two-fold increase in the ratio of human plasma oleic acid to stearic acid was shown to be accompanied by a four-fold increase in plasma TG concentrations (Attie, et al., 2002).

In this study, the fatty acid profiles in healthy adolescents are consistent with previous studies involving either obese children or middle aged and older adults showing that plasma medium chain SFA are positively associated with insulin resistance level and MUFA palmitoleic acid is positively associated with adiposity level (Okada, et al., 2005)(Warensjö, et al., 2005)(Warensjö, et al., 2006). In line with previous human studies (Clifton & Nestel, 1998)(Wang, et al., 2003), positive association was observed between plasma medium chain SFA and HOMA-IR in the present work. As compared to PUFA, SFA is less likely to be oxidized and more likely to accumulate in cells (Clarke, et al., 2002)(Manco, et al., 2004). Hence, the insulin resistant state could result in preferential oxidation

of PUFA, leading to the observed altered plasma profile of decreased PUFA and increased medium SFA. The association of dietary SFA intake with elevated insulin resistance is reported to be due to an altered membrane saturation degree, leading to altered bio-membrane fluidity and associated trans-membrane signaling pathways (Manco, et al., 2004). In the current study, the association of Δ9 with HOMA-IR was lost after the adjustment of SFA intake using pentadecanoic acid, further supporting the concept that dietary SFA is an important modifier of insulin resistance.

In this study, no significant association was observed between specific plasma n-6 fatty acids and insulin resistance indices such as HOMA-IR. On the other hand, a negative association between LA and insulin resistance indices was observed originally that was no longer seen after adjustment for waist girth (Table 3.2.), which was likely due to a strong inverse correlation between LA and waist girth (data not shown). The strong and negative association seen between the LA and adiposity in the present work is consistent with previously reported studies (Okada, et al., 2005)(Warensjö, et al., 2005)(Warensjö, et al., 2006). Increasing HOMA-IR accompanied by lower blood LA levels has been reported by other studies (Okada, et al., 2005)(Wang, et al., 2003)(Warensjö, et al., 2005)(Warensjö, et al., 2006); however, no mechanistic explanation has been given regarding this association. A possible explanation might be due to the indications that LA is an indicator of total fat consumption as seen from clinical trials comparing low fat vs. median fat diets (King, et al., 2006). Low LA intake has been related to relatively low total fat intake, which would lead to increased synthesis of endogenous medium chain saturated fatty acids, such as palmitic acid (King, et al., 2006). Therefore, the apparent negative association between low plasma LA and insulin resistance indices may be driven by the positive association of these indices with medium chain saturated fatty acids.

In contrast to LA, the originally non-significant negative associations of AA with both HOMA-IR and fasting insulin became marginally significant and significant respectively after adjustment of waist girth. These latter observations

are consistent with previous human studies that have reported a negative association of fasting insulin or HOMA-IR with AA levels in fasting serum, erythrocyte membrane and skeletal muscle membrane (Borkman, et al., 1993)(Clifton & Nestel, 1998)(Rodríguez & Christophe, 2005)(Samuelson, et al., 2001). As a critically important physiological PUFA fatty acid, AA has many unique physiological functions including its role as an ecosanoid precursor and the most abundant fatty acid in bio-membranes (Shils, et al., 2006). Besides dietary intake, it can be derived in *de novo* synthesis from homo gamma linoleic acid via Δ5, whose activity is positively associated with insulin sensitivity and negatively associated with insulin resistance (Vessby, et al., 2002). In the present study, Δ5 could not be computed by the ratio AA / homo gamma linoleic acid since homo gamma linoleic acid was non-detectable. The observed negative associations of AA with both HOMA-IR and fasting insulin, however, indicate a direct association between AA and insulin action.

n-3 fatty acids did not show significant associations with HOMA-IR or adiposity measures in this study population, apart from a positive association of ETA with HOMA-IR, which was not observed after adjustment for adiposity. On the other hand, a negative association was observed between fasting glucose and n-3 PUFA, which was largely based upon an inverse association between fasting glucose and EPA (Table 3.2.). n-3 PUFA supplementation trials by fish oil did not shown significant fasting plasma glucose lowering effects (Farmer, et al., 2001)(Nettleton & Katz, 2005). More studies are warranted to determine whether the observed association in the present study came by chance or it indicated that lower fasting glucose is a physiological condition that is modulated by altered endogenous n-3 PUFA metabolism.

A limitation of the present study was that the causal relationships between the variables of interest cannot be determined based on the cross-sectional data. Besides adiposity, insulin resistance and dietary factors, there could be other hormonal, genetic or metabolic factors that could impact upon fatty acid profiles and the activity of Δ9. Thus, there are a variety of other endogenous factors that

could account for the observed positive association between ETA and adiposity. In addition, as the current study was conducted in adolescent females, more studies are needed in order to confirm whether conclusions from the present study could be generalized to other populations. Furthermore, the impact of mother's gestational diabetes status on the daughter's fatty acid metabolism or on the fatty acid metabolism among mothers themselves is worthwhile for future study as available data from the current study were insufficient to detect such effects. Measures that could be improved for future studies include: (1) the use of glucose clamp or frequently sampled intravenous glucose tolerance tests which are more accurate than HOMA-IR to detect insulin resistance level and insulin sensitivity; and (2) application of dual energy X ray scanning (DXA), which is a more precise measure of body composition. Additionally, the state of puberty could be considered more closely to examine because it is an important determinant of insulin action during growth spurt. Another option for future studies would be the use of the fatty acid profiles of erythrocyte membranes as a surrogate marker of relatively long term dietary intake modified by *de novo* synthesis (Sun, et al., 2007).

In summary, the results of the current study suggest that, independent of insulin resistance level and dietary medium chain SFA intake, increased activity of Δ9 leading to higher endogenous synthesis of MUFA is reflective of higher BMI whereas dietary PUFA appear to inhibit Δ9 activity. On the other hand, high intake of saturated fat as assessed by plasma 15:0 content is associated with increased insulin resistance. Additionally, Δ9 is positively and strongly associated with circulatory TG levels, independent of adiposity, insulin resistance and dietary saturated fat intake. Overall, the present findings indicate the importance of maintaining a healthy body weight and balanced fat intake as early as adolescence in the maintenance of a healthy metabolic profile related to insulin activity and lipoprotein and fatty acid metabolism.

3.6. Acknowledgement

This study was supported by Canadian Institute of Health Research (CIHR) grant. We thank Sina Gallo and Donna Leggee for their contribution in fatty acids analysis. The participation of our subjects and the cooperation of their parents are also greatly appreciated.

Table 3.1. Mean proportion (% of total fatty acids) of fatty acids in fasting plasma of the study population [a]

	Control (n=96)	Case (n=82)	P
SFA	36.78 (1.79)	36.02 (2.10)	<.05
Lauric acid (C12:0)[b,c]	0.07 (0.08)	0.06 (0.05)	<.1
Myristic acid (C14:0)[b]	1.02 (0.61)	0.94 (0.65)	NS
Pentadecanoic acid (C15:0)[b]	0.27 (0.07)	0.26 (0.08)	NS
Palmitic acid (C16:0)[b]	24.11 (2.03)	23.33 (1.64)	<.05
Heptadecanoic acid (C17:0)	0.39 (0.05)	0.37 (0.06)	.05
Stearic acid (C18:0)	8.27 (0.78)	8.21 (0.86)	NS
MUFA	24.66 (2.53)	24.70 (2.29)	NS
Palmitoleic acid (C16:1 *n-7*)[b]	1.82 (0.85)	1.84 (0.63)	NS
Oleic acid (C18:1 *n-9*)	19.59 (2.15)	19.53 (1.97)	NS
TFA[b]	1.00 (0.58)	0.90 (0.40)	<.1
C18:1 *trans*[b,d, f]	0.82 (0.44)	0.74 (0.33)	<.05
C18:2 *trans*[e,f]	0.21 (0.11)	0.20 (0.07)	NS
n-6 PUFA[b]	32.15 (3.54)	32.64 (3.08)	NS
LA (C18:2 *n-6*)	23.57 (2.69)	24.07 (2.56)	NS
AA (C20:4 *n-6*)	7.70 (1.56)	7.70 (1.38)	NS
n-3 PUFA[b]	5.37 (1.22)	5.53 (0.82)	NS
ETA (C20:3 *n-3*)[b]	1.65 (0.43)	1.82 (0.50)	<.05
EPA (C20:5 *n-3*)[b]	0.53 (0.32)	0.53 (0.27)	NS
DHA (C22:6 *n-3*)[b]	1.64 (0.80)	1.72 (0.81)	NS
Δ9-16 [b]	0.08 (0.03)	0.08 (0.02)	NS
Δ9-18 [b]	2.34 (0.43)	2.35 (0.47)	NS

[a] All values are presented as mean (SD) unless otherwise indicated. NS, not significant.

[b] Data are not normally distributed, values are median (interquartile range).

[c] Control n=89, case n=72.

[d] Control n=95, case n=82.

[e] Control n=90, case n=78.

[f] C18:1 trans is the sum of all C18 trans fatty acids with one double bond, C18:2 trans is the sum of C18:2 ct, C18:2 tc and C18:2 tt.

Table 3.2. Glucose homeostasis indices as predictor of plasma fatty acids after adjustment for waist girth [a]

	Fasting glucose		Fasting insulin		HOMA-IR	
	β	± SE	β	± SE	β	± SE
SFA	0.028	0.409	1.973	0.715**[b]	1.823	0.690**
Lauric acid (C12:0)	-0.004	0.018	0.035	0.032	0.034	0.031
Myristic acid (C14:0)	-0.016	0.096	0.571	0.166***	0.527	0.160**
Pentadecanoic acid (C15:0)	5.0×10^{-4}	0.014	0.079	0.025**	0.074	0.024**
Palmitic acid (C16:0)	0.005	0.332	1.226	0.589*	1.123	0.568*
Stearic acid (C18:0)	0.137	0.170	0.148	0.310	0.173	0.299
MUFA	0.044	0.496	0.555	0.872	0.526	0.840
Palmitoleic acid (C16:1 *n-7*)	0.009	0.117	0.299	0.204	0.279	0.196
Oleic acid (C18:1 *n-9*)	0.105	0.430	0.547	0.759	0.535	0.730
TFA	0.058	0.090	0.051	0.145	0.059	0.139
C18:1 *trans*	0.068	0.077	0.024	0.123	0.037	0.119

C18:2 *trans*	-0.010	0.021	0.027	0.037	0.022	0.036
n-6 PUFA	0.429	0.635	-2.459	1.097*	-2.166	1.058*
LA (C18:2 *n-6*)	0.203	0.541	-1.144	0.945	-0.996	0.910
AA (C20:4 *n-6*)	0.301	0.308	-1.176	0.542*	-1.015	0.523#
n-3 PUFA	-0.492	0.204*	-0.169	0.371	-0.268	0.357
ETA (C20:3 *n-3*)	-0.057	0.072	0.161	0.130	0.135	0.125
EPA (C20:5 *n-3*)	-0.189	0.067**	-0.091	0.122	-0.131	0.117
DHA (C22:6 *n-3*)	-0.183	0.117	-0.116	0.212	-0.143	0.203

[a] All indices (except fasting glucose) are tested with the log-transformed values. HOMA-IR, homeostasis model assessment; C181t, the sum of trans fatty acids with C18 and one double bonds; C182t, the sum of C18:2ct and C18:2tc.

[b] # .05 ≤P < .10, *P < .05, **P < .01, ***P < .001.

Table 3.3. Spearman correlation coefficients of Δ9 ratios with indices of adiposity and insulin resistance and PUFA before and after adjustment of dietary SFA intake [a]

	Δ9-16 (adjusted)	Δ9-16	P [e]	Δ9-18 (adjusted)	Δ9-18	P
BMI	0.268*** [f]	0.204**	<.05	0.185*	0.147[#]	<.1
% Body fat	0.251***	0.197**	<.10	0.093	0.071	NS
Waist girth	0.227**	0.160*	<.05	0.138[#]	0.106	NS
HOMA-IR[b,c]	0.109	0.210**	<.01	0.050	0.096	<.05
Fasting glucose	0.059	0.068	NS	-0.032	-0.030	NS
Fasting insulin[b,c]	0.093	0.195[#]	<.01	0.051	0.099	<.05
PUFA[d]	-0.500***	-0.682***	<.001	-0.450***	-0.568***	<.001
LA (C18:2 *n-6*)	-0.495***	-0.593***	<.001	-0.259***	-0.321***	<.01
AA (C20:4 *n-6*)	-0.127[#]	-0.269***	<.001	-0.348***	-0.445***	<.001
EPA (C20:5 *n-3*)	-0.075	-0.046	NS	-0.231**	-0.212**	NS
DHA (C22:6 *n-3*)	-0.187*	-0.274***	<.01	-0.118	-0.166*	<.05

[a] n=178 unless otherwise indicated. Δ9-16 (adjusted) and Δ9-18 (adjusted) are residuals of Δ9-16 and Δ9-16 corrected for pentadecanoic acid.

[b] n=170.

[c] Data are logistic transformed.

[d] PUFA polyunsaturated fatty acids.

[e] Significant difference between the unadjusted and adjusted correlation coefficients.

[f] Actual significances of spearman correlations of Δ9 with the parameters on the left hand side of the table. [#] .05 ≤ P< .10, *P < .05, **P < .01, ***P < .001.

Table 3.4. Δ9 ratios as predictor of fasting plasma TG and main lipoprotein and Apo B[a]

	Δ9-16 (adjusted)			Δ9-16			Δ9-18 (adjusted)			Δ9-18		
	β	± SE	R^2	β	± SE	R^2	β	± SE	R^2	β	± SE	R^2
TG[b]	0.066	0.014***[d]	0.15	4.222	0.656***	0.23	0.096	0.012***	0.31	0.254	0.027***	0.38
Apo B[c]	0.022	0.013[#]	0.03	1.242	0.634[#]	0.04	0.047	0.012***	0.10	0.115	0.028***	0.11
LDL	0.075	0.049	N/A	3.913	2.342[#]	0.02	0.108	0.045*	0.04	0.266	0.105*	0.04
HDL	-0.006	0.021	N/A	-0.790	1.00	N/A	-0.070	0.019***	0.07	-0.173	0.044***	0.07
CH	0.121	0.054*	0.03	6.480	2.572*	0.04	0.118	0.050*	0.04	0.302	0.117*	0.04

CH=cholesterol

[a] n=170. All values are β estimates ± SEs, adjusted for waist girth and HOMA-IR.

[b] Data are logistic transformed.

[c] n=167.

[d] [#].05 ≤ P < .10, *P < .05, **P < .01, ***P < .001.

Bridge 2

Manuscript 1 showed that the positive correlation between waist circumference and Δ9 is independent of dietary saturated fat intake among apparently healthy female adolescents. This result supports the argument that adiposity is a regulator of Δ9, a desaturase catalyzing the synthesis of MUFA. Also, **Manuscript 1** demonstrated that the positive correlation between waist circumference and Δ9 is independent of the correlation between Δ9 and insulin resistance level (HOMA-IR) as the latter relationship was driven by dietary saturated fat intake. The above findings are in agreement with results from rodent models showing that Δ9 activity is directly related to adiposity via adipose derived hormone leptin, which regulates Δ9 transcription (Cohen & Friedman, 2004). In addition, our findings showing that Δ9 is a predictor of plasma TG levels, independently of dietary saturated fat intake, adiposity and insulin resistance, are also consistent with the concept that Δ9 is a mediator in the leptin-regulated energy homeostasis pathway, Taken together, data presented in **Manuscript 1** strongly support the independent regulation of adiposity on Δ9. In addition to Δ9, results from a limited number of rodent studies have indicated that adiposity and insulin resistance can regulate the activity of Δ5, a desaturase enzyme catalyzing the synthesis of HUFA. Human studies are warranted to evaluate whether the regulation of adiposity on Δ5 can be extended to the human context. The Cree of James Bay, who reside in northern Québec, traditionally consumed a variety of fish and terrestrial mammals rich in HUFA n-3. Reduced consumption of the traditional food has been noted among the Cree in recent decades together with an emergence of health risks such as obesity and T2D. The following study examined the interplay between obesity, insulin resistance and the activity of Δ5 in terms of HUFA n-3 status of Cree, which might provide new insights regarding the health transition occurring in this population.

CHAPTER 4. MANUSCRIPT 2

Decreased activity of desaturase 5 in association with obesity and insulin resistance aggravates declining long chain omega 3 fatty acid status in Cree undergoing dietary transition

Yuan E. Zhou[a], Stan Kubow[a], Eric Dewailly[b], Pierre Julien[b], Grace M. Egeland[a*]

Author affiliation:

[a] School of Dietetics and Human Nutrition, McGill University, H9X 3V9 Montreal, Canada

[b] Unite de Recherche en Sante publique. Centre de Recherche du CHUQ, Universite Laval, G1V 2M2 Québec, Canada

This manuscript has been accepted by the *British Journal of Nutrition*

Zhou YE, Kubow S, Dewailly E, Julien P, Egeland GM. Decreased activity of desaturase 5 in association with obesity and insulin resistance aggravates declining long chain omega 3 fatty acid status in Cree undergoing dietary transition. Br J Nutr. 2009; 102(6): 888-894

This project was funded by the Niskamoon Corporation.

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4.1. Abstract

Objective: Emerging evidence shows that Δ5, the key regulator in the synthesis of highly unsaturated long chain fatty acids (HUFA), is modulated by factors including adiposity, diet and insulin resistance.

Design: We explored the association of these factors in a cross-sectional study within a high risk Cree population. Anthropometric measures and fasting blood glucose and insulin were analyzed. Δ5 was estimated as the ratio of AA to homo gamma linoleic acid on erythrocyte membrane.

Setting: Mistissini community, Cree Territory of Québec, Canada

Subjects: 98 female, 68 male, 20-88 years of age.

Results: Obesity (BMI≥30 kg/m^2) was prevalent across age groups. Δ5 was inversely associated with BMI [Spearman's correlation coefficient (r_s) = -0.175)] and positively associated with age (r_s=0.593, P<0.0001), which was driven by age-related increases in dietary intake of n-3 fatty acids and decreases in homo gamma linoleic acid. HOMA-IR was significantly inversely associated with Δ5 in age-adjusted linear regression analyses in normoglycemic individuals (β=-.127 (0.059), P=0..03), whereas no association was observed among glucose intolerant individuals (interaction term P=.03). In contrast, there were no significant interactions indicating differences in the slope for each of the adiposity measures in their associations with Δ5.

Conclusions: This study indicates that the dietary transition of reduced consumption of fish among younger Cree may compound the effects of obesity and emerging insulin resistance which, in turn, could reduce bioavailability of HUFA n-3 (through reduced Δ5 activity). Also, the study suggests that disease progression is an important consideration when evaluating correlates of Δ5 activity in observational studies.

