Relationships of Vitamin D Status and Markers of Skeletal Health to Metabolic Syndrome and Markers of Glucose Homeostasis among Inuit Adults: The International Polar Year Inuit Health Survey 2007-2008

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ABSTRACT

In Canada, Inuit are facing a rapid nutrition transition away from consumption of traditional food (TF) which coincides with a higher prevalence of obesity, type 2 diabetes mellitus (DM2) and lower vitamin D status. Vitamin D status is inversely associated with the risks of developing prediabetes, metabolic syndrome (METS) and DM2. Cross talk between energy metabolism and bone through osteocalcin is implicated in the pathophysiology and etiology of DM2. Epidemiological studies show that osteocalcin associates with reduced insulin resistance and enhanced pancreatic secretion of insulin. This thesis consists of 4 studies using data from Inuit adults (≥18 y) who participated in the cross-sectional 2007-2008 International Polar Year Inuit Health Survey conducted in three arctic regions, Nunavut, Nunatsiavut and Inuvialuit Settlement Region. Measurements included fasting plasma glucose (FPG), serum 25-hydroxyvitamin D (25(OH)D), insulin, serum lipids, blood pressure, leptin, adiponectin, osteocalcin, red blood cell fatty acids (RBC FA) and forearm bone mineral density (FaBMD). Dietary data were obtained from a 24-h dietary recall and a food frequency questionnaire (FFQ).

Study 1: The primary aim (n=1418) of this study was to examine whether vitamin D status among Inuit adults is associated with fasting plasma glucose (FPG) and homeostatic model assessment of insulin resistance (HOMA-IR) after adjustment for covariates. A secondary aim (n=1250) was to explore if adipokines further explained FPG and HOMA-IR. Serum 25(OH)D (per 30 nmol/L) was associated with a lower HOMA-IR (β= -0.058; 95% CI: -0.111 to -0.005, $P< 0.05$), but not FPG (β= -0.031; 95% CI: -0.14 to 0.080, $P> 0.05$) after adjustment for age, sex, arctic region, body mass index (BMI) and variables representing TF intakes and RBC FA. There was a positive association between marine mammal intake and FPG and HOMA-IR. Arctic region modulated both FPG and HOMA-IR. Adiponectin was

inversely related to HOMA-IR, leptin was positively related to both FPG and HOMA-IR, and RBC-Omega 3 was inversely related to FPG.

Study 2: The objective was to assess whether increases in 25(OH)D and TF intake were associated with a reduction in METS (n=1725) and its components or DM2 (n=1857) after adjustment for covariates. It was found that greater intake of total TF was not associated with lower odds of METS (OR: 0.88; 95% CI: 0.74 to 1.05, *P*= 0.158), and the association between serum 25(OH)D and METS was inconclusive (OR: 0.86; 95% CI: 0.71 to 1.03, *P*= 0.107). Higher serum 25(OH)D lowered the odds of serum high triglyceride (TG) and low serum high density lipoproteins (HDL), whereas higher intakes of fish lowered the odds of a high ratio of serum low density lipoprotein (LDL) to HDL. Being from Nunatsiavut relative to Nunavut and greater marine mammal intake were associated with higher odds for METS. Neither serum 25(OH)D or TF were associated with DM2, but arctic region, obesity and RBC-Sat modulated the odds of DM2.

Study 3: The objective was to assess the association between serum osteocalcin and HOMA-IR and FPG (n=256). Although serum osteocalcin was inversely correlated with HOMA-IR (r = -0.17, *P*= 0.005) and FPG (r = -0.14, *P*= 0.023), osteocalcin was not associated with HOMA-IR (β= 0.001, 95% CI: -0.023 to 0.025, *P*= 0.913) or FPG (β= 0.008; 95% CI: -0.146 to 0.162, *P*= 0.917) in the fully adjusted models.

Study 4: The objective was to examine bone health in Inuit women with impaired fasting plasma glucose (IFG) and DM2. FaBMD was not different between cases of DM2 versus control and cases of IFG versus controls, but being free of osteoporosis was more common in cases of DM2 versus controls (OR: 12.8; 95% CI: 1.5 to 109.9, *P*= 0.016). Fish and marine mammal intakes were positively associated with FaBMD.

Conclusions: In summary, in Inuit adults serum 25(OH)D, RBC-Omega3 and fish are protective against insulin resistance and metabolic abnormalities. Serum osteocalcin was not

related to glucose regulation in this study, whereas DM2 could be associated with higher FaBMD. It appears that metabolic abnormalities in Inuit adults are related to geographical distribution, with people in Nunatsiavut and Inuvialuit Settlement Regions having higher rates of metabolic abnormalities relative to Nunavut. Furthermore, differences were noted regarding how dietary factors modulated diseases, for example serum 25(OH)D was inversely associated with insulin resistance and dyslipidemia, but not IFG and DM2, suggesting that serum 25(OH)D is protective before DM2 is evident. These results indicate that fish is metabolically a more neutral source of nutrients including vitamin D and that traditional lifestyles remain protective against chronic diseases.

RÉSUMÉ

Au