4.2. Introduction

Consequences of obesity include insulin resistance, dyslipidemia, hypertension and the development of overt diabetes and cardiovascular disease with reduced life expectancy (Olshansky, et al., 2005). There are multiple obesity-mediated pathways that are known and/or suspected to contribute to the influence of adiposity on metabolic disturbances such as excessive free fatty acid released from adipose tissue and its inhibitory effect on tissue insulin sensitivity, or a low grade inflammation related to obesity (Ronti, et al., 2006). Fatty acids, particularly highly unsaturated fatty acids (HUFA), are considered as important factors associated with a variety of obesity-related illnesses. HUFA are important for the maintenance of normal bio-membrane structure and function (Torrejon, et al., 2007) and are precursors of eicosanoids that have important physiological properties affecting numerous cardiovascular, immune and cellular secretory functions (Shils, et al., 2006). Additionally, HUFA are key regulators of many genes involved in controlling lipid homeostasis and may thereby help to lessen dyslipidemia (Torrejon, et al., 2007). Further, the anti-arrhythmic action of n-3 HUFA is considered to be protective against myocardial infarction and ischemic heart disease via increasing cell membrane fluidity (Harris, et al., 2008), influencing membrane ion channel function and regulating cytosolic sodium ion and L type Ca ion levels in cardiac myocytes (Harris, et al., 2008) (Russo, 2009).

As HUFA are derived from both exogenous and endogenous sources, both dietary intake of fatty acids and factors influencing endogenous synthesis could contribute to disease risk. Desaturase 5 is the key enzyme in the metabolic pathways of n-3 and n-6 HUFA. Although there is no direct measurement of Δ5, its activity can be estimated by the ratio of precursor fatty acid to the subsequent "daughter" fatty acids present in serum (Warensjö, et al., 2006)(Warensjö, et al., 2005), plasma (el Boustani, et al., 1989), erythrocytes (Rodríguez & Christophe, 2005) and skeletal muscle membrane (Pan, et al., 1995). In that regard, the plasma ratio of homo gamma linoleic acid to AA in human studies is typically used as a surrogate measure of Δ5 activity. The ratio of EPA to eicsoatetraenoic acid is also reflective of enzymatic Δ5 activity; however, this latter ratio is not used in human

studies due to the low or undetectable amounts of eicsoatetraenoic acid in human plasma.

Indigenous peoples residing in the Canadian North have a paradoxical co-existence of obesity with low cardiovascular disease risk (Young, et al., 1993), which has been related to a relatively high intake of n-3 fatty acids from traditional foods. Foods which are traditionally consumed by Cree include lake trout, whitefish, burbot, walleye, and sturgeon which contain significant amount of DHA and EPA (Blanchet, et al., 2000)(Health Canada, 2007)(United States Department of Agriculture, 2009). Also, Cree typically consume wild fish which has higher level of n-3 fatty acids than fish from aquaculture source (Dewailly, et al., 2001). On the other hand, Indigenous Peoples are undergoing rapid dietary transition that could diminish n-3 HUFA intake leading to increased chronic disease risk (Brassard, et al., 1993)(Robinson, 1988). Thus, assessment of plasma HUFA status among Indigenous Peoples undergoing dietary transitions is important. The present study has focused upon the Cree of James Bay as they have continued to undergo dietary transition away from traditional food rich in n-3 fatty acids (Dewailly, et al., 2002).

Among the endogenous regulatory factors of Δ5, insulin is of particular interest as its administration in a wide age range of healthy subjects increases activity of Δ5 (el Boustani, et al., 1989). Also, an inverse association has been observed between insulin resistance levels and Δ5 activity in cross-sectional studies of apparently healthy people (Pan, et al., 1995)(Warensjö, et al., 2006) and in a 20-year prospective follow-up study of middle-aged men (Warensjö, et al., 2005). The data from these latter studies suggest that, in addition to dietary intake of HUFA, altered insulin resistance levels can modify HUFA tissue profile via regulation of Δ5 activity. Independent of insulin action, adiposity could also impact upon Δ5 activity and tissue HUFA profiles as clinical studies have demonstrated strong negative correlations between adiposity measures and Δ5 activity (Pan, et al., 1995)(Warensjö, et al., 2006)(Warensjö, et al., 2005). In that regard, obese Zucker rats demonstrated reduced affinity of hepatic Δ5 to its

substrates as compared to their lean rat counterparts, which was not explainable by differences in insulinemia (Blond, et al., 1989).

In the present study, we explored the associations of Δ5 activity and erythrocyte levels of HUFA with measures of adiposity and insulin resistance in a James Bay Cree population undergoing dietary transition away from traditional food.

4.3. Methods

4.3.1. Location and subjects

A cross-sectional study was conducted in the community of Mistissini, Québec in the summer of 2005 and was coordinated by the Cree Board of Health in collaboration with McMaster, Laval, and McGill universities. The community survey was the first in an ongoing study entitled "Nituuchischaayihitaau Aschii: A Multi-Community Environment-and-Health Longitudinal Study in Iiyiyiu Aschii". Background information on selected aspects of the methodology and study population is provided elsewhere (Egeland, et al., 2008a). Briefly speaking, local radio announcements and local publicity were employed to raise public awareness on the project. A stratified random sampling consisting of 4 age strata was used to select the residents from municipal list of the community of Mistissini. Qualified local bilingual research assistants worked as recruiters. Among 359 adults (age ≥ 20 year) who were randomly selected, 79 of them were out of town or not able to be contacted. For the 280 potential participants, 62% of them participated in the study (n=172). Among these participants, 166 of them had their fasting blood samples taken. Ethics approval was obtained from participating institutions. The recruitment and interviews were conducted with the assistance from bilingual Iiyiyiuch interviewers. Participants were randomly selected from a municipal list, while pregnant women were excluded. While the community study included children, the current analyses were restricted to adults.

Among the 172 adult participants, fasting blood samples were available from 166 individuals (98 women and 68 men) aged 20 to 88 years for the measurement of erythrocyte membrane fatty acids, serum glucose and insulin levels. Weight and body fat percentage were measured using a bioelectrical impedance scale (Tanita). Height was measured without shoes to the nearest centimeter using a stadiometer with the patient standing on a hard surface. Body mass index (BMI) was calculated (kg/m^2). Waist circumference was measured at the end of exhalation with the tape placed horizontally between the last floating rib and the iliac crest. Obesity was defined by BMI (BMI $\geq$ 30 kg/m^2) and abdominal obesity was defined by the waist (waist $\geq$ 102 cm in men and $\geq$ 88 cm in women) (Health Canada, 2003).

4.3.2. Laboratory Analyses

4.3.2.1. Fatty acids

Blood concentrations of fatty acids were determined in red blood cell (RBC) membranes by gas-liquid chromatography. RBCs (300 µL) were thawed and lysed in 1 mL water. Membranes were isolated by centrifugation (21,000 g for 15 minutes) and washed twice with a 0.9% NaCl solution. The pellet was spiked with an internal standard of phosphatidylcholine pentadecanoic acid (Avanti Polar Lipids, Alabaster AL) and lipids were liquid-liquid extracted using chloroform and methanol (2:1 v/v) according to a modified Folch method (Shaikh and Downar, 1981). Fatty acids from membrane phospholipids were methylated in methanol/benzene (4:1 v/v) mixed with acetyl chloride according to previously described methods (Lepage & Roy, 1986). Fatty acid profile were obtained by capillary gas chromatography using a temperature gradient on a HP5890 gas chromatograph (Hewlett Packard, Toronto, Canada) equipped with a HP8823 capillary column coupled with a flame ionization detector (FID). Helium was used as an elution gas (split ratio 1:72). Fatty acids were identified according to their retention time on the column, using a standard mixture of 37 fatty acids as a basis for comparison (FAME 37 mix, Supelco Inc., Bellefonte PA), which contained the fatty acid standard pentadecanoic acid, as well as a mixture of 31

fatty acids GLC-411 (NuCheck Prep Inc. Elysian, MN) and a mixture PUFA-3 (Matreya Inc, Ontario, Canada). Results were expressed as percent total fatty acids.

4.3.2.2. Blood glucose and insulin

Plasma glucose was measured enzymatically, and fasting insulin concentrations were measured with a commercial double-antibody radioimmunoassay as described by Dewailly et al. 2001 (Dewailly, et al., 2001).

4.3.3. Statistical analysis

Data for continuous variables are presented as mean ± SD if normally distributed and as the geometric mean (95% CI) if not normally distributed. Categorical variables were calculated as proportions. Fatty acid content in erythrocyte membranes was expressed as a percentage of total fatty acids. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-3 polyunsaturated fatty acids (PUFA), n-6 PUFA, and trans fatty acids (TFA) were calculated by summing the concentrations of individual acids if they were detectable. The activity of Δ5 was estimated by the AA / homo gamma linoleic acid ratio. Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR), calculated as follows: HOMA-IR = [Fasting insulin (μU/mL)×fasting plasma glucose (mmol/L)/22.5].

As the age span of the current study population ranged from 20 to 88 years, subjects were divided into four age groups. Linear regression was applied to detect linear trends of the anthropometric measures, HOMA-IR, fatty acid profiles of erythrocyte membranes and Δ5 activity across age groups. Interactions between HOMA-IR and anthropometric measures were tested with general linear models. General linear model was conducted to test linear trends of continuous variables across age groups. Generalized regression model was used to test linear trends of categorical variables across age groups. As there were no observed interactions of gender on Δ5 related associations, the results of associations of Δ5 with anthropometric measures and HOMA-IR were presented in a combined

manner for all subjects. Spearman correlations were conducted for the correlations of Δ5 with adiposity measures, HOMA-IR and age. Age-adjusted multivariable linear regression was used to: (1) separately evaluate the associations of Δ5 with HOMA-IR; and (2) separately assess the associations of Δ5 with BMI, body fat% and waist circumference. Because disease state may influence the association of HOMA-IR with Δ5, the interaction term was included in the age-adjusted linear regression analyses to test the interaction of fasting glucose status with HOMA-IR and Δ5. All P values were obtained from 2-sided tests. Data were analyzed with the SAS software (version 9.1; SAS Institute, Cary, NC, USA).

4.4. Results

4.4.1. Subject characteristics

The current study population was composed of 98 females [mean age 40.1 yrs (SD=15.8), mean BMI 34.9 (SD=6.8) kg/m^2] and 68 males [mean age 40.7 yrs (SD=16.1), mean BMI 31.5 (SD=5.5) kg/m^2]. There were no significant differences among age groups in adiposity measures (P for trend, not significant) (Table 4.1.). The geometric mean HOMA-IR increased slightly and non-significantly (P<0.1) with increasing age, whereas the proportion of subjects with impaired fasting glucose (i.e., fasting glucose defined as ≥ 5.7 mmol/L) (25) increased significantly with advancing age (P<.0001).

4.4.2. Erythrocyte Fatty Acid Content

Fatty acid classes of erythrocyte membranes showed distinctive associations with age (Table 4.2.). In particular, total n-3 fatty acids increased significantly across age groups (P<0.0001). The latter observed association was driven mainly by two HUFA fatty acids, EPA and DHA, which consistently and significantly increased with age (P<0.0001). Conversely, total n-6 fatty acids decreased significantly across age groups (P<0.0001). Almost all individual n-6 fatty acids showed the same strong and inverse associations with age (P<0.0001) except for AA whose percentage in fatty acid profile of erythrocyte membrane differed only slightly between different age groups (P = 0.05).

As expected, n-3 fatty acids were inversely correlated with n-6 fatty acids (r_s= -0.716, P≤0.0001). Similarly, homo gamma linoleic acid negatively correlated with EPA (r=-0.463, P<0.0001) and DHA (r=-0.503, P<0.0001). In terms of the numerator and denominator of the ratio representing Δ5 activity, the numerator (AA) increased with age (p=.05) while the denominator (homo gamma linoleic acid) decreased with age (P<0.0001), resulting in significant increases in Δ5 across all age groups (P<0.0001) (Table 4.2.).

The proportion of total SFA increased with age (P<0.05), but the increase was subtle and there was no significant difference in individual SFA level by age. In terms of other fatty acid classes, i.e., MUFA and TFA, no significant differences were observed across age groups for either the total fatty acid class or for any individual fatty acid.

4.4.3. Δ5, HOMA-IR and Adiposity

Negative correlations were observed between Δ5 and adiposity measures, particularly BMI (r_s= -0.175, P<0.05) and % body fat (r_s= -0.168, P<0.05). The activity of Δ5, was not correlated significantly with HOMA-IR in the unadjusted analyses (Table 4.3.). Upon further analyses of fasting glucose status, however, a significant interaction term showed differences in the slope of HOMA-IR with Δ5 as the dependent variable (P=0.03). In that regard, a strong and significant inverse relationship of HOMA-IR with Δ5 was observed among those with normal fasting glucose but not those with impaired fasting glucose. There was no observed significant interaction of fasting glucose status on the associations between adiposity and Δ5. Thus, all the subjects were combined together in separate age-adjusted models evaluating BMI, %body fat, and waist circumference for their associations with Δ5. The adjusted model using Δ5 as the dependent variable showed for BMI: β=-0.037, SE=0.019, P=0.05; for % body fat: β=-0.041, SE=0.013, P=0.002; and for waist: β=-0.020, SE=0.009, P=0.03.

4.5. Discussion

In the current study, HUFA n-3 fatty acids, especially DHA, increased significantly with age, while n-6 fatty acids showed an opposite trend. These latter findings are consistent with previous observations that older Cree consume more traditional food rich in HUFA n-3 fatty acids, while young Cree consume more market foods that are rich in n-6 fatty acids (Dewailly, et al., 2002). In that regard, plasma EPA and DHA were shown to increase significantly with age in an earlier study of James Bay Cree (Dewailly, et al., 2002). High consumption of traditional food, including fish, has been reported among older community members throughout northern indigenous communities in the three Canadian territories (Kuhnlein, et al., 2004). In the current study, homo gamma linoleic acid negatively correlated with the two n-3 fatty acids, EPA and DHA, that are abundant in fish and sea food (Appavoo, et al., 1991)(Kuhnlein, et al., 1991). As only trace amounts of homo gamma linoleic acid are present in food, this fatty acid is primarily indicative of endogenous metabolic activity. Since n-3 fatty acids and n-6 fatty acids compete with each other within membrane phospholipids (Khan, et al., 2003) and for the desaturases (Escudero, et al., 1998), increased intake of fatty acids from one class leads to the reduced presence of fatty acids in membranes from the other classes. Hence, a reasonable explanation is that higher consumption of fish in older Cree inhibits elongation and desaturation of n-6 fatty acids in these subjects leading to the negative relationship between n-3 HUFA and homo gamma linoleic acid observed in the erythrocyte membrane fatty acid profiles.

Fatty acid composition of erythrocyte membrane reflects dietary intake of fatty acids that are mainly exogenously originated, such as HUFA, TFA and odd number fatty acids. Although the fatty acid profile of erythrocyte membranes is modulated by both endogenous pathways and dietary intake, the efficiency of endogenous synthetic pathway of n-3 HUFA fatty acids is recognized to be rather low. Based on clinical trials using isotopically labeled tracer fatty acids or analyzing blood fatty acid profile after dietary supplementation, a recent review concluded that only about 5% of ALA consumed is transformed to EPA and less

than 0.5% of ALA is converted into DHA (Plourde & Cunnane, 2007). Hence, the erythrocyte membrane n-3 HUFA content reflects primarily dietary intake. The measurement of fatty acid profiles of erythrocyte membranes used in the present study has been extensively tested in dietary PUFA intervention studies using dietary sources of EPA and DHA (Brown, et al., 1990)(Kuriki, et al., 2003) and is commonly used as an evaluation of HUFA intake in epidemiological studies (Baylin, et al., 2005). Normally the endogenous pathway of HUFA biosynthesis exerts a relatively minor influence on the HUFA content of erythrocyte membranes. When dietary HUFA intake is low, however, the contribution of endogenous HUFA biosynthesis towards erythrocyte HUFA content becomes physiologically significant. Since dietary fatty acid intake was closely related to age in the present study, an adjustment for age was performed to eliminate the confounding influence of fatty acid intake on Δ5 activity, which was estimated by AA to homo gamma linoleic acid ratio in erythrocyte membranes. Thus, the impact of other metabolic influences on Δ5 activity, such as obesity and insulin resistance, was assessed by adjusting for the confounding effects of age and dietary fatty acid intake.

Interestingly, in our study population, there was no significant difference of obesity prevalence between age groups in both genders. Obesity was prevalent in the study population as shown by the mean BMI of > 33 kg/m^2, including more than one third of subjects having BMI of ≥ 35 kg/m^2 (data not shown) and a mean waist circumstance > 106 cm in all of the age groups. Both prevalence of obesity and the average BMI level among all age groups were much higher than previously reported in a recent Canadian national survey of the non-indigenous population (Health Canada, 2004) or data from an earlier national survey of multiple ethnic populations in the United States (Centers for Disease Control and Prevention, 2005).

A significant proportion of Cree subjects had impaired fasting glucose level (≥5.7 mmol/L) and this proportion increased with age. At normal fasting glucose condition (<5.7 mmol/L), a positive association exists between levels of

insulin and glucose (r=0.417, P=0.0001, data not shown) among the Cree. This correlation, however, disappeared in the subgroup with impaired fasting glucose, indicating that insulin's regulation was disrupted. Hence, it was not surprising that an inverse association between HOMA-IR and Δ5 was observed among subjects with normal fasting glucose, while this association became non-significant among subjects with impaired fasting glucose.

In contrast, no significant interaction was observed for the effects of adiposity on Δ5 between normoglycemic and impaired glucose tolerant individual suggesting that gradients of adiposity continue to play an important role in Δ5 activity regardless of the degree of disease progression. In the present study, body fat percentage was a more significant negative predictor of Δ5 than waist girth, which may be related to the unusually high adiposity of the Cree study population. In the presence of high adiposity, effects of abdominal adiposity on metabolic consequences may be diluted, since Health Canada suggests that waist girth is not to be considered as an additive risk for people with BMI $\geq$ 35 kg/m^2 (Health Canada, 2003). Similar to the Cree, inverse associations between adiposity level and Δ5 have been reported in several studies including Pima Indian (Pan, et al., 1995), obese Hungarian children (Decsi, et al., 1996) and Caucasians (Warensjö, et al., 2006). Among Pima Indians, the ratio of AA to homo gamma linoleic acid in skeletal muscle membrane was significantly and strongly inversely related to adiposity measures (Pan, et al., 1995). A study on Hungarian children showed that ratio of AA to homo gamma linoleic acid in plasma phospholipids was reduced in obese children compared to normal controls and this ratio was lowest among obese subjects with the metabolic syndrome (Decsi, et al., 1996). The latter observation may suggest that obesity and other metabolic syndrome components such as insulin resistance may have additive effects on suppressing Δ5 activity. A recent study on apparently healthy middle-aged Caucasians reported a negative correlation between the ratio of AA to homo gamma linoleic acid in serum cholesteryl ester and sagittal abdominal diameter, that was independent of age (Warensjö, et al., 2006). Evidence from in vitro studies and rodent models also suggests a direct link between adiposity and Δ5

activity. In one in vitro study, fresh microsomes taken from obese and lean Zucker rats were incubated with the substrate homo gamma linoleic acid at physiological concentrations. Desaturase 5 activity, measured as AA formed per time unit per weight unit of liver microsomal protein, was lower in freshly isolated microsomes from obese Zucker rat at all age stages, which correlated with in vivo observations of Δ5 activity (as assessed by hepatic ratios of AA to homo gamma linoleic acid) being lower in obese than in lean Zucker rats (Blond, et al., 1989). The lower Δ5 activity in the hepatic microsomes in the obese Zucker rats was not accountable by the hyperinsulinemic condition of the obese Zucker rats. Similar findings have been reported (Cunnane, et al., 1985) in some but not all (Hughes & York, 1985) studies involving the ob/ob mice. In the present work, no significant interaction was observed for the effects of adiposity on Δ5 between normoglycemic and impaired fasting glucose individuals suggesting that gradients of adiposity continue to play an important modulatory role in Δ5 activity regardless of the degree of disease progression involving glucose intolerance.

In most populations, advancing age typically leads to increasing adiposity and reduced insulin sensitivity with a consequent decrease in Δ5 activity. Among the Cree, however, adiposity was not related to age and age was only weakly and non-significantly related to insulin resistance. Therefore, age cannot account for the observed relationships of the adiposity measures with Δ5 seen among the studied population of Cree subjects or the observed association between HOMA-IR with Δ5 observed among Cree subjects with normal fasting glucose. Additionally, the statistical adjustment for age used in the current study adjusts for the observed age-related differences in dietary intake. To date, animal studies show inconsistent findings regarding the importance of age on Δ5 activity (Maniongui, et al., 1993)(Takahashi & Horrobin, 1988) indicating that future studies are warranted in this regard.

Several limitations should be recognized in the present study. The causal relationships between the variables of interest cannot be determined based on the cross-sectional data. In addition, more precise measurements of body composition

such as dual energy X ray absorptiometry (DXA) could be considered to detect subcutaneous adipose tissue and visceral adipose tissue in future studies, which would clarify which part of adipose tissue has more significant influence on fatty acid metabolism. Future studies are needed to observe whether the results from the current studies can be generalized to other populations.

The results reported herein indicate that the high prevalence of obesity among Cree of James Bay concomitant with a low HUFA n-3 fatty acid intake could exacerbate chronic disease risks through reduction in Δ5 activity. The activity of Δ5 may be important for disease prevention as this enzyme is critical for synthesis of optimal n-3 HUFA tissue profiles that are associated with anti-inflammatory and anti-arrhythmic cardioprotective effects. These findings also illustrate that several disease-related factors such as dietary changes, obesity and insulin resistance can work in tandem to elevate disease risk in Indigenous Peoples undergoing rapid transitions.

4.6. Acknowledgment

This study was supported by the Niskamoon Corporation. The authors thank the community research staff and participants. The authors' responsibilities were as follows-Yuan E. Zhou: analyzed the data and drafted the manuscript; Stan Kubow: supervised the manuscript drafting; Eric Dewailly: designed the study; Pierre Julien: carried out the fatty acid analysis; Grace M. Egeland: designed the study and supervised the data analysis; and all authors: contributed to the revision of the manuscript. None of the authors had any financial or personal conflict of interest to disclose.

Table 4.1. Clinical characteristics of the study population

Age (year)	20-29 N=51		30-39 N=46		40-49 N=30		50+ N=39		P for trend
BMI (kg/m^2)*	33.1	(7.3)	34.1	(6.7)	33.8	(5.0)	33.3	(6.5)	NS
Body Fat (%)*	38.7	(11.5)	39.9	(9.4)	40.4	(8.4)	40.5	(8.0)	NS
Waist girth (cm)†	107.0	(15.7)	110.2	(14.4)	109.3	(11.0)	111.2	(14.7)	NS
Proportion of obesity by BMI (%)‡	58.3		71.1		82.8		62.9		NS
Proportion of obesity by waist girth(%)§	72.9		82.2		79.3		89.7		NS
Proportion of female (%)	56.9		63.0		56.7		59.0		NS
HOMA-IR	5.6	(4.7-6.8)	6.4	(5.1-7.9)	6.7	(5.1-8.7)	7.6	(5.9-9.7)	<0.1
Proportion of impaired fasting glucose (%)	25.5		47.8		66.7		82.1		<0.0001

Data are mean (standard deviation) or %, geometric mean (95% CI) for HOMA-IR.

* Age group 18-29 (yr) n=48, 30-39 (yr) n=45, 40-49 (yr) n=29, 50+ (yr) n=35.

† Age group 18-29 (yr) n=48, 30-39 (yr) n=45, 40-49 (yr) n=29, 50+ (yr) n=39.

‡ Proportion of subjects with BMI ≥ 30 kg/m^2. § Proportion of subjects with waist girth ≥ 88 cm (female) or ≥ 102 cm (male).

Table 4.2. Mean proportion (% of total fatty acids) of fatty acids in erythrocyte of the study population

Age (year)	20-29 N=51	30-39 N=46	40-49 N=30	50+ N=39	P for trend
SFA	41.89 (0.74)	41.83 (0.77)	42.29 (0.74)	42.31 (0.85)	<0.05
Myristic acid (C14:0)	0.31 (0.04)	0.32 (0.04)	0.32 (0.04)	0.29 (0.07)	NS
Palmitic acid (C16:0)	19.66 (0.77)	19.82 (0.61)	19.76 (0.71)	19.88 (0.70)	NS
Stearic acid (C18:0)	14.75 (0.46)	14.76 (0.49)	14.88 (0.70)	14.78 (0.62)	NS
MUFA	18.79 (0.84)	18.75 (0.93)	18.36 (0.62)	18.77 (1.18)	NS
Palmitoleic acid (C16:1 *n-7*)	0.32 (0.20)	0.37 (0.15)	0.32 (0.16)	0.29 (0.27)	NS
Oleic acid (C18:1 *n-9*)	11.40 (0.60)	11.40 (0.77)	11.29 (0.69)	11.74 (1.04)	NS
Vaccenic acid (C18:1 *n-7*)	1.46 (0.35)	1.50 (0.35)	1.30 (0.36)	1.30 (0.36)	NS†
Nervonic acid (C24:1 *n-9*)	4.96 (0.40)	4.84 (0.31)	4.84 (0.40)	4.89 (0.47)	NS
n-6 PUFA	31.96 (0.80)	31.47 (0.92)	31.14 (0.87)	29.43 (1.60)	<0.0001
n-6 HUFA	18.60 (0.88)	18.65 (0.89)	18.63 (0.68)	18.23 (1.14)	=0.05
LA	10.94 (0.65)	10.51 (0.69)	10.40 (0.78)	9.50 (1.15)	<0.0001
Homo gamma linoleic acid (C20:3 *n-6*)	2.12 (0.29)	2.00 (0.27)	1.86 (0.31)	1.46 (0.34)	<0.0001

AA (C20:4 *n-6*)	14.32	(0.74)	14.54	(0.67)	14.59	(0.49)	14.61	(0.84)	=0.05
Adrenic acid (C22:4 *n-6*)	3.39	(0.40)	3.24	(0.34)	3.17	(0.35)	2.85	(0.37)	<0.0001
Docosapentaenoic acid (C22:5 *n-6*)	0.89	(0.12)	0.87	(0.13)	0.88	(0.10)	0.77	(0.17)	<0.0001
n-3 PUFA	5.89	(0.70)	6.47	(0.69)	6.84	(0.64)	8.11	(1.39)	<0.0001
n-3 HUFA	5.40	(0.63)	5.88	(0.62)	6.38	(0.66)	7.70	(1.31)	<0.0001
ALA (C18:3 *n-3*)	0.15	(0.10)	0.15	(0.11)	0.15	(0.10)	0.13	(0.16)	NS
EPA (C20:5 *n-3*)	0.37	(0.09)	0.43	(0.11)	0.47	(0.11)	0.73	(0.29)	<0.0001
DPA (C22:5 *n-3*)	0.20	(0.15)	0.24	(0.13)	0.18	(0.15)	0.15	(0.17)	NS
DHA (C22:6 *n-3*)	2.97	(0.54)	3.44	(0.54)	3.82	(0.62)	4.80	(1.03)	<0.0001
Δ5*	6.89	(1.08)	7.43	(1.22)	8.06	(1.45)	10.60	(2.62)	<0.0001

Data are mean (standard deviation).

*Δ5 is estimated as ratio of AA to homo gamma linoleic acid.

†No clinical significance, because though P value <0.01 for the general linear model, R^2 is only 4% and β is too small (-0.005)

Table 4.3. Spearman correlation coefficients among adiposity HOMA-IR and age

	Body fat %	BMI	Waist	HOMA-IR	Age
BMI	0.777****				
Waist	0.650****	0.907****			
HOMA-IR	0.557****	0.599****	0.568****		
Age	0.013	0.012	0.073	0.145†	
Δ5	-0.168*	-0.175*	-0.116	-0.112	0.593****

†0.1<p<0.05, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

Table 4.4. Age-adjusted regression coefficients (SE) of adiposity measures and HOMA-IR and their individual associations with Δ5[*]

Independent variables	β	SE	P	R^2_{adj} [*]
Body fat%[†]	-0.041	0.013	0.002	0.515
BMI[†]	-0.037	0.019	0.05	0.496
Waist[‡]	-0.020	0.009	0.03	0.479
Fasting glucose < 5.7mmol/L				
HOMA-IR[§]	-2.110	0.566	<0.001	0.511
Fasting glucose ≥ 5.7mmol/L				
HOMA-IR[§]	-1.015	0.674	0.14	0.419

[*] Adjusted for age and separately assessed, R^2_{adj} refers to the whole model.

[†] n=157.

[‡] n=161.

[§] n=79 for fasting glucose < 5.7mmol/L and n=87 for fasting glucose ≥ 5.7mmol/L.

Bridge 3

Manuscript 2 demonstrated that dietary transition is occurring among Cree as HUFA n-3 status was strongly and positively association with age. Moreover, obesity was prevalent among Cree and adiposity was associated with inhibited Δ5 activity, which might exacerbate the risk of chronic diseases among this population. Reduced intake of HUFA n-3 together with depressed biosynthesis of HUFA n-3 via the inhibition of Δ5 mediated by high adiposity could decrease the cardioprotective anti-inflammatory and anti-arrhythmic effects of HUFA n-3. Similar to the Cree population, the Inuit have benefited from the health benefits of their traditional foods in terms of high intake of long chain n-3 fatty acids. In the last few decades, however, the Inuit have been experiencing a shifting dietary pattern away from traditional foods to market foods, which are generally high in saturated fat and *trans* fat (Kuhnlein, et al., 2004). Available knowledge about native populations in northern Canada in terms of n-3 PUFA intake has been mostly derived from fractured information collected from studies conducted in separate regions and time periods. Therefore, the International Polar Year Inuit Health Survey provided a unique opportunity to evaluate the HUFA n-3 status among Inuit residing in a vast area from easternmost to westernmost Canada.

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Highly unsaturated n-3 fatty acids status of Canadian Inuit: International Polar Year Inuit Health Survey, 2007-2008

Yuan E. Zhou[a], Stan Kubow[a] , Grace M. Egeland[a]

Author affiliation:

[a] School of Dietetics and Human Nutrition and the Centre for Indigenous Peoples' Nutrition and Environment, McGill University, Macdonald Campus, 21,111 Lakeshore Rd., Ste.Anne-de-Bellevue, Québec, , Canada, H9X 3V9

This manuscript is submitted.

This study was supported by Canadian Institute of Health Research (CIHR) grant

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5.1. Abstract

Background:

Previous studies suggest that dietary patterns and the extent of reliance upon traditional food vary among Inuit communities. Dietary transition away from traditional foods, an important source of nutrients for Inuit, is occurring with younger generations consuming less traditional food.

Methods:

Utilizing erythrocyte membrane fatty acid composition as an indicator of diet, we explored the regional and generational variability of highly unsaturated n-3 fatty acids (HUFA n-3) in the International Polar Year Inuit Health Survey of 3 Inuit jurisdictions in Canada. Participants were recruited through random sampling of households. Fatty acid data were available among 2200 adults (≥ 18 yr).

Results:

HUFA n-3 levels in the Eastern Arctic were significantly higher than in the Western Arctic with Nunatsiavut and Baffin showing the highest HUFA n-3 status compared to Kivaliq, Kitikmeot and Inuvialuit Settlement Region (ISR) (both P<.0001). Fatty acid proportion in erythrocyte membranes showed pronounced differences between coastal communities and three inland communities, including a much higher HUFA n-3 status among the former (P<.0001). Additionally, the HUFA n-3 status showed a strong positive association with age, particularly among Baffin and Kivalliq Inuit. HUFA n-3 were inversely associated with saturated (r_s[spearman correlation coefficient]=-0.648, P<.0001) and *trans* fatty acids (r_s=-0.331, P<.0001).

Conclusion:

The present study results provided biochemical support for varying dietary patterns and dietary transition among Inuit across the Canadian Arctic. The analyses also suggested multifactorial determinants to HUFA n-3 status among

Canadian Arctic Inuit. A nutritional intervention strategy with multiple approaches may be needed to improve and maintain their HUFA n-3 status.

5.2. Introduction

The majority of Canadian Inuit reside in a vast geographic area that includes the Northwest and Nunavut Territories, northern Québec and Labrador: the areas are collectively known as the Inuit homeland or Inuit Nunaat. While all areas have undergone rapid changes in the social, cultural and economic environment, particularly in recent decades, available nutritional and health data represent fragmented assessments over time and diverse geographic areas. The nutritional and lifestyle transition being observed has become a public health concern with epidemiologic transitions being noted in obesity and chronic disease risk (Gracey & King, 2009), which were considered to be virtually non-existent in the past (Young, et al., 2007) (Young, et al., 1990) (Kjaergaard, et al., 2009) (Friborg & Melbye, 2008). Dietary transition away from traditional foods, which are rich in a variety of nutrients (Kuhnlein, et al., 2008), is one critical factor linking the lifestyle changes with the shifting pattern in health and disease. In that regard, alterations in dietary fat intake are of interest given their importance in the development of chronic disease risk (Dewailly, et al., 2001) (Counil, et al., 2009).

While dietary transition among arctic indigenous peoples has been addressed by different research teams, most evidence has been obtained from 24 hour dietary recalls or food frequency questionnaires (Kuhnlein, et al., 2004) (Kuhnlein, et al., 1996b) (Blanchet, et al., 2000) (Egeland, et al., 2004) as well as food component analysis (Deutch, et al., 2007), while analyses of biomarkers of nutritional exposures have been under-represented in the literature. The measurement of fatty acid composition of erythrocyte membranes used in the current study has been commonly used to assess highly unsaturated fatty acids (HUFA) and *trans* fatty acids (TFA) intake in epidemiologic studies (Baylin, et al., 2005). In an earlier study that evaluated plasma fatty acid profiles in the Iglookik community of Baffin Island in the mid-1990s, a strong positive

association between age and plasma EPA and DHA (Rode, et al., 1995) was noted. Similarly, in the 2004 Nunavik Health Survey, n-3 fatty acids of erythrocyte membrane phospholipids among 888 adult Inuit from 14 communities in Nunavik increased with age (Counil, et al., 2008). Similar associations between n-3 fatty acids of erythrocyte membrane phospholipids and age were also observed among 524 Inuit from Disko Bay in Greenland (Counil, et al., 2008). Furthermore, in the 2004 Nunavik Health Survey, TFA levels of erythrocyte membrane phospholipids were high especially among the Inuit youth, and TFA levels among Nunavik Inuit was relatively higher than TFA levels observed among Greenland Inuit (Counil, et al., 2008). A health survey in a small community in the Baffin Region showed that plasma n-3 fatty acids were inversely related to TFA, indicating that traditional food is replaced by market food of poorer quality: a dietary transition which would contribute to chronic disease risk (Egeland, et al., 2009). A previous health survey measuring plasma phospholipid fatty acids was done in Kivalliq (previously known as "Keewatin") nearly two decades ago whereby an increase of EPA and a decrease of n-6 including AA was reported among Inuit (Young, et al., 1999).

For indigenous peoples, food choice is influenced by multiple factors (Kuhnlein & Receveur, 1996a) (Willows, 2005). Age is a strong determinant of traditional food consumption, with younger indigenous people in the arctic consuming less traditional food than older individuals within the same communities, which has been supported by dietary questionnaires (Kuhnlein, et al., 2004) (Counil, et al., 2008) as well as by fatty acid profiles of plasma and erythrocyte membranes (Rode, et al., 1995) (Zhou, et al., 2009). Other factors including road access, latitude and population size were also proposed as being determinants of dietary habits (Whiting & Mackenzie, 1998). Dietary assessment data from 24-hour recalls in a cross-sectional survey conducted in 16 communities in Canadian Northwest Territories showed that communities with higher intakes of traditional foods were more likely located at higher latitudes (Receveur, et al., 1997).

The current analyses are based upon data from the International Polar Year (IPY) Inuit Health Survey: a comprehensive health survey of Inuit residing in three jurisdictions within Canada conducted in 2007 and 2008. Since a similar health survey was conducted in Nunavik, northern Québec, in 2004, this region was not included in the survey. A representative sample of Inuit residents were chosen from 36 communities in the survey area, with latitudes ranging from 54° to 76° N and longitude ranging from 58° to 135° W. Such a vast landscape offers the opportunity to evaluate the extent to which HUFA n-3 status varies across the Canadian Arctic and the extent of dietary transition by region.

5.3. Methods:

5.3.1. Location and subjects

IPY Inuit Health Survey was conducted in the late summer and fall of 2007 and 2008, with Baffin and Kivalliq Regions of Nunavut Territory participating from August to September 2007 and Nunatsiavut, Kitikmeot Region of Nunavut, and Inuvialuit Settlement Region (ISR) participating in the same time period in 2008. A total of 36 communities were included, 3 of which were inland communities. Participants, age 18 years or older, who identified themselves as Inuk were recruited through random selection of households stratified by community. Pregnant women were excluded from the health survey. A total of 2,595 adults participated, representing approximately 11% of the Inuit population in the geographic areas surveyed. After completing informed consent forms, participants were transported to the Canadian Coast Guard Ship research ice-breaker, CCGS Amundsen, where the survey team of nurses and bilingual interviewers completed the assessments. The project was reviewed and approved by McGill Institutional Review Board and by all 3 jurisdictions. Among the 2595 participants, fasting red blood cell samples were available from 2200 individuals aged 18-90 years for the measurement of erythrocyte membrane fatty acids.

5.3.2. Laboratory analysis

5.3.2.1. Anthropometric measures

Weight and body fat percentage were measured using a bioelectrical impedance scale (Tanita, Tokyo, Japan). Height was measured without shoes to the nearest millimeter using a stadiometer with the patient standing on a hard surface. BMI was calculated (kg/m^2). Waist circumference was measured at the end of a normal expiration with the tape placed horizontally between the last floating rib and the top of the hip, and the measurement taken to the nearest millimeter. Obesity was defined by BMI $\geq$ 30 kg/m^2 and abdominal obesity was defined by the waist circumference (waist $\geq$ 102cm in men and $\geq$ 88cm in women) (Health Canada, 2003).

5.3.2.2. Fatty acid analysis

Blood concentrations of fatty acids were determined in erythrocyte membrane by gas-liquid chromatography (Lipid Analytical Laboratories Inc, Guelph, Canada). Fatty acid composition of red blood cells was determined based on previous studies (Dewailly, et al., 2001)(Stark & Holub, 2004). Lipids were extracted from the blood samples according to the method of Folch et al. (Folch, et al., 1957). The fatty acid methyl esters were prepared by the method of Morrison and Smith and were analyzed on a Varian 2400 gas-liquid chromatograph (Palo Alto, CA) with a 60-metre DB-23 capillary column (0.32 mm internal diameter) (Morrison & Smith, 1964).

5.3.3. Statistical analysis

Fatty acid concentrations, as a percent of total fatty acids, were evaluated by geographic region and coastal and non-coastal communities using generalized linear regression model (GLM). If the overall difference was significant, pairwise comparisons were conducted with Bonferroni adjustment. Chi-square tests were used for categorical variables, and if the overall difference was significant, multiple comparison procedure was conducted by the methods established by Zar and COMPPROP macro in SAS, which was applied for the test (Duncan, et al.,

2009). Fatty acid content in erythrocyte membrane was expressed as a percentage of total fatty acids. SFA, monounsaturated fatty acids (MUFA), n-3 PUFA, n-6 PUFA, PUFA and TFA were calculated by summing the concentrations of individual fatty acids from the same class if they were detectable. Fatty acid data are presented as median (and interquartile ranges: 25^{th} - 75^{th}%ile). Since there were pronounced differences in fatty acid composition of erythrocyte membranes between coastal and inland Inuit, they were analyzed separately. GLM was used to compare fatty acid profile of erythrocyte membranes among coastal regions, and Mann-Whitney test were used to compare one inland community Baker Lake with the other two inland communities Inuvik and Aklavik. Highly skewed variables were log transformed before being entered into the GLM, and Bonferroni adjustment for pairwise comparisons were made. As the age span of the present study population ranged from 18 to 90 years, subjects were divided into four age groups to evaluate the inter-generational differences in fatty acid concentrations. Alcohol consumption (no versus current) and smoking (no *vs.* current) were examined but not included in the final analyses, since they were not significant predictors of HUFA n-3 status. Interaction of gender and geographic location on associations between fatty acids and factors of interest were tested. Since gender did not have a significant effect on the fatty acid related associations, data on men and women were combined for analyses. Spearman correlations of n-3 fatty acids with SFA and TFA were adjusted for age. In further examinations, spearman correlations of Δ5 (estimated by the AA/ homo gamma linoleic acid ratio) with BMI, body fat% and waist circumference were conducted after adjustment for age. The activity of Δ6 (gamma linoleic acid/LA), an enzyme that catalyzes the first step in the endogenous synthesis of PUFA, was not estimated in the current study since gamma linoleic acid was under detection limit among approximately one third of participants. All *P* values were obtained from 2-sided tests. Data were analyzed with the SAS software (version 9.2; SAS Institute, Cary, NC).

5.4. Results

5.4.1. Subject characteristics of each survey region

The mean age of survey participants ranged from 41.0 yrs (SD=15.0) in Kitikmeot to 44.5 yr (SD=14.0) in Nunatsiavut (Table 5.1). Although differences in age among regions were found to be significant (P=0.012), it was largely due to the large survey sample size, rather than biologically meaningful age differences. Female participants were more prevalent than male participants in each region, from 58.0% in coastal communities in Kivalliq to 69.7% in Aklavik and Inuvik in ISR. However, the gender distribution did not significantly vary by survey region (Table 5.1). Differences in adiposity by region were more pronounced (Table 5.1.). Baffin Inuit had the lowest adiposity levels, with a BMI at 26.8 (SD=6.0) and a mean waist circumference of 88.5 cm (SD=14.3). At the same time, Inuit from Inuvialuit, especially coastal Inuvialuit, appeared to have the highest adiposity level, with BMI at 29.8 (SD=6.3) and a waist circumference of 100.0 cm (SD=16.6). Obesity is prevalent among all survey regions with abdominal obesity showing particularly high rates, which ranged from 35.7% in Baffin to 74.3% among the inland communities of ISR. Regional difference regarding obesity prevalence was significant (P<.0001). In all survey regions, a large proportion of Inuit were self-reported to be current smokers and to drink alcohol with significant regional difference noted in these habits (both P<.0001) (Table 5.1.). Smoking prevalence ranged from 54.8% in Nunatsiavut to 73.5% in Kitikmeot, and alcohol consumption ranged from 40.2% in Baker Lake (inland community of Kivalliq) to 69.9% in coastal community of Inuvialuit. In the current survey, 88.0% Baffin Inuit and 82.4% Kivalliq Inuit reported to speak Inuktitut at home, respectively. Only 19.8% Kitikmeot, 12.1% Inuvialuit Inuit and 1.3% Nunatsiavut Inuit reported to speak their Inuit language at home.

5.4.2. Fatty acid composition of erythrocyte membranes of Inuit in each survey region

Regional differences in fatty acid composition of erythrocyte membranes were observed, particularly between inland communities and coastal communities

(Table 5.2) (Figure 5.1). The n-3 status of inland communities was pronouncedly lower than that of coastal regions. Within inland regions, HUFA n-3 status, particularly EPA level, was lower in Aklavik and Inuvik of ISR. Among coastal communities, Kitikmeot and Kivalliq of Nunavut and ISR had lower HUFA n-3 status, while Nunatsiavut and Baffin had higher HUFA n-3 status. EPA was lowest in Kivalliq and highest in Baffin. DHA was lowest in Kivalliq and coastal Inuvialuit, but highest in Nunatsiavut. A much lower n-6 fatty acid status was also noted in inland communities. Within inland communities, LA, homo gamma linoleic acid and AA were all lower in Baker Lake. Among coastal regions, n-6 level was similar with the exception of Nunatsiavut, which had higher n-6 levels, particularly for AA. At the same time, SFA levels for the inland communities, in particular palmitic acid, were considerably higher than that of coastal regions. Among coastal communities, coastal Inuvialuit showed slightly higher SFA, driven by its palmitic acid. MUFA of inland communities was also significantly higher than that of coastal communities, which was mainly driven by Oleic acid. Among coastal communities, relatively low Oleic acid was found among Nunatsiavut Inuit. Moreover, higher TFA was observed among inland Inuit, especially their C18:1 TFA which was the primary TFA detected. Among coastal communities, TFA was highest in Kitikmeot and appeared to be relatively low in Baffin where the primary C18:1 t was lowest.

Similarly, fatty acids derived ratios showed significant regional discrepancy. N-6/n-3 ratios were higher in inland communities, particularly in Aklavik and Inuvik where the ratio was more than two times higher than that observed in other survey regions. Among coastal regions, n-6/n-3 ratio was higher in Kivalliq than in other coastal communities. The other ratio DPA/EPA was lower in the inland compared to that in the coastal communities. Among coastal regions, DPA/EPA was relatively higher in Kivalliq and Nunatsiavuit. SFA/PUFA of inland communities was noticeably higher than that of coastal regions. Among the coastal regions, highest SFA/PUFA was observed in Kitikmeot and coastal Inuvialuit while the ratio was lower in Nunatsiavut.

5.4.3. Spearman correlations of n-3 fatty acids with SFA and TFA and spearman correlations of Δ5 with adiposity

HUFA n-3 fatty acids, EPA, DPA and DHA all were inversely correlated to SFA (r_s [spearman correlation coefficients] = -.648, P<.0001 for total HUFA n-3 and total SFA) (Table 5.3.). The strongest associations between HUFA n-3 and the individual SFA were those between HUFA n-3 and the median chain SFA palmitic acid (r_s = -0.587, P<.0001 for HUFA n-3 and palmitic acid). HUFA n-3 fatty acids also showed inverse correlation with TFA (r_s = -0.331, P<.0001 for total HUFA n-3 and total TFA). The associations of HUFA n-3 with the primary TFA C18:1t was rather strong (r_s = -0.311, P<.0001) (Table 5.3.). Adiposity measures BMI, waist and body fat% were significantly and inversely related toΔ5 after adjustment for age (Table 5.4.).

5.4.4. Fatty acid composition across age groups

HUFA n-3 fatty acids correlated strongly with age (r_s= 0.622 in Baffin and Kivalliq, r_s= 0.256 in other regions, both P<.0001, data not shown). The regional factor showed a significant interaction regarding the association between age and HUFA n-3, as the trend of an increasing HUFA n-3 status with age was more evident in Baffin and Kivalliq than that observed in other regions (Figure 5.2.).

5.5. Discussion:

The fatty acid profiles of erythrocyte membrane shifted across the Canadian Arctic area. Generally speaking, HUFA n-3 status was highest in Nunatsiavut to the east and Baffin in the northeast and lower in the western regions. In contrast, TFA, n-6 to n-3 ratio, and SFA to PUFA ratio demonstrated an opposite trend regarding the above regional differences. Overall, coastal Inuit and inland Inuit appeared to have dietary fat intake patterns very distinct from each other with the latter more dependent on market foods containing high SFA and TFA but low HUFA n-3. Further, HUFA n-3 fatty acids showed a strong positive association with age among survey participants from Baffin and Kivalliq,

indicating that dietary transition is occurring in these communities. In contrast, the age-associated differences in fatty acids in other regions were relatively minor, suggesting a more uniform dietary pattern in the population. Among the Ganasan of Siberia, Russia there was no age related association with n-3 and n-6 levels. The authors suggested the Ganasan population had adapted to reach a stable dietary pattern as the "acculturation" process had already been completed (Rode, et al., 1995).

Regional differences of fatty acid composition of erythrocyte membranes among Inuit reflect the relative extent of reliance upon traditional and market foods. The ratio of n-6/n-3 in indigenous peoples' diets or historical diets with current western diets has been used as an indicator of dietary westernization, with higher ratios indicating lower consumption of traditional foods (Simopoulos, 2008). Similarly, TFA and SFA also indicate a greater reliance upon market food. Interpretation of the n-6/n-3 ratios without other dietary indicators, however, should be viewed with caution. Although marine mammals, such as seal and whale as well as marine and fresh water fish are all part of the Inuit traditional food system, wild land animals such as game animals and birds are also consumed (Egeland, et al., 2009). Analyses of arctic food items showed that the fatty acid composition of meat from marine mammals and land animals differ (Kuhnlein, et al., 2002). Compared to land animals, marine mammals and marine fish are the good sources of HUFA n-3, particularly of EPA and DHA, and are low in SFA and n-6 fatty acids. Land animals, on the other hand, were found to be high in DPA (Innis & Kuhnlein, 1987) (Appavoo, et al., 1991), and LA (Innis & Kuhnlein, 1987) (Kuhnlein, et al., 2002). Thus, a high consumption of land animals relative to marine mammals or fish would also result in a high n-6/n-3 ratio. However, when land animal meat was compared to market meat, the former would still be a better choice for health in terms of its fatty acid profile. For example, the National Nutrient Database of the United States Department of Agriculture showed that even lean beef has a pronouncedly higher proportion of SFA, which would account for about half of the beef fat, depending on which part of the body was evaluated (United States Department of Agriculture, 2009). In

contrast, beef only contains negligible PUFA, which mostly are n-6 (United States Department of Agriculture, 2009). Similar reports can be also viewed in the National Nutrient Database of Canada (Health Canada, 2009). The above results were consistent with reports from an earlier Alaskan study which measured LA and AA to be 15.4% and 5.6%, respectively in caribou muscle, as compared to 2.5% LA and 0.5% AA in beef (Wo & Draper, 1975). Interestingly, beef from grass-fed cows had increased HUFA n-3 fatty acids (United States Department of Agriculture, 2009). Also, wild Atlantic salmon was reported to be "leaner" but had proportionally higher HUFA n-3 compared to farmed Atlantic salmon (Health Canada, 2009). The above findings suggest that when evaluating the intake of traditional foods versus market foods in a given Inuit region, a variety of indicators need to be considered rather than only relying upon one specific biomarker. Based on available food nutrient data, one would expect that when dietary pattern shifts from high consumption of marine mammals or fish, to high consumption of land animals, to high consumption of stored-bought meat, the fatty acid composition of erythrocyte membranes would change accordingly (Figure 5.3.). Furthermore, if market meat has been highly processed or if market food choices also include rich sources of hydrogenated oils there would be higher levels of TFA (Figure 5.3.). Current food nutrient databases only report TFA data of very few foods items given that only recently have there been methodological advances allowing for greater ease in TFA analyses.

In the current study, fatty acid profiles of erythrocyte membranes between inland Inuit and coastal Inuit demonstrated strikingly pronounced differences. Technically, Baker Lake community of Kivalliq is the only inland arctic community in Canada (Government of Nunavut, 2009), located by the Thelon river on the shore of Baker Lake, a fresh water lake. Aklavik and Inuvik of Inuvialuit are lying on the Mackenzie Delta, which is Canada's largest fresh water delta (Town of Inuvik, 2009) and so these communities were also categorized as inland communities in the analysis. Inland Inuit would have less access to marine mammals and marine fish than coastal Inuit. Moreover, petroleum industry is the main economic source in Inuvik and Aklavik, while hunting, fishing and trapping

are still major economic activities in other communities in ISR (The Legislative Assembly Northwest Territories, 2009). Aklavik and Inuvik are rather modernized towns with quite stable municipal infrastructure (The Inuvik Interagency Committee, 2009). The low PUFA status including both HUFA n-3 and n-6 observed among inland Inuit along with their relatively high TFA, SFA and SFA/PUFA levels indicate that they have a more westernized dietary pattern than Inuit residing along the coast. At the same time, the n-6/n-3 ratio of Aklavik and Inuvik was much higher than that of the Baker Lake. This latter observation along with the low DPA/EPA ratio among Aklavik and Inuvik Inuit further suggests that these Inuit communities have a higher consumption of market foods than Baker Lake Inuit.

Ranging from Eastern Arctic to the Central and Western Arctic, a decreasing HUFA n-3 status was observed in the coastal communities in the regional analyses. Nunatsiavut, the easternmost region where Canadian Inuit reside, showed the highest HUFA n-3 status among the three arctic administrative regions, particularly in terms of DHA. Multi-species fisheries are still the main economic source and subsistence food for Nunatsiavut Inuit (Nunatsiavut Government, 2009). On the western side of Canadian Arctic, ISR had the lowest HUFA n-3 status. Inuvialuit is a rather modernized region with mining and oil production being an important industry (Town of Inuvik, 2009) (Inuvialuit Regional Corporation, 2009). Wage earning employment provided current Inuvialuit Inuit with much more reliable livelihood than traditional hunting (Inuvialuit Regional Corporation, 2009). At the same time, the wage employment could consequently set restraint on time and/or energy for community members to engage in hunting or fishing for obtaining their traditional foods. Instead, local Inuit would have become more dependent on store-bought foods.

In addition, the fatty acid profile of erythrocyte membranes of survey participants showed a shifting pattern in the north to south dimension from Baffin to Kitikmeot to Kivalliq. Among the three regions, Baffin Inuit showed highest HUFA n-3 status and the lowest ratios of DPA/EPA and n-6/n-3. At the same

time, the three regions also displayed rather similar SFA levels. The above observations suggest higher consumption marine mammals and marine fish among Baffin Inuit relative to consumption of wild land animals.

Among Inuit across the Canadian Arctic, the fatty acid profile clearly demonstrated generational differences. Young indigenous people are consuming less traditional food than older individuals, indicating dietary transition is occurring consistent with previous reports on lower consumption of country food among young indigenous people residing in different arctic and sub-arctic regions, such as Nunavik (Blanchet, et al., 2000), Baffin island (Kuhnlein, et al., 1996b), Belcher island (Wein, et al., 1998), and more recently among the Cree of James Bay Québec (Zhou, et al., 2009). The associations between age and fatty acid classes in erythrocyte membranes in the present study showed very similar patterns to those observed in the Cree of James Bay (Zhou, et al., 2009). Both James Bay Cree and Canadian Arctic Inuit had pronounced increases in HUFA n-3 with age, while fatty acids from the n-6 family all showed decreases with advancing age. The opposite associations of HUFA n-3 and n-6 with age may be due to the high consumption of marine fish and/or marine mammals rich in n-3 among community members and the competition between fatty acids from n-3 and n-6 families for the enzymes in the endogenous fatty acid synthesis pathway and for binding-sites on erythrocyte membranes. The only primary n-3 that did not show age related association in both studies was ALA, which primarily comes from plant sources. Both the present work and the James Bay Cree study (Zhou, et al., 2009) observed that SFA, MUFA and TFA levels were similar across age groups, which could be attributed to membrane homeostasis (Zhou, et al., 2009) or to similar dietary intake levels. Age related dietary transition is driven by the interplay among multiple factors, such as age-differences in food preference (Wein, et al., 1996), greater representation of younger adults in the workforce with less time for hunting and harvesting activities (Wein, et al., 1996) and customs of providing traditional food to an elder as an expression of respect (Wein, et al., 1998).

The significant inter-generational difference of HUFA n-3 status among Canadian Arctic Inuit suggests differences due to the proportionally different consumption levels of traditional foods and market foods and provides evidence of ongoing nutrition transition. At the same time, between-regional differences are more difficult to interpret. When comparing regions, it is important to recognize that market food choices but not market food per se (Kuhnlein, et al., 2008) influence fatty acid profiles. Further, a comprehensive evaluation of different dietary indicators is needed to assess the relative intake of traditional and market food. Moreover, traditional foods are harvested locally and nutrient concentration of traditional foods from the same species could vary in different geographic locations. Additionally, household economy varies between regions, which could also influence market food choices. Thus, interpreting regional differences in fatty acid status is more difficult than within region comparisons.

Interestingly, the association between HUFA n-3 and age in the Central Arctic Baffin and Kivalliq was much stronger than that in other arctic regions. The reasons for the latter observation are not clear. Coincidently or not, according to 2006 Canada census, Baffin and Kivalliq are the two regions that reported the highest proportion of people speaking non-official languages (presumably Inuit traditional languages) at home rather than English, 65% and 60%, respectively (Statistics Canada, 2006). The proportion of non-English speaking at home was only 24% in Baker Lake, 14% in Kitikmeot, and as low as 2-3% in Inuvik and Aklavik (Statistics Canada, 2006). Similarly, in our survey, a large proportion of Baffin Inuit and Kivalliq Inuit reported to speak Inuit languages only at home, in contrast to only a minority of Inuit in Kitikmeot, ISR and Nunatsiavut reported so. Language is the core of a culture. The predominant and primary language spoken at home likely reflects the degree of western influence and would be related to traditional food harvesting activities and consumption and fatty acid status.

Data from the IPY Inuit Health Survey strongly suggests that Inuit foods that are rich in HUFA n-3 are being replaced by low quality market foods or other local foods with inferior HUFA n-3 level. These trends are particularly evident in

inter-generational differences and the difference between inland and coastal communities. These latter findings are consistent with previous study reports (Counil, et al., 2008) (Egeland, et al., 2009). Consequently, a deteriorating fatty acid profile characterized as reduced protective factor (HUFA n-3) and elevated detrimental factors (SFA and TFA) is occurring among Inuit, particularly the young, and those living inland.

Our data confirms that obesity is prevalent among Canadian Arctic Inuit. Obesity is a major contributor to a variety of chronic diseases including cardiovascular disease and type 2 diabetes mellitus (Smith, 2007). In line with a recently published study conducted among Cree of James Bay (Zhou, et al., 2009), other human observational studies (Warensjö, et al., 2006) (Pan, et al., 1995) (Decsi, et al., 1996) and animal experiments (Blond, et al., 1989) (Cunnane, et al., 1985), an inverse relationship was observed between adiposity and Δ5 (waist: $r_s = -.162$, $P<.0001$, adjust for age, data were not shown), a critical enzyme in the biosynthesis of HUFA. Obesity is also related to insulin resistance whose inhibition on Δ5 has been well documented in the literature (Vessby, et al., 2002). Thus, obesity among Inuit could reduce biosynthesis of long-chain fatty acids and, thereby, exacerbate the effects of reduced n-3 intake associated with dietary transition. Dietary transition and obesity may be further exacerbated by a hypothesized genetic impairment in Δ5 activity proposed by Gibson and Sinclair (Gibson & Sinclair, 1981). A few studies among Canadian Central Arctic Inuit, Canadian west coastal Inuit and Greenland Inuit also observed the disproportionally lower AA, the immediate Δ5 product, which was not explained by the diet (Stark, et al., 2002) (Young, et al., 1999) (Bates, et al., 1985). While further research is warranted, it can be hypothesized that dietary transition compounded by a high prevalence of obesity among Inuit could eventually increase disease burden in this population.

There are a few limitations of the current study. As a cross-sectional survey, no causality can be established from the observed association. Moreover, there were no data available at the time of data analysis and manuscript drafting

about the absolute calorie intake of food items consumed. Furthermore, fatty acids of different families were expressed as percentages of total fatty acids and not as absolute amounts. Consequently, the proposed mechanisms of the observed regional discrepancies cannot be tested in the present study. Additionally, the survey was conducted in the later summer and early fall and may therefore not reflect circumstances in other seasons. Furthermore, insulin is an important regulator of Δ5, however, insulin data were not available for incorporation into the analyses. The adjustment for waist, however, is a well-recognized variable that is highly correlated with insulin, which could at least partly correct any bias. The relationships observed, however, are consistent with previous metabolic studies examining the impact of western-style high fat, SFA and TFA on fatty acid biomarkers. Future studies are needed to examine the external validity of the findings observed from the current study. Also, compositional analysis of meal samples is recommended in future studies, which could provide a more accurate assessment of regional differences than dietary studies alone. Moreover, comparison of the composition of meals collected over time could help in the evaluation of temporal changes in diet.

According to the best knowledge of the authors, the present study is the first to describe the fatty acid composition of erythrocyte membranes in Inuit across the Canadian Arctic. In contrast to popular perceptions of a homogeneous population, considerable differences in fatty acid profiles were observed among Canadian Arctic Inuit, reflective of the heterogenous dietary intake patterns between regions and generations. The current study provides additional evidence of dietary transition among Arctic Inuit, which may unfortunately exacerbate chronic disease risk. These findings strongly call for action to formulate multiple approaches to maintain and restore HUFA n-3 status among Canadian Arctic Inuit.

5.6. Acknowledgement

This study was supported by the Canadian Institute of Health Research (CIHR) and Inuvialuit Settlement Region, Nunavut and Nunatsiavut IPY Inuit Health Survey Steering Committees. The authors thank the International Polar Year Inuit Health Survey research staff and participants. The authors' responsibilities were as follows-Yuan E. Zhou: analyzed the data and drafted the manuscript; Stan Kubow: supervised the manuscript drafting; Grace M. Egeland: designed the study and supervised the data analysis and drafting; and all authors: contributed to the revision of the manuscript. None of the authors had any financial or personal conflict of interest to disclose.

Table 5.1. Population characters of survey regions (mean ± SD)

Variable	Nunatsiavut	Baffin	Kitikmeot	Kivalliq	Inuvialuit	Baker Lake (Kivalliq)[1]	Aklavik, Inuvik (Inuvialuit)[1]	P
Age (yr)	44.5±14.0 n=265	41.8±14.9 n=778	41.0±15.0 n=360 L2	41.1±15.9 n=407	44.2±17.2 n=163	44.5±16.2 n=107	44.2±17.9 n=129	.0012
BMI	29.2±5.7 n=259	26.8±6.0 n=751 L	29.4±6.8 n=335	28.6±6.9 n=392	29.8±6.3 n=145	28.2±5.9 n=106	29.4±6.5 n=112 H	<.0001
Waist (cm)	96.5±14.2 n=260	88.5±14.3 n=750 L	95.7±17.1 n=334	92.5±16.4 n=385	100.0±16.6 n=147	93.4±14.5 n=99	98.4±17.2 n=114 H	<.0001
Body fat(%)	33.1±10.1 n=257	28.0±10.9 n=750 L	31.0±11.3 n=332	30.0±11.0 n=392	33.4±10.6 n=145	30.3±10.6 n=105	32.0±10.7 n=112 H	<.0001
Female%	62.6	61.5	60.0	58.0	63.6	58.9	69.7	ns
Obesity%[3]	39.6	26.8 L	38.1	35.1	40.3	29.9	49.3 H	<.0001
Obesity%[4]	58.1	35.7 L	48.3	42.0	55.8	45.8	74.3 H	<.0001
Smoking%[5]	54.8 L	73.0	73.5	72.0	70.9	71.0	62.2	<.0001
Drinking%[5]	69.7	61.6	67.1	50.7	69.9	40.2 L	64.1	<.0001
Speaking Inuit language at home(%)	1.3	88.0	19.8	82.4	12.1	39.5	3.6	<.0001

[1]Baker Lake is an inland community in Kivaliq, Aklavik and Inuvik are inland communities in Inuvialuit Settlement Region.

[2]"L" refers to lowest and "H" refers to highest among regions.

[3]Obesity: BMI≥30

[4]Obesity: waist>88cm for women and waist>102 for men

[5]The remaining regions tied regarding the prevalence of smoking and drinking

Table 5.2. Median (interquartile ranges) of selected fatty acids as a percent of total fatty acids of erythrocyte membranes: International Polar Year Inuit Health Survey, 2007-2008.

Fatty acids	Nunatsiavut	Baffin	Kitikmeot	Kivalliq	Inuvialuit	Baker Lake (Kivalliq)[1]	Aklavik,Inuvik (Inuvialuit)[1]
	n=230	n=749	n=358	n=438	n=163	n=110	n=152
Myristic acid (C14:0)[2]	0.25 [L]	0.48 [n=778]	0.23 [L2]	0.67 [H]	0.28 [L]	0.37	0.42
	(0.21-0.33)	(0.37-0.62)	(0.16-0.33)	(0.54-0.83)	(0.22-0.34)	(0.28-0.53)	(0.32-0.51)
Pentadecanoic acid (C15:0)[2]	0.18 [L]	0.21	0.20 [L]	0.27 [H]	0.23	0.30	0.24
	(0.14-0.22)	(0.16-0.26)	(0.16-0.23)	(0.19-0.38)	(0.19-0.26)	(0.24-0.37)	(0.16-0.31)
Palmitic acid (C16:0)	25.41 [L]	28.66	26.09	27.97	29.94 [H]	38.64	37.87
	(24.43-27.09)	(26.86-30.51)	(24.12-29.71)	(26.89-29.52)	(26.73-32.16)	(31.49-40.92)	(35.80-39.41)
Stearic acid (C18:0)	12.77	11.45 [L]	13.05 [H]	12.42	12.91	14.10	13.72
	(12.06-13.41)	(10.65-12.45)	(12.26-14.07)	(11.65-13.29)	(11.51-13.90)	(12.87-15.78)	(12.86-14.58)
Total SFA	39.72 [L]	41.45 [n=778]	41.41	42.69	43.89 [H]	54.99	53.53
	(38.62-41.63)	(39.73-43.69)	(38.42-46.67)	(40.82-44.35)	(41.38-48.20)	(48.54-58.31)	(51.11-55.36)
Palmitoleic acid (C16:1 *n-7*)[2]	1.23	0.71 [L]	1.51[H]	0.79	0.79	1.34 [H]	1.10
	(1.01-1.67)	(0.52-0.93)	(1.19-1.95)	(0.64-0.97)	(0.58-1.02)	(0.88-1.97)	(0.83-1.46)

Oleic acid (C18:1 *n-9*)	18.62 [L]	22.49 [n=778]	20.96	23.31 [H]	22.52	27.53	26.95
	(17.79-20.07)	(21.04-24.00)	(19.59-23.07)	(22.26-24.44)	(20.37-24.55)	(24.78-29.19)	(25.40-28.16)
Total MUFA	21.46 [L]	24.18 [n=778]	24.56 [H]	25.16 [H]	24.74 [H]	30.46 [H]	29.51
	(20.24-23.23)	(22.65-25.69)	(22.68-27.09)	(24.17-26.41)	(22.73-27.22)	(27.56-31.76)	(27.69-30.67)
C16:1 *trans*[2]	0.03	0.05	0.02	0.09 [H]	0.01 [L]	0.10	0.15 [H]
	(< 0.01-0.10)	(0.02-0.09)	(< 0.01-0.10)	(0.04-0.19)	(< 0.01-0.02)	(0.01-0.18)	(0.03-0.20)
C18:1 *trans*[2]	0.87	0.82 [L]	1.20 [H]	0.90	1.10 [H]	1.59 [H]	1.29
	(0.70-1.06)	(0.50-1.28)	(0.95-1.51)	(0.55-1.31)	(0.91-1.46)	(1.27-2.07)	(1.04-1.59)
C18:2 *trans*[2]	0.16	0.13 [L]	0.17	0.13 [H]	0.17	0.17	0.14
	(0.14-0.19)	(0.07-0.19)	(0.12-0.21)	(0.04-0.30)	(0.13-0.21)	(0.12-0.21)	(0.11-0.18)
Total trans[2]	1.08	1.04 [L]	1.43 [H]	1.22	1.29 [H]	1.89 [H]	1.58
	(0.93-1.32)	(0.64-1.55)	(1.15-1.79)	(0.75-1.74)	(1.10-1.67)	(1.53-2.54)	(1.32-1.88)
LA (C18:2 *n-6*)	14.79	15.91 [H]	13.67 [L]	14.99 [L]	13.82	7.61	10.74 [H]
	(13.12-16.23)	(13.69-17.68)	(11.16-15.57)	(13.20-16.59)	(12.21-15.70)	(5.62-10.96)	(9.52-11.92)
Homo gamma linoleic acid	1.58 [H]	1.29 [L]	1.35 [L]	1.41	1.08 [L]	0.36	0.66 [H]

(C20:3 *n-6*)	(1.33-1.91)	(0.95-1.68)	(0.91-1.76)	(1.13-1.68)	(0.85-1.56)	(0.24-0.88)	(0.55-0.82)
AA (C20:4 *n-6*)	10.95 [H]	7.46	8.29 [L]	8.16	6.66 [L]	1.44	2.27 [H]
	(9.17-12.49)	(6.41-8.75)	(5.75-9.97)	(7.13-9.12)	(4.70-9.01)	(0.89-4.54)	(1.78-3.07)
Total *n-6*	30.62 [H]	26.05	25.26 [L]	25.58	23.63 [L]	9.83	14.22 [H]
	(27.11-32.70)	(22.65-29.05)	(19.22-29.22)	(23.46-27.91)	(19.91-27.51)	(7.01-17.06)	(12.67-16.91)
HUFA *n-6*	12.83 [H]	8.36	9.54 [L]	9.06	7.87 [L]	1.51	2.53 [H]
	(10.73-14.87)	(7.13-9.86)	(6.39-11.53)	(7.86-10.36)	(5.40-10.50)	(0.93-5.44)	(1.93-3.93)
ALA (C18:3 *n-3*)	0.23 [H]	0.18	0.20 [L]	0.18	0.14 [L]	0.01	0.01
	(0.18-0.27)	(0.11-0.25)	(0.09-0.30)	(0.13-0.24)	(0.08-0.23)	(< 0.01-0.06)	(< 0.01-0.05)
EPA (C20:5 *n-3*)	1.02	1.68 [H]	1.13	0.83 [L]	0.93	0.85 [H]	0.39
	(0.71-1.48)	(0.87-3.19)	(0.72-1.82)	(0.49-1.64)	(0.59-1.63)	(0.49-1.44)	(0.22-0.66)
DPA (C22:5 *n-3*)	1.62 [H]	1.56 [H]	1.54 [L]	1.42	1.23 [L]	0.03 [H]	0.01
	(1.34-1.81)	(1.22-1.98)	(0.99-1.99)	(1.19-1.71)	(0.67-1.61)	(<.01-0.15)	(<.01-0.12)
DHA (C22:6 *n-3*)	3.73 [H]	2.72	2.83	1.75 [L]	2.21 [L]	0.11	0.19
	(2.84-4.81)	(1.78-3.71)	(1.65-4.41)	(1.10-2.69)	(1.01-3.78)	(0.05-0.60)	(0.09-0.41)
Total *n-3*	6.61 [H]	6.31 [H]	5.84	4.30 [L]	4.53 [L]	1.39 [H]	0.75

	(5.38-8.26)	(4.25-9.23)	(3.75-8.25)	(3.17-6.13)	(2.41-7.11)	(0.79-3.23)	(0.44-1.27)
HUFA *n-3*	6.32 [H]	6.13 [H]	5.52 [L]	4.05 [L]	4.17 [L]	1.33 [H]	0.68
	(5.02-8.03)	(4.01-9.05)	(3.53-7.97)	(2.88-5.91)	(2.30-6.82)	(0.76-3.06)	(0.41-1.13)
n-6/*n-3*	4.58 [L]	4.17 [L]	4.47 [L]	6.14 [H]	5.82 [H]	7.01	18.91 [H]
	(3.47-5.58)	(2.58-6.74)	(3.05-6.01)	(4.07-8.47)	(3.73-8.27)	(4.80-12.37)	(10.65-29.60)
DPA/EPA	1.52 [H]	0.93	1.12 [L]	1.66 [H]	1.09	0.06	0.04
(C22:5*n-3* / C20:5*n-3*)	(1.06-2.11)	(0.56-1.48)	(0.67-1.84)	(0.95-2.62)	(0.57-1.69)	(<.01-0.41)	(0.01-0.43)
SFA/PUFA	1.05 [L]	1.24	1.27 [H]	1.39	1.45 [H]	4.91 [H]	3.53
	(0.98-1.21)	(1.13-1.42)	(1.04-1.88)	(1.25-1.54)	(1.20-2.09)	(2.39-6.90)	(2.90-4.21)

[1] Baker Lake is an inland community of the Kivalliq Region of Nunavut; Aklavik and Inuvik are inland communities in Inuvialuit.

[2] "L" refers to statistically lowest and "H" refers to statistically highest among regions. Comparison was conducted within coastal communities and within inland communities separately.

Table 5.3. Partial spearman correlations of n-3 fatty acids with SFA and TFA[1]

	Myristic acid (C14:0)	Palmitic acid (C16:0)	Stearic acid (C18:0)	SFA	C16:1 *trans*	C18:1 *trans*	C18:2 *trans*	TFA
EPA (C20:5 *n-3*)	-0.012	-0.260****[2]	-0.304****	-0.350****	-0.149****	-0.209****	-0.091****	-0.222****
DPA (C22:5 *n-3*)	-0.116****	-0.641****	-0.357****	-0.688****	-0.199****	-0.321****	-0.113****	-0.338****
DHA (C22:6 *n-3*)	-0.285****	-0.662****	-0.320****	-0.699****	-0.250****	-0.302****	-0.042*	-0.324****
HUFA *n-3*	-0.163****	-0.587****	-0.365****	-0.648****	-0.233****	-0.311****	-0.084***	-0.331****

[1] Adjusted for age, n=2197.

[2] *<.05,**<.01,***<.001,****<.0001

Table 5.4. Partial spearman correlations of estimated Δ5 desaturase activity with adiposity[1]

	BMI (m/kg^2)	Waist (cm)	Body fat (%)
AA / homo gamma linoleic acid (C20:4 *n-6*/C20:3 *n-6*, Δ5 estimate) [2]	-.128****[2]	-.162****	-.125****

[1] Adjusted for age, n=2067.

[2] *< .05, **<.01, ***<.001, ****<.0001

Figure 5.1. Median level of fatty acid classes of erythrocyte membranes of Inuit from coastal regions and inland communities

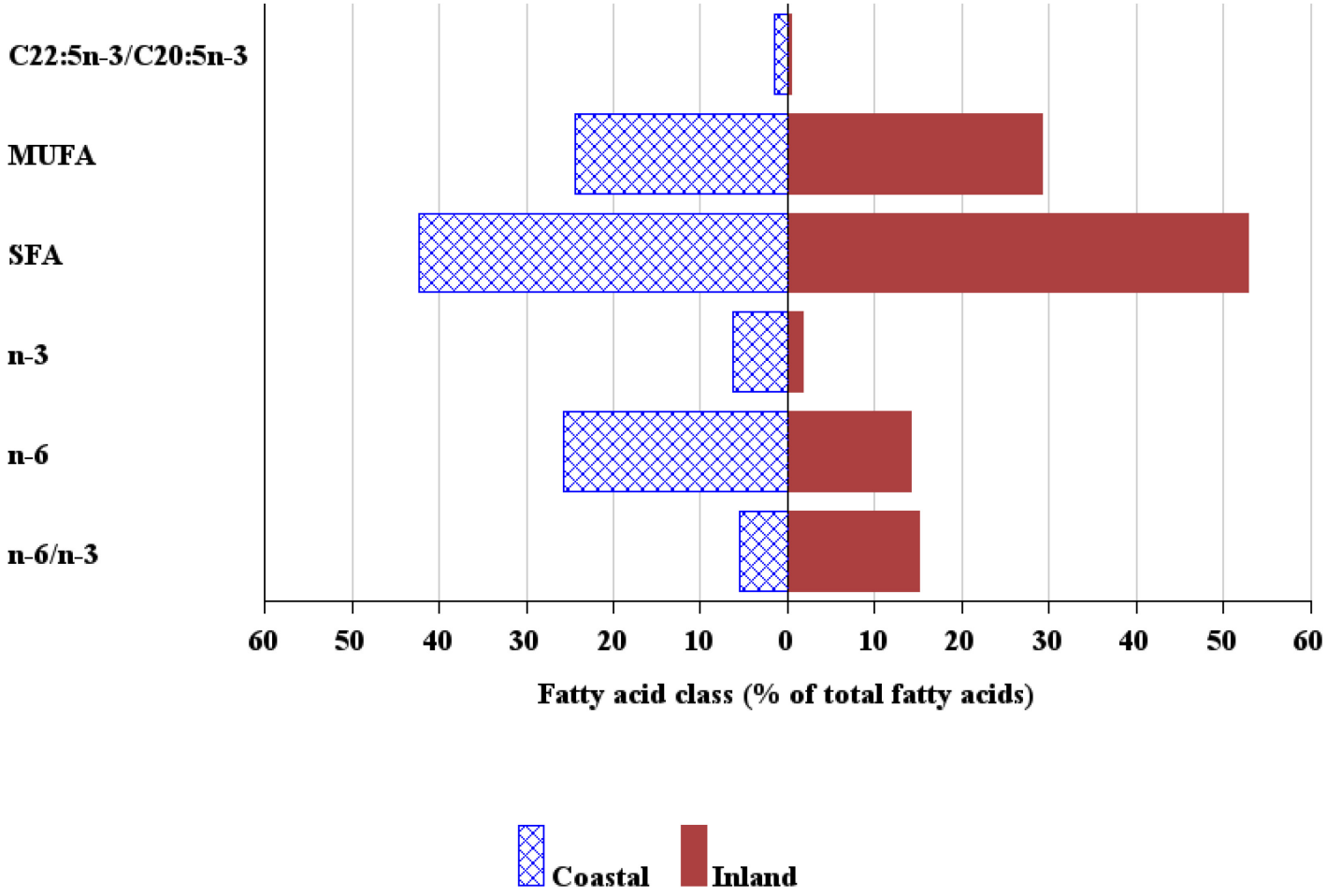


Figure 5.2. HUFA n-3 of erythrocyte membranes of Inuit from Baffin & Kivalliq (excluding Baker Lake) and other regions (median and interquartile range)

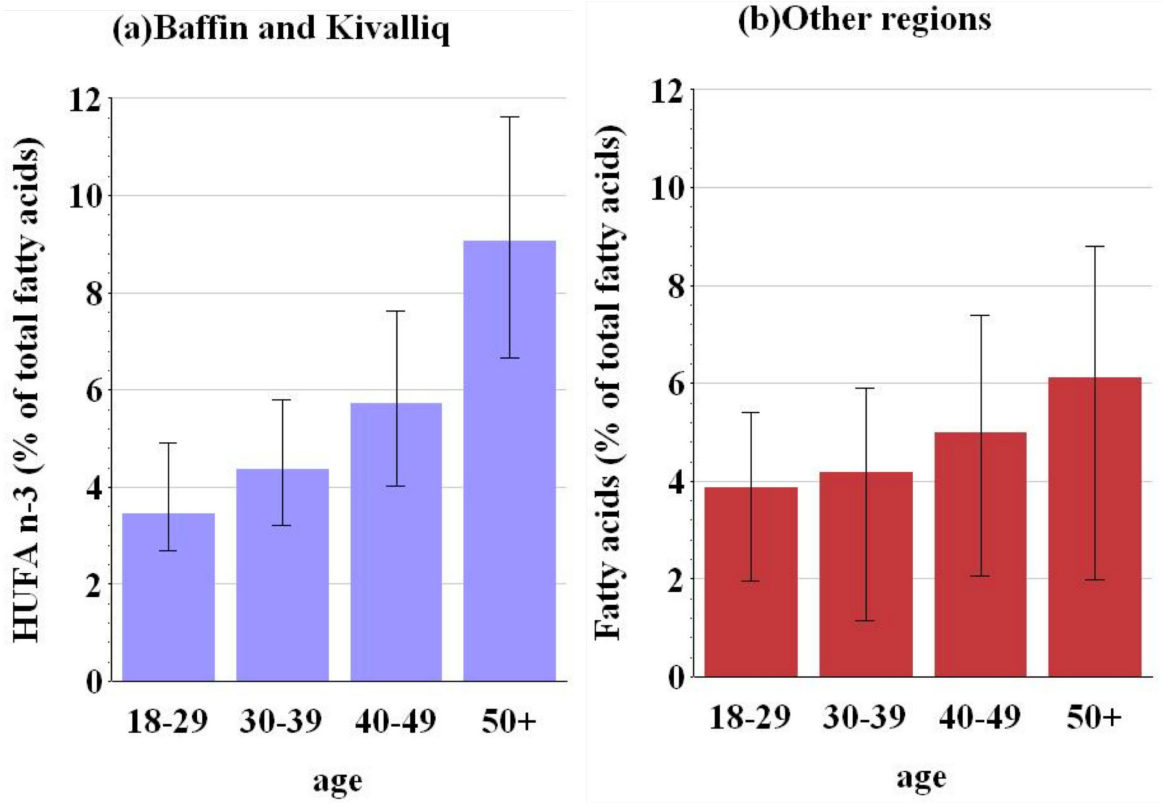
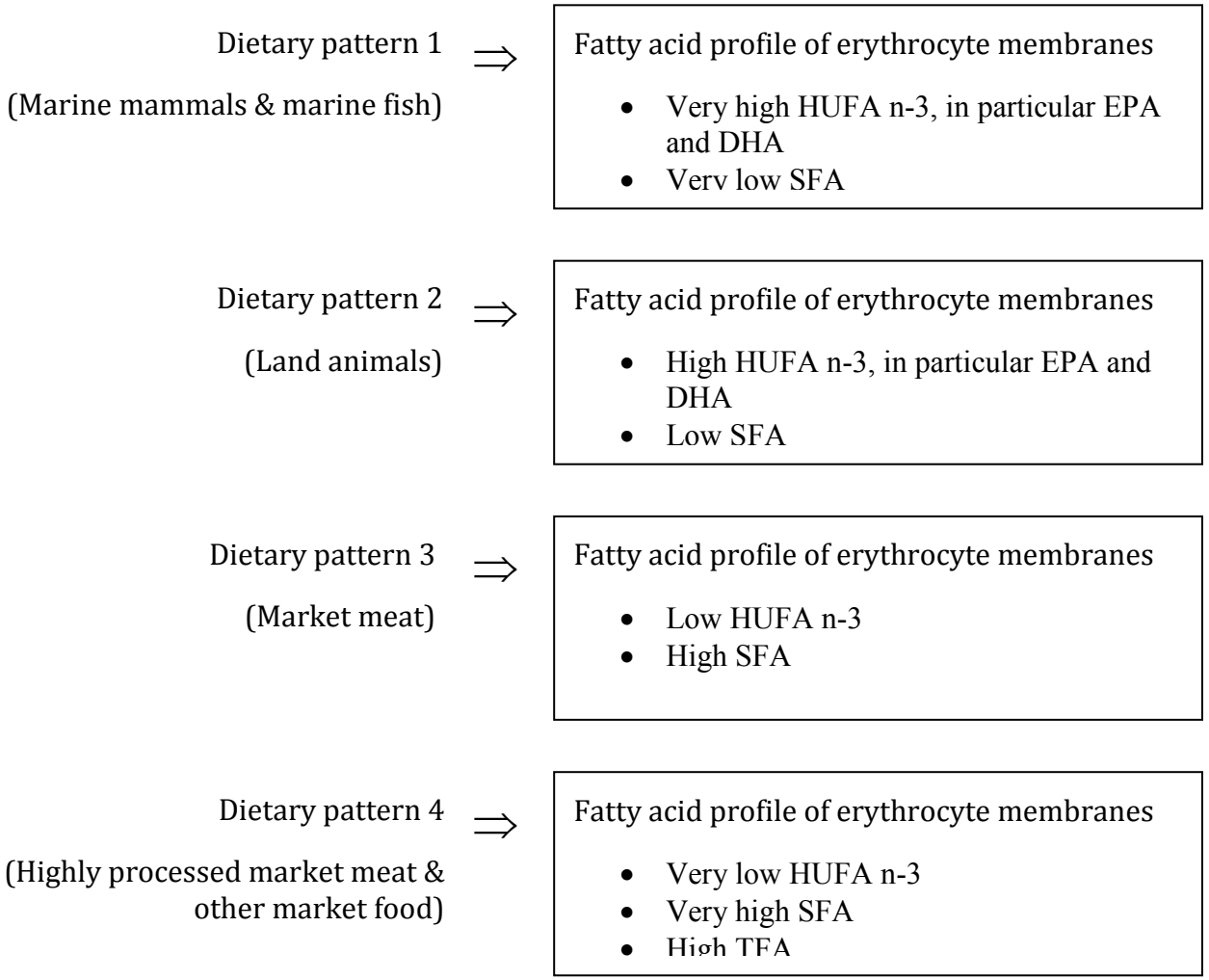


Figure 5.3. Diagram of theoretical fatty acid profiles of erythrocyte membranes with different dietary patterns



Bridge 4

Manuscripts 1 and 2 focused on defining the role of adiposity and its related metabolic consequences such as insulin resistance on the regulation of Δ9 among adolescent females and the regulation of Δ5 among James Bay Cree. **Manuscript 3** examined whether the inverse association between adiposity and Δ5 noted among Cree could also be observed among Canadian Arctic Inuit, and whether the inter-generational differences in HUFA *n-3* levels seen among the Cree also exists in the Inuit population. **Manuscript 3** further examined the inter-regional differences in HUFA *n-3* status among the Canadian Arctic Inuit. Previous animal studies, however, had suggested that besides hormonal regulators, fatty acid metabolism was also subject to nutritional regulators. For instance, mild iron deficient male Sprague-Dawley rats showed decreased AA to LA ratios in plasma phospholipids (Cunnane & McAdoo, 1987), indicating impaired fatty acid metabolism under the condition of moderately low iron status. However, information from human studies is limited regarding whether iron status would impact fatty acid metabolism. Low serum iron levels were related to the reduced conversion of short chain *n-3* fatty acids into long chain *n-3* among adults, reflecting a decrease of the activity of Δ5 and/or Δ6 (Krajcovicova-Kudlackova, et al., 2004). The presence of ID among Inuit residing in Canadian Arctic has been suggested to be a public health concern (Jamieson & Kuhnlein, 2008). Therefore, data obtained from Inuit Health Survey could provide an opportunity to explore the interplay between long chain *n-3* status, desaturase activity and ID status among Canadian Arctic Inuit.

CHPATER 6. MANUSCRIPT 4

Is iron status associated with highly unsaturated fatty acid status among Canadian Arctic Inuit?

Yuan E. Zhou[a], Stan Kubow[a] , Grace M. Egeland[a]

Author affiliation:

[a] School of Dietetics and Human Nutrition and the Centre for Indigenous Peoples' Nutrition and Environment, McGill University, Macdonald Campus, 21,111 Lakeshore Rd., Ste.Anne-de-Bellevue, Québec, Canada, H9X 3V9

This manuscript is to be submitted.

This study was supported by Canadian Institute of Health Research (CIHR) grant

Manuscript outline

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6.1. Abstract

Background:

Impaired unsaturated fatty acid synthesis was noted in iron deficient (ID) animal models, suggesting that iron may have an impact on fatty acid *de novo* lipogenesis. However, limited information is available from human study populations. Traditional food of Canadian Arctic Inuit is considered to be an important source of nutrients such as highly unsaturated n-3 fatty acids (HUFA) and iron.

Methods:

Utilizing erythrocyte membrane fatty acid composition as an indicator of unsaturated fatty acid status, we explored whether the presence of ID would affect fatty acid status and an estimate of the activity of desaturase 5 (Δ5), which is crucial in the bio-synthesis of HUFA. Data analyzed were collected among 1528 Canadian Arctic Inuit (≥ 18 years old) who participated in the International Polar Year Inuit Health Survey, 2007-2008.

Results:

Only 13.7% of survey participants had ID. Serum ferritin showed a moderate positive association with HUFA n-3 after adjusting for age, waist and C-reactive protein ($r=0.169$, $P<.0001$). Serum ferritin correlated significantly with Δ5 after further adjustment for HUFA n-3 ($r=0.126$, $P<.0001$). HUFA content increased with elevated ferritin levels across the study population. The activity of Δ5, however, was not lower in the presence of ID.

Conclusion:

Given the existence of mild ID together with a high intake of iron and HUFA among Inuit, the link between serum ferritin and Δ5 might be obscured in the current study population. Future studies are recommended in other populations

that experience more severe ID to evaluate iron status in relation to the activity of Δ5 and HUFA status, in combination with either moderate or low levels of HUFA consumption.

6.2. Introduction

Despite well-documented hormonal regulation of endogenous fatty acid metabolism, there is relatively little knowledge regarding nutritional regulators, particularly micronutrients. Experimental rat studies suggest that reduced iron status could be related to altered fatty acid metabolism as moderate diet-induced iron deficiency (ID) resulted in reduced conversion of short-chain polyunsaturated fatty acids to highly unsaturated fatty acids (HUFA) (Cunnane & McAdoo, 1987) (Stangl & Kirchgessner, 1998). These latter findings demonstrated decreased activity of iron-containing desaturases including Δ5, which are crucial in the *de novo* lipogenese of HUFA (Nakamura & Nara, 2004b). The association between ID and HUFA status, however, has not been fully explored in the human context. For Canadian Arctic Inuit, their traditional HUFA *n-3* rich diet is considered to be a protective factor against cardiovascular diseases. Despite their putatively high consumption of red meat, anemia has been considered to be prevalent among Inuit (Jamieson & Kuhnlein, 2008). Given the suggested prevalence of ID among Inuit and the potentially important physiological function of iron on PUFA *n-3* metabolism in humans, the impact of iron status on HUFA status among Canadian Arctic Inuit was investigated.

6.3. Method

6.3.1. Location and subjects

The current analyses are based upon data from the International Polar Year (IPY) Inuit Health Survey conducted in the late summer and fall of 2007 and 2008. It was a comprehensive health survey of Inuit residing in three jurisdictions covering a vast territory from Canadian Eastern Arctic Western Arctic. Participants, age 18 years or older, who self-identified as Inuk were recruited

through random selection of households stratified by community with pregnant women being excluded. The current analyses was restricted to 1528 individuals who had available data on erythrocyte membrane fatty acids, serum ferritin and hemoglobin and whose C-reactive protein (CRP) level was less than 3.0 mg/L. The project was reviewed and approved by McGill Institutional Review Board and the three Inuit jurisdictions.

6.3.2. Laboratory analyses

6.3.2.1. Anthropometric measures

Weight and body fat percentage were measured using a bioelectrical impedance scale (Tanita Inc., Tokyo, Japan). Height was measured without shoes using a stadiometer with the patient standing on a hard surface. BMI was calculated (kg/m^2). Waist circumference was measured at the end of a normal expiration with the tape placed horizontally between the last floating rib and the top of the hip.

6.3.2.2. Fatty acids

Erythrocyte membrane fatty acid concentrations were determined by gas-liquid chromatography (Lipid Analytical Laboratories Inc, Guelph, Canada) as described previously (Stark & Holub, 2004). Lipids were extracted from the blood samples according to the method of Folch et al (Folch, et al., 1957). The fatty acid methyl esters were prepared by the method of Morrison and Smith (Morrison & Smith, 1964), and were analyzed on a Varian 2400 gas-liquid chromatograph (Palo Alto, CA) with a 60-m DB-23 capillary column (0.32 mm internal diameter).

6.3.2.3. Hemoglobin, ferritin and CRP

Hemoglobin concentrations in freshly collected whole blood samples were measured by HemoCue 201+ analyzers (HemoCue, Inc., Lake Forest, CA, U.S.A.). Serum samples were separated from fasting blood samples and stored at -

80°C until analysis. CRP was measured by a high sensitive Near Infrared Particle Immunoassgy rate methodology with SYNCHRON Systems (Beckman Coulter, Mississauga, Canada) with detection limit at 0.2 mg/L. Ferritin was measured by a sandwich chemiluminescence immunoassay on a LIAISON Analyzer (DiaSorin S.p.A., Saluggia (Vercelli, Italy) with detection limit < 0.5 ng/mL.

6.3.3. Statistical analysis

As there were no gender related differences regarding the fatty acid composition of erythrocyte membranes and no observed interactions of gender and geographic location on the associations among variables of interest, the results were presented in a combined manner for all subjects. Spearman correlations were conducted, including the correlation between Δ5 and ferritin adjusted for age, waist, CRP and HUFA n-3. The age, waist and region adjusted fatty acid and desaturase differences between subjects across categories of serum ferritin (i.e., those with ID and 3 tertiles of serum ferritin among those without ID) was evaluated by Generalized Linear Model (GLM) and post-hoc comparisons were conducted with Bonferroni adjustment. The activity of Δ5 was estimated by the ratio of AA/ homo gamma linoleic acid. ID was defined according to recommended cut-off values derived from NHANES (Looker, et al., 1997). In order to avoid the results being biased by inflammation, which is known to elevate serum ferritin levels, the data were evaluated by adjusting for serum CRP in correlation analyses and by excluding participants with a CRP greater than 3.0 mg/L in all analyses (Pearson, et al., 2003). All P values were obtained from 2-sided tests. Data were analyzed with the SAS software (version 9.2; SAS Institute, Cary, NC).

6.4. Results

6.4.1. Subject characteristics

Overall 26.3% of women and 3.4% of men were defined as having ID. Overweight and obesity was prevalent in both genders (BMI: 25.5±5.0

[mean±SD] and 27.4±5.6 for participants with ID and without ID, respectively). Serum ferritin showed marked differences between participants with and without ID: the median (Q1-Q3) ferritin level was 6.60 (4.70-9.40) μg/L and 35.70 (21.60-72.50) μg/L for participants with ID and without ID, respectively.

6.4.2. Spearman correlation coefficients of fatty acid composition of erythrocyte membranes and Δ5 with serum ferritin and hemoglobin among Inuit

In contrast to other fatty acids, *n-3* fatty acids showed strong and positive associations with age (r=0.420 for HUFA *n-3*, *P*<.0001)(Table 6.1.). Similarly, Δ5 and serum ferritin also demonstrated strong correlations with age (both r >0.3, both *P*<.0001). The correlation between hemoglobin and age, however, was too weak to be considered clinically important (r=-0.069, P<.01). After adjustment for age, waist and CRP, ferritin showed moderately strong correlations with HUFA *n-3* (r=0.169, *P*<.0001) and Δ5 (r=0.194, P<.0001)(Table 6.1.). After further adjusting for HUFA *n-3*, ferritin and Δ5 still displayed a significant although weak correlation (r=0.126, *P*<.0001)(Table 6.1.).

6.4.3. Fatty acid composition of erythrocyte membranes of Inuit

The HUFA composition of erythrocyte membranes for both n-3 and n-6 appeared to be lowest in the presence of ID and the HUFA status increased consistently with the elevation of iron status (both P <.0001)(Table 6.2.). Such differences were not evident on the activity of Δ5, however, estimates of Δ5 were non-significantly higher with the highest iron status (0.05< P <.1)(Table 6.2.). Erythrocyte membrane fatty acid profiles of the other fatty acid classes were similar at different levels of ferritin.

6.5. Discussion

The findings of the current study indicate that iron status, as measured by serum ferritin, may have independent associations with Δ5 and thus the synthesis of long chain unsaturated fatty acids. The latter associations, however, were not

strong enough to lead to markedly significant differences in the fatty acid profiles of erythrocyte membranes.

The higher consumption of traditional food in older age groups among Canadian Arctic Inuit has been detailed in the literature (Kuhnlein, et al., 2004), and was reflected in the results of the current study. The traditional food of arctic Inuit is mainly composed of meat from marine fish, marine mammals, land animals and birds, which are generally rich in HUFA *n-3* and heme iron (Kuhnlein, et al., 2008). The strong association of age with both HUFA *n-3* in erythrocyte membranes and serum ferritin reflected the increased consumption of traditional Inuit food sources among the elderly Inuit. The competition between fatty acids from *n-3* and *n-6* classes likely resulted in the inverse associations of *n-6* with age. The association between age and the estimated activity of Δ5 was driven by correlations of age with fatty acids. Despite the rather strong correlation with serum ferritin and age ($r=0.399$, $P<.0001$), hemoglobin was weakly correlated with age indicating that hemoglobin levels are a less relevant biomarker of traditional food consumption than ferritin.

The findings of the present study also indicate an independent but weak association between ferritin and Δ5. This relationship remained significant after adjustment for HUFA *n-3*, suggesting that it is not attributable to diet. The presence of ID was related to the lower levels of *n-3* and *n-6* HUFA. Interestingly, in a study of children, 6-11 yrs of age, with ID (ferritin 8.2±5.4 μg/L), iron supplementation for 15-weeks was associated with significant increases in the *% n-3* of erythrocyte membranes, but not in estimates of desaturase activity (Smuts, et al., 1994). In a cross-sectional study, adults with low iron status (serum iron <12 μmol/L for men, <10 μmol/L for women) were shown to have lower ratios of HUFA to short chain PUFA than adults with normal iron levels (Krajcovicova-Kudlackova, et al., 2004). More information regarding other dietary or metabolic variables are needed to evaluate the lack of a significant relationship of ID status with Δ5 in the present study.

Cautious interpretation of the results from the current study and earlier studies is needed as a variety of confounding factors could limit data interpretation in the present work. The two previous human studies discussed above had small sample sizes. For the current study, only a small proportion of individuals had ID. The sample size of Inuit with ID may not have been sufficiently large to detect significant differences. Moreover, the use of the conservative approach using Bonferroni adjustment for post-hoc comparison in the present work might have led to an inflated type 2 error. The cross-sectional nature of the current study cannot establish causal relationship between iron status and fatty acid metabolism.

In summary, the current Inuit health survey results provide only weak evidence regarding a relationship between serum ferritin and erythrocyte membrane HUFA status. As Inuit have an iron-rich animal based diet, future studies in other populations might be warranted to evaluate the role of severe ID on HUFA status.

6.6. Acknowledgement

This study was supported by the Canadian Institute of Health Research, and Inuvialuit Settlement Region, Nunavut and Nunatsiavut IPY Inuit Health Survey Steering Committees. The authors thank the IPY Inuit Health Survey research staff and participants. The authors' responsibilities were as follows-Yuan E. Zhou: analyzed the data and drafted the manuscript; Stan Kubow: supervised the manuscript drafting; Grace M. Egeland: designed the study and supervised the data analysis and manuscript drafting; and all authors: contributed to the interpretation and revision of the manuscript. None of the authors had any financial or personal conflict of interest to disclose.

Table 6.1. Spearman correlation coefficients (r_s) of fatty acid classes of erythrocyte membranes and Δ5 with serum ferritin and hemoglobin adjusted for age, waist and CRP

	Age			Ferritin *			Ferritin †	
	r_s	P		r_s	P		r_s	P
Δ5	0.316	<.0001	Δ5	0.194	<.0001	Δ5	0.126	<.0001
SFA	0.040	ns	SFA	-0.084	<.01			
MUFA	-0.057	<.05	MUFA	-0.142	<.0001			
TFA	0.010	ns	TFA	-0.134	<.0001			
n-6	-0.293	<.0001	*n-6*	0.072	<.01			
HUFA *n-6*	-0.116	<.0001	HUFA *n-6*	0.150	<.0001			
n-3 ‡	0.586	<.0001	*n-3* ‡	0.194	<.0001			
HUFA *n-3*	0.420	<.0001	HUFA *n-3*	0.169	<.0001			
Ferritin	0.358	<.0001						
Hemoglobin	-0.069	<.01						

* Adjustment for age, waist circumference and CRP (n=1505).

† Adjustment for age, waist circumference, CRP and HUFA *n-3*(n=1505).

‡ n=847 for spearman correlation with age, n=838 for partial spearman correlation with ferritin.

Table 6.2. Fatty acid composition of erythrocyte membranes of Inuit Health survey participants adjusted for age, waist, CRP and region (least square mean ± standard error)

	ID [*]	Non-ID			
		Ferritin tertile 1	Ferritin tertile 2	Ferritin tertile 3	P
	(n=210)	(n=439)	(n=440)	(n=439)	
Ferritin [†]	6.60	17.80	35.70	92.20	
(µg/L)	(4.70-9.40)	(14.70-21.60)	(30.00-45.65)	(72.50-132.80)	
Age (yr)	33.6±11.4	36.4±12.0	40.0±13.9 [n=439]	47.1±15.1	
Fatty acids					
SFA	45.93±0.29	45.59±0.20	45.16±0.21	45.57±0.22	ns
MUFA	26.29±0.18	25.80±0.13 [ab‡ 2§]	25.56±0.13 [ac4]	25.62±0.13 [ad4, bd1]	<0.01
LA (C18:2 *n-6*)	13.43±0.19	13.51±0.13	13.57±0.14	12.87±0.14 [ad4, bd4, cd4]	<0.001
Homo gamma linoleic acid (C20:3 *n-6*)	1.21±0.03	1.19±0.02	1.23±0.02	1.13±0.02 [ad4, bd4, cd4]	<0.01
AA (20:4 *n-6*)	6.29±0.17	6.53±0.12	6.91±0.12 [ac3, bc1]	7.04±0.13 [ad3, bd2]	<0.001

HUFA *n-6*	7.23±0.20	7.48±0.14	7.93±0.14 [ac3, bc1]	8.07±0.15 [ad2, bd1]	<0.001
EPA (C20:5 *n-3*)	0.95±0.08	1.12±0.06 [ab2]	1.19±0.06 [ac4, bc2]	1.90±0.06 [bd4, cd2]	<0.0001
DHA (C22:6 *n-3*)	1.77±0.08	2.00±0.06 [ab4]	2.08±0.06 [ac4, bc3]	2.12±0.06 [ad4, bd4, cd4]	<0.01
HUFA *n-3*	3.70±0.18	4.19±0.13 [ab4]	4.39±0.13 [ac4, bc3]	4.71±0.13 [ad4, bd4, cd4]	<.0001
Δ5	6.24±0.39	5.60±0.28	5.85±0.29	6.60±0.30	ns

* ID: iron deficiency (ferritin<12 µg/L).

† ferritin: median (the 25th percentile-the 75th percentile).

† [1] P<.05, [2]P<.01, [3]P<.001, [4]P<.0001.

‡ a for individuals with ID, b, c and d for non-ID individuals at the 1st, 2nd and 3rd tertile of ferritin, respectively; ad indicates significant difference between individuals with ID and non-ID individuals at the 3rd ferritin tertile, and so on.

§ [1] P<.05, [2]P<.01, [3]P<.001, [4]P<.0001.

CHAPTER 7. OVERALL SUMMARY AND CONCLUSION

The present thesis firstly evaluated the regulation of adiposity on fatty acid metabolism via its impact on the activity of rate limiting desaturases for the biosynthesis of unsaturated fatty acids and the related metabolic implications as observed in a healthy and young population. The current thesis work further examined whether obesity affects HUFA n-3 status adversely in a Canadian Cree population with possible consequent adverse health consequences that may be worsened by decreased n-3 intake associated with nutrition transition in the Cree. The proposed metabolic linkages of adiposity with disruptions in desaturase pathways and the associated metabolic abnormality were summarized in figure 7.1. The present dissertation also assessed the HUFA n-3 status among Canadian Arctic Inuit who reside in separate regions with rather distinct natural and social environments that may be at different stages of acculturation. Additionally, the current thesis explored whether iron status was associated with HUFA n-3 status via the activity of rate limiting desaturase in the biosynthesis of HUFA.

The acknowledgement of disturbed lipid metabolism as a fundamental component of insulin resistance syndrome has led to an examination of the associations of tissue fatty acid composition with insulin resistance syndrome components, particularly levels of insulin resistance. The landmark study of Borkman et al. demonstrated that Δ5 could modulate the action of insulin by altering the C20-C22 PUFA composition of biomembrane phospholipids (Borkman, et al., 1993). In contrast to Δ5, subsequent studies indicated that Δ9 and Δ6 were positive predictors of insulin resistance indices (Brenner, 2003) (Vessby, et al., 2002). Additionally, the associations of adiposity with Δ9, Δ6 and Δ5 were also noted in epidemiologic studies (Warensjö, et al., 2007) (Okada, et al., 2005) (Warensjö, et al., 2006) (Warensjö, et al., 2005), which supported *in vitro* experiments and animal studies (Cohen & Friedman, 2004) (Blond, et al., 1989)(Cunnane, et al., 1985). The above studies, however, did not address a few key issues: (a) how insulin, the only anabolic hormone that stimulates the bio-synthesis of energy nutrients including fatty acids, could impact the activities of

desaturases; (b) whether the associations of adiposity with desaturases were independent of insulin; and (c) whether the associations observed in animal models, in terms of leptin-regulated energy homeostasis being mediated by Δ9, could be replicated in the human context.

Manuscript 1 is the first part of the thesis work that investigated the associations of fasting plasma fatty acid profiles and Δ9 with insulin resistance indices, blood lipids, adiposity and dietary SFA intake indicators among female adolescents. These apparently healthy teenage girls came from a cross-sectional survey in a Montreal based gestational diabetes mellitus (GDM) cohort. Young adolescents were originally chosen to potentially provide new insights regarding the early mechanisms that underlie the development of metabolic disorders in later life. The adolescent population also offered an opportunity to largely avoid the influences of illness and aging, which may be a source of bias in other studies.

Initially the study intended to test whether a mother's GDM history influenced risk factors of daughters such as disturbed lipid metabolism, including the disturbed activity of desaturases and altered plasma fatty acid composition. Girls with and without GDM mothers, however, were not significantly different in terms of their fasting plasma fatty acid profiles. In addition, GDM status of mothers was not an effect modifier of the associations examined. It is noteworthy that girls with GDM mothers tended to have higher adiposity, particularly their waist circumference (81.2±12.4 cm for daughters with GDM mothers, 75.3±8.7 cm for daughters with control mothers, P<.001) (Egeland & Meltzer, 2008b).

As expected, adiposity measures showed significant correlations with Δ9 ratios. The positive associations between adiposity measures and Δ9 ratios were consistent with "leptin resistance" among the majority of obese individuals that was proposed in the literature (Considine, et al., 1996). The use of ratios (palmitoleic acid/palmitic acid and oleic acid/stearic acid) to estimate the activity of Δ9 can be confounded as the observed associations may result from intake of SFA rich diet rather than being derived from endogenous synthesis. After adjustment for SFA intake using pentadecanoic acid, which is exogenously

derived and whose food sources mainly include diary products and ruminant meant, as a biomarker, however, the association between adiposity measures with Δ9 ratios was significantly strengthened. This latter evidence gave strong support to the hypothesized regulatory role of adipose tissue on Δ9 activity, previously observed in animal models (Cohen & Friedman, 2004). In concert with the above findings, rodent models showed that leptin up-regulated *SCD-1* (equivalent to Δ9) regardless of the level of insulin (Biddinger, et al., 2006). On the other hand, adjustment for SFA intake greatly weakened the association between Δ9 and HOMA-IR. High SFA diets are suggested to increase insulin resistance risk, possibly through an increased degree of bio-membrane saturation leading to disrupted membrane functions, such as trans-membrane signaling. Thus, dietary SFA intake appears to confound evaluation of the associations of HOMA-IR with fatty acid metabolism. Indeed, this finding was consistent with results from the present study, suggesting that the regulation of leptin on Δ9 is not only independent of that of insulin, but may even be stronger than the latter.

The above study observed pronounced positive associations of Δ9 with TG and Apo B, independent of adiposity, insulin resistance level and dietary SFA. These associations in the human context were consistent with previous animal studies showing that Δ9 is a key factor for hepatic VLDL synthesis by facilitating the synthesis of hepatic cholesterol ester and TG (Cohen, et al., 2002)(Miyazaki, et al., 2000). The above finding, taken together with the independent associations of Δ9 with adiposity discussed above, is consistent with the concept that Δ9 is a mediator in leptin regulated homeostasis. The main contribution of **Manuscript 1** was the provision of support of the latter hypothesis in the human context, which was originally developed using animal models.

To the knowledge of the authors, the present study showed for the first time in humans the presence of inverse associations of Δ9 with PUFA, particularly LA, AA and DHA. Further adjustment for HOMA-IR did not change the significance and magnitude of these latter associations. Given the key roles of Δ9 in TG and hepatic VLDL synthesis described above, the observed inverse

relationship between PUFA and Δ9 is consistent with an inhibitory effect of PUFA on lipogenesis. These results are also consistent with animal studies showing that PUFA inhibited lipogenesis to counteract the action of insulin (Ntambi, 1992). Molecular biological studies and animal studies have shown that PUFA can inhibit the nuclear transcript factor SREBP-1c that is involved in activating lipogenesis (Sampath & Ntambi, 2004).

Manuscript 2 is the second part of present thesis work that investigated the associations of the Δ5 ratio derived from fatty acid composition of erythrocyte membrane with adiposity and insulin resistance indices among Cree of James Bay. The study participants are Cree residents from Mistissini community in the Eastern James Bay Cree in northern Québec and enrolled in the health screening. The Cree of James Bay provided an example of how evolving life styles have increased life-style related diseases, which have only emerged within the past few decades among the Cree.

Consistent with a previous report regarding the prevalence of obesity among Cree children (Downs, et al., 2008), the current thesis work showed that obesity among Mistissini Cree adults was remarkably high, particularly abdominal obesity. The prevalence of obesity was high across all age groups. More strikingly, the prevalence of obesity seemed to start at very early life stage, since for the 20-29 year group, 58.3% people had BMI ≥ 30 and 72.9% people had abdominal type of obesity.

A steep age-related positive association with the Δ5 ratio was shown among Cree. In addition, long chain n-3 fatty acid composition of erythrocyte membranes, particularly that of EPA and DHA that are rich in fish sources, were strongly and positively related to age. These results were consistent with the higher consumption of traditional foods among older age groups as shown by 24-h dietary recall data collected for this study (data not shown) and other studies regarding other indigenous populations living in circumpolar arctic area (Counil, et al., 2008). The current study, being a part of the pilot study of Multi-Community Environment-And-Health Longitudinal Study in the Cree region of

northern Québec, suggested that dietary transition and rising obesity is occurring in Cree communities, which is in concert with the rise of chronic diseases such as T2D among Cree as previously discussed.

Upon further examination, Δ5 inversely correlated with adiposity measures but unexpectedly, was not significantly related to HOMA-IR. Since about half of study participants had impaired fasting glucose, this study had the statistical power to evaluate whether impaired fasting glucose status would impact upon the above association between Δ5 and HOMA-IR. A significant interaction was observed as a significant inverse association was shown between Δ5 and HOMA-IR among Cree with normal fasting glucose, but not among Cree with impaired fasting glucose status. On the other hand, adiposity was a significant predictor of Δ5 among Cree with impaired fasting glucose whereas adiposity was a weaker predictor of Δ5 in Cree with normal fasting glucose. Taken together, the latter results indicate that the impact of adiposity on Δ5 is likely influenced by insulin status under normal fasting glucose conditions, whereas obesity might exert an independent influence on Δ5 during disturbed insulin regulation. Although *in vitro* experiments and obese rodent models have shown the latter described relationship between obesity and reduced Δ5 activity (Blond, et al., 1989)(Cunnane, et al., 1985), there is still very limited information defining the underlying mechanism(s) involved.

An implication of the current thesis is that obesity and dietary transition both impact upon tissue HUFA n-3 levels and consequent health status. High prevalence of obesity and the emerging insulin resistance among the young generation could decrease the endogenous synthesis of HUFA n-3. The reduced consumption of traditional food items among the Cree youth further contributes to the lower long chain n-3 status in the younger age groups. As long chain n-3 are associated with anti-inflammatory and anti-arrhythmic protective effects against inflammatory diseases such as cardiovascular diseases, their reduced bioavailability would likely aggravate disease risk. The above findings thus illustrate the concept that disease-related factors such as dietary transition, obesity

and insulin resistance can work in tandem to elevate disease risk in indigenous peoples undergoing rapid lifestyle transitions.

Manuscript 3 is the third part of present thesis work that examined inter-generational and geographic differences regarding long chain n-3 status among Inuit populations. The study was part of International Polar Year Inuit Health Survey, which covered Nunatsiavut, Nunavut (Baffin, Kivalliq and Kitikmeot), and ISR, located in the Canadian Eastern Arctic, Central Arctic and Western Arctic, respectively.

Obesity was prevalent among Inuit, ranging from 26.8% in Baffin to 49.3% in Inuvialuit coast communities, and abdominal obesity ranged from 35.7% in Baffin to 74.3% Inuvialuit inland communities. These results reflect similar data in our previous Cree study, although the prevalence of obesity among Cree was even higher (Zhou, et al., 2009). Similar to the findings among Cree, an inverse association between adiposity and $\Delta 5$ was observed among Canadian Arctic Inuit.

The inter-regional difference of long chain n-3 between Inuit populations was pronounced, particularly between coastal Inuit and inland Inuit. The latter showed strikingly low levels of HUFA n-3 status, less than one third of coastal community Inuit. On the other hand, the SFA level, TFA level, SFA/PUFA and n-6/n-3 of inland Inuit were pronouncedly higher than those of coastal Inuit. In addition, eastern coastal Inuit had higher HUFA n-3 status than western coastal Inuit. These observations suggested that, instead of being a homogenous ethnic group as being commonly perceived, Canadian Arctic Inuit residing in separate regions are distinct from each other, at least in terms of n-3 food consumption.

The inter-regional difference could be attributed to a variety of factors, ranging from physical environment to social environment. Inuit foods are composed primarily of meat, either from sea mammal such as whale and seal, or from fish such as arctic char, or from land animal such as caribou or birds. The distribution of animal species differs across Arctic or sub-Arctic regions, which

could lead to differences in dietary fatty acid composition (Kuhnlein, et al., 2002). For example, sea mammals and fish contain higher HUFA EPA and DHA, but less DPA, total n-6 and SFA than land animals. Furthermore, although Inuit were traditionally dependent on wildlife as food sources, Inuit in different regions could have different dietary preference to the species they would consume. Depending on region, Inuit can also have differential access to highly processed market meat and raw market meat from farmed animals, which contains higher SFA and n-6 and lower n-3 than wild meat even for the same species (United States Department of Agriculture, 2009) (Health Canada, 2009). Different economic development and wage employment opportunities could also play a role as employed Inuit have less time and energy to engage in hunting or fishing activities, or they opt to engage in hunting activities that demands less time. Employment could also increase purchasing power to buy market foods that are high in sugar, fat, SFA or TFA. Future studies are warranted for a better understanding of the various factors that could contribute to the regional differences observed in the present thesis study.

The current study also studied inter-generational differences as higher HUFA n-3 status had been found among older age groups in our previous Cree study. Interestingly, the Baffin and Kivalliq regions demonstrated a stronger age-related positive association with HUFA n-3 as compared to other regions. Given that the consumption of traditional foods is a central part of Inuit culture, the crude approach of the use of language as a surrogate indicator was applied. In that regard, consistent with our findings, a large proportion of residents in Baffin and Kivalliq were reported to speak their traditional language at home (Statistics Canada, 2006), in contrast to other regions. This report was consistent with data collected from the current study. Future work is needed to evaluate language as an indicator of westernization on health transition among Inuit.

Manuscript 4 is the fourth part of the thesis work and evaluated whether there was a significant association between ID status and long chain HUFA n-3 status among Canadian Arctic Inuit who participated the International Polar Year

Inuit Health Survey. Previous animal studies had suggested that even moderately low iron status resulted in decreased activity of desaturases (Cunnane & McAdoo, 1987)(Stangl & Kirchgessner, 1998). The current study showed weak positive associations between ferritin levels and erythrocyte membrane HUFA status, which did not seem to be attributable to diet. The estimated activity of Δ5 did not seem to be related to ID status. Despite previous literature reports (Jamieson & Kuhnlein, 2008), only a mild occurrence of ID was observed in the current survey, with an ID prevalence at 26.3% and 3.4% among females and males, respectively. The mild ID prevalence along with access to the iron rich Inuit traditional foods may explain in part the lack of a strong association between iron status and Δ5. Further exploration of the relationship between iron status and HUFA n-3 status is needed in populations with a higher risk of ID.

There were a few limitations of the studies of the present dissertation:

First, due to the nature of the study, the cross-sectional associations between the variables of interest cannot be constructed as causal but can provide an impetus for future studies to establish causal relationship.

Secondly, because it is not feasible to directly measure the activity of desaturases in humans, the ratios of daughter fatty acids to parent fatty acids are the universally applied approaches to estimate the activity of Δ9, Δ6 and Δ5 in human studies. Though convenient, this method does have its limitations. For instance, dietary intake could severely bias the ratios and the observed associations between ratios and factors of interest. It was worthy noting that in previous human studies regarding the hormonal regulation of the activity of desaturases, the reported associations were not adjusted for dietary intake data. This latter limitation could limit the generalization of previous findings.

Thirdly, each part of the thesis work also had its own limitations. In first part of the current dissertation work, only fasting plasma fatty acid data were available. Fatty acid composition in adipose tissue would much more accurately reflect long-term dietary intake and the activity of relevant metabolic pathways. A

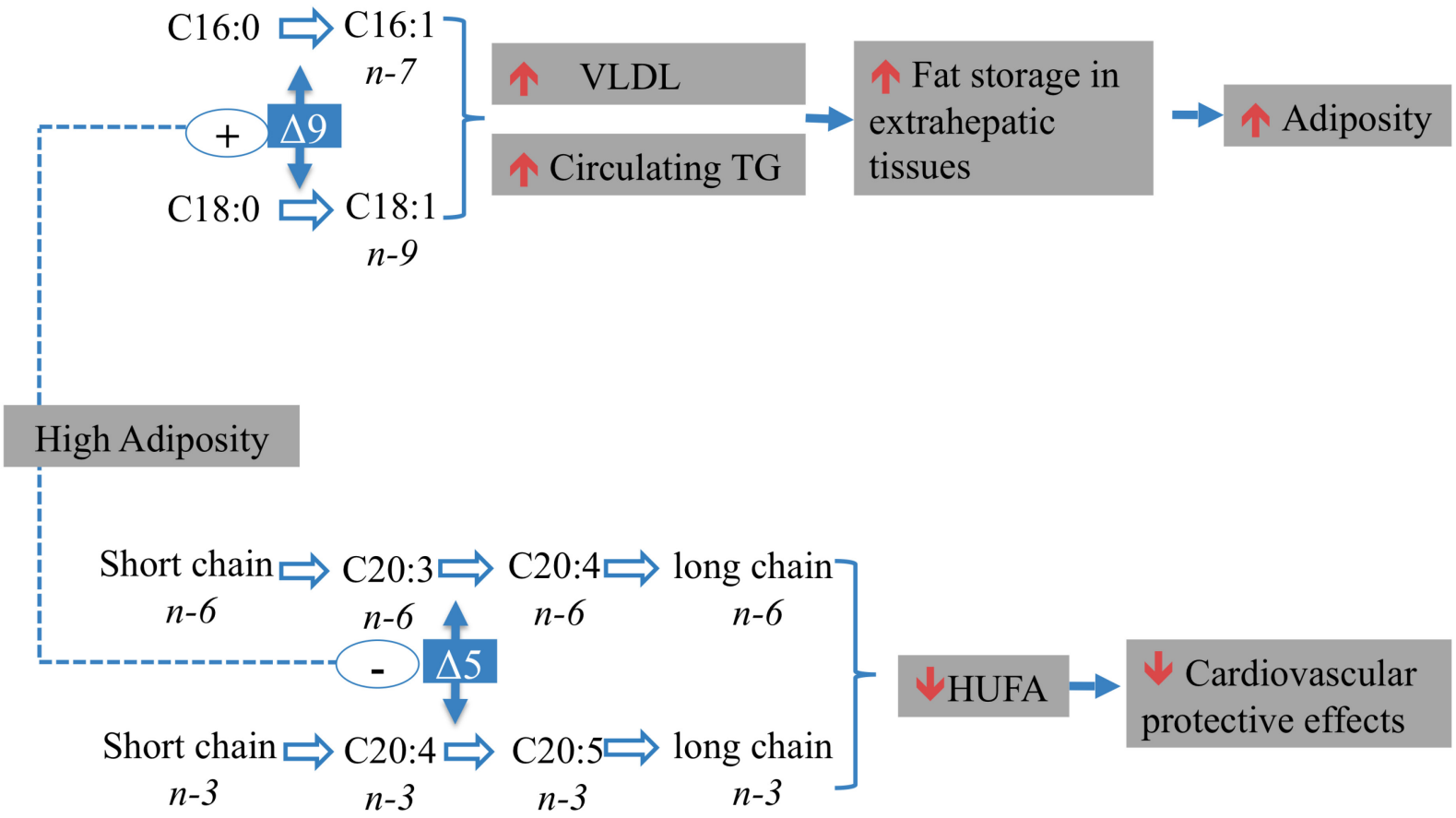
more convenient option for future studies is utilization of erythrocyte membrane fatty acid profiles as a surrogate measure for long-term dietary intake. Also, in the first part of the thesis, besides adiposity, insulin resistance and dietary intake, there could be other hormonal influences, genetic or metabolic factors that have an impact on fatty acid profiles and Δ9, which could account for the observed positive association between ETA and adiposity. Further mechanistic feeding studies would be needed to provide further evidence of the findings uncovered in the present work. Furthermore, leptin measures were not planned prior and during the implementation of the study. In the second part of the present dissertation work, bioelectrical impedance scale was used to give a crude measure for overall fat mass, but this measurement could not distinguish subcutaneous adipose tissue and visceral adipose tissue and therefore was unable to identify the impact of different parts of adipose tissue on fatty acid metabolism. In the third and fourth parts of the thesis, no dietary data were yet available to examine the fatty acid-related associations observed in the present study at the time of the completion of the dissertation.

Future studies are recommended: (a) leptin should be measured to help assess the hypothesized leptin regulated energy homeostasis pathway discussed previously in the thesis; (b) more precise measurements such as dual energy X ray scanning (DXA) are suggested to be used to examine subcutaneous adipose tissue and visceral adipose tissue in order to identify how different parts of adipose tissue influence fatty acid metabolism; (c) compositional analysis of meal samples collected among Arctic and sub-Arctic indigenous people should be conducted. These additional studies could provide valuable information upon which to further examine the fatty acid-related associations among Inuit and Cree reported in the present thesis. Moreover, meal samples collected at different periods of time and from different regions, could provide an indication of how the diets of arctic and sub-arctic indigenous people evolve over time and across regions.

In conclusion, the present thesis work suggests that adiposity could play a critical role in regulating the activity of desaturases and thus influence fatty acid

metabolism. The up-regulation of adiposity on Δ9 that catalyzes the synthesis of MUFA, which was previously supported by robust evidence from *in vitro* experiments and animal studies, received further support from the thesis data using a healthy female adolescent population. The thesis work thus has supported the concept that besides the previously well-described mechanism of leptin regulation of energy homeostasis via central nervous system, there is another leptin regulated pathway of energy homeostasis for which Δ9 is a critical mediator. The current thesis work has also suggested that adiposity could exert an independent inhibition on Δ5, which is key to HUFA biosynthesis. The Canadian Indigenous People thus could experience a compounded health risk in terms of HUFA n-3 status related to the emergence of obesity together with reduced intake of HUFA n-3 due to decreased traditional food consumption. The present work only observed a weak association between iron status and HUFA n-3 status; however, final conclusions in that regard are still premature due to the study population characteristics and the study design limitations. The current thesis also demonstrated pronounced inter-generational differences regarding HUFA n-3 status among Cree of James Bay, and among Canadian Arctic Inuit. Moreover, the present work displayed considerable inter-regional difference across Inuit Health Survey area, which could indicate that HUFA n-3 status might be related to a variety of factors, physiological, environmental, social-economical and cultural factors. Nutritional intervention strategies with multiple approaches are thus necessary to improve and maintain HUFA n-3 status among Canadian indigenous peoples.

Figure 7.1. Metabolic linkages of adiposity with disruptions in fatty acids pathway via disturbed Δ9 and Δ5 and abnormal metabolic consequences



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APPENDIX

Appendix 1. Study setting and methodology for manuscript 1

In the present doctoral thesis, the data came from a follow-up evaluation of the mother-daughter pairs affected or unaffected by GDM or T2D that resulted in the singleton term deliveries at the Royal Victorial Hospital in 1989-1991. Eligible mother-daughter pairs were identified from Royal Victoria patient record and the McGill Obstetrics and Neonatal Database (MOND). Controls were selected by frequency matching, which was based on postal code at the time of delivery. There were 4 income and 4 maternal age groupings and, thus, in total, 16 distinct sampling units. Bilingual letters and telephone calls were used to recruit

subjects. A trained bilingual program coordinator was responsible for the contact with the family. Post-term (> 42 weeks) and pre-term (< 37 weeks) pregnancies were excluded, so were pregnancies with pre-existing or pregnancy-related medical complications to ensure no confounding influence of other diseases.

Sample size was calculated based on the following considerations: If even only 60% of identified GDM cases were assumed to be willing to participate in this program, then a recruitment of 75 mother-daughter pairs from one birth year would be reached. Because only subtle differences were expected in clinical indicators of T2D, the majority of data would be examined as continuous variables. According to the data reported in previous literature, the standard deviation of fatty acids in erythrocyte membrane was set at 1.7. When the ratio of mother-daughter pairs in the two groups was 1:1, the proposed sample size gave us more than 90% statistical power at an alpha of 0.05 to test the difference (±) of 1% for fatty acid composition (SD=1.7) between the two groups. Among eligible case mothers being contacted by the study team, the overall participation rate was 51.6%, and for control, it was 24.4%. Upon the termination of enrollment, 88 pairs in case group and 99 pairs in control group were recruited. Information including anthropometric measures and dietary intake was collected from all the subjects. Blood samples were taken from 82 mother-daughter pairs in the case group and 96 mother-daughter pairs in the control group, which met the original goal of sample size.

Subjects who agreed to participate in the study were invited to the Royal Victoria Hospital in the morning after an overnight fast. A 75-g fasting oral glucose tolerance test (OGTT) was performed by a bilingual nurse, and blood samples at 0, 30 min and 2 hours were collected for the measurement of serum glucose and insulin level. Fasting serum was used for the routine biochemical tests, such as the concentration of cholesterol, TG, HDL-C and LDL-C, Apo B and HbA1c, and Thyroid Stimulating Hormone (TSH). Fasting plasma was frozen at -80°C for other biochemical analysis including plasma fatty acids.

Besides, information of the following items was collected: the measurement of anthropometrics; dietary investigation via qualified semi-quantitative food frequency questionnaires on the dietary intake over the last one year; behavior and life style investigation via modifiable questionnaires containing demographic characteristics, physical activity, medication history, reproductive and family history. Physical activities within the past two weeks and past year were assessed.

Whole blood samples were collected in evacuated tubes containing EDTA. Then plasma are separated and stored at -80°C for further analysis. The analysis of fatty acids profile of the fasting plasma was performed in CINE, Macdonald campus, McGill. A one-step extraction and transesterification method was applied instead of a traditional fatty acid analysis procedure which requires multiple steps. Briefly speaking, 50 μL plasma samples were added into 10 mL screw cap tubes containing 1.9 mL run solution which consisted of 1.7 mL BHT-methanol, 100μL acetyl chloride and 100 μL internal standard. After waterbath heating at 100°C for 1 h, 1.0 mL hexane was added to each tube. The tubes were then vortexed for 30 sec and the upper organic phase was collected. The extract was evaporated under nitrogen, re-dissolved in hexane, and transferred to GC vial inserts for the test on a 100m × 0.25mm I.D. × 0.25μm Varian CP-select CB for FAME fused-silica capillary column in a Varian 3400 CX gas chromatograph equipped with a flame ionization (Varian Inc., Pala Alto, CA). The temperature program was as follows: initial, 80ºC with a 1 min hold; ramp: 30ºC/min to 180ºC, 1ºC/min to 196ºC, 20ºC to 230ºC with a 15 min hold and 30ºC/min to 270ºC with a 8 min hold. The detector temperature was set at 275ºC and injector temperature at 250ºC . The carrier gas was Helium set at a flow rate of 2 mL/min. The signal:noise ratio was set at 3 allowing a method detection limit of 8.6 x 10-4 mg/mL. The fatty acids were identified by comparing each peak's retention time with those of methyl ester standards (GLC-6923 containing 31 FAMEs plus added lauric acid and C18:3 FAMEs, Nu-Check Prep Inc.). SeronormTM lipid (SERO AS, Billingstad, Norway) containing 23 fatty acids of animal origin was used as an additional

external control. Data was collected using the Chromatographic Star Ver 3 (Instrument) and Saturn W/S Ver 5.4.1 (Integration) software (Varian Inc., Pala Alto, CA). The relative amount of each fatty acid was quantified by integrating the area under the peak and dividing the results by the total area for all fatty acids. Due to the fact that the C18:1 trans isomers appeared as a series of overlapping peaks which the Saturn program was unable to separate to quantify, thus, the C18:1 trans isomers (t6, t9 and t11) were identified and integrated as one broad peak (C18:1t total).

Appendix 2. Study setting and methodology for manuscript 2

Diabetes screening in the summer 2006 in Mistissini Cree community in Cree Territory of Northern Québec, Canada as part of a pilot study of a long-term multi-institutional, multi-community longitudinal project "Nituuchischaayihitaau Aschii: A Multi-Community Environment-and-Health Longitudinal Study in Iiyiyiu Aschii".

Sampling of the population followed a stratified sampling design considering the following age categories: children between 0 and 7 years old, children between 8 and 14 years old, adults between 15 and 39 years old and

adults over 40 years old. Within each age stratum, participants were selected by simple random sampling without replacement to draft the lists of potential enrollment to be contacted by recruiters. A first list of 300 participants was selected for recruitment, all being contacted. Based on refusals, a second backup list of participants was randomly selected and contacted in order to meet the enrollment goal in each age category.

The recruitment was facilitated by a municipal list, while pregnant women were excluded. Community radio and meetings were used to raise the public awareness of this project. The recruitment and interview were conducted with the assistance from bilingual Iiyiyiuch interviewers. For adults (age≥ 20 year old) being selected and able to be reached, 62% of them participated in the study (n=172).

Though children were included in this pilot project, the current thesis work was restricted to adults. Blood samples were taken from 98 adult women and 68 adult men aged 20 to 88 years old, for the measurement of erythrocyte membrane fatty acids, the serum glucose and insulin levels at 0 and 2h in OGTT, and the routine biochemical tests in fasting serum.

Weight and body fat percentage was measured using a bioelectrical impedance scale (Tanita). Height was measured without shoes to the nearest centimeter using a stadiometer with the patient standing on a hard surface. Body mass index (BMI) was calculated (kg/m^2). Waist circumference was measured at the end of exhalation with the tape placed horizontally between the last floating rib and the iliac crest.

Blood concentrations of fatty acids were determined in red blood cell (RBC) membranes by gas-liquid chromatography in Unité de Recherche en Santé Publique, Centre de Recherche du CHUQ, Universite Laval. RBCs (300 μL) were thawed and lysed in 1 mL water. Membranes were isolated by centrifugation (21,000 g for 15 minutes) and washed twice with a 0.9% NaCl solution. The pellet was spiked with an internal standard of phosphatidylcholine pentadecanoic acid

(Avanti Polar Lipids, Alabaster AL) and lipids were liquid-liquid extracted using chloroform and methanol (2:1 v/v) according to a modified Folch method (Shaikh and Downar, 1981). Fatty acids from membrane phospholipids were methylated in methanol/benzene (4:1 v/v) mixed with acetyl chloride according to previously described methods (Lepage and Roy, 1986). Fatty acid profile were obtained by capillary gas chromatography using a temperature gradient on a HP5890 gas chromatograph (Hewlett Packard, Toronto, Canada) equipped with a HP8823 capillary column coupled with a flame ionization detector (FID). Helium was used as an elution gas (split ratio 1:72). Fatty acids were identified according to their retention time on the column, using a standard mixture of 37 fatty acids as a basis for comparison (FAME 37 mix, Supelco Inc., Bellefonte PA), which contained the fatty acid standard pentadecanoic acid, as well as a mixture of 31 fatty acids GLC-411 (NuCheck Prep Inc. Elysian, MN) and a mixture PUFA-3 (Matreya Inc, Ontario, Canada). Results were expressed as percent total fatty acids.

Appendix 3. Study setting and methodology for manuscript 3 and 4

International Polar Year Inuit Health Survey was a cross-sectional 2-year survey program. The first year survey was conducted in Nunavut during a 12 week period in the later summer and early fall (August to September) in 2007. The second year survey was implemented in Nunatsiavut and Inuvialuit during the same period of time in 2008. Two villages in Nunavut were also included in the 2008 survey because they locate far away from the 2007 survey route. Totally there were 35 communities were covered by the survey, 26 communities in Nunavut, 5 communities in Nunatsiavut and 4 communities in Inuvialuit. Two

separate research crews, one based on land and the other on ship, coordinated with each other.

The land-based crew visited the hamlets ahead of arrival of the Canadian Coast Guard ship (CCGS) Amundsen, selected private Inuit households by stratified random sampling, and then recruited participants. The survey design was a stratified random sampling of private Inuit households. The survey regions were stratified by municipality and lists of municipal households where Inuit reside were used to facilitate the random sampling. Once a household was selected, all adults ≥ 18 years old were invited to participate. The estimated sample size was about 2000 adult Inuit permanent residents in survey regions in order give the power to estimate the prevalence of selected health indicators within an acceptable margin or error and enables analytic power for evaluating determinants of health. To reach the target sampling size, recruiters attempted to recruit 12% adult Inuit ≥ 18 years old or 40 participants if less than 40 eligible residents in the communities. Eventually, 2595 adult Inuit were recruited, accounting for about 11% of Inuit population in the survey area. Among them, 2200 people's fatty acid data were available.

Adult Inuit who agreed to participate the survey were invited to the Ship to attend health examination, and were interviewed by the ship based crew for information for demographic information and health behavior such as dietary intake, physical activity, medical histories, etc. Height was measured without shoes to the nearest millimeter using a stadiometer with the patient standing. Waist circumference was measured to the nearest millimeter at the end of exhalation with the tape placed horizontally on the midpoint between the top of the hip and the last loose rib. Weight and body fat percentage was measured using a bioelectrical impedance scale (Tanita). Body mass index (BMI) was calculated (kg/m^2).

On board, blood samples were also taken from the participants for blood biochemical measures including erythrocyte membrane fatty acids. Briefly speaking, fasting blood was collected in vacutainers with EDTA-coating. The

vacutainers were centrifuged with their capes on at 2400 rpm for 20 minutes at 4ºC. Plasma was gently transferred and the layer of white blood cells was carefully remove from the top of the remaining RBC pellet. Ice cold saline was added to the tube until it was near the top of the tube, and the tube was covered with parafilm and mixed well by inversion, followed by centrifuge for 15 minutes at approx. 2400 rpm. Then supernatant was discarded. The rinse procedure was repeated again. Then 200 ul ice cold water/methanol/BHT solution was added to the RBC. About 200 ul RBC was transferred to a 1.5 ml cryovial. The cryovial was flushed with nitrogen and capped tightly with a BROWN cap. One ml of remaining RBCs was transferred to each of two 1.5 ml cryovials. Blood samples were immediately frozen at -80ºC and shipped to Center for Indigenous Nutrition and Environment (CINE) and stored at -80ºC before being shipped at -80ºC to Lipid Analytical Laboratories Inc, Guelph, Canada for fatty acid analysis. The fatty acids methyl esters were prepared by the method of Morrison and Smith and were analyzed on a Varian 2400 gas-liquid chromatograph (Palo Alto, CA) with a 60-metre DB-23 capillary column (0.32 mm internal diameter).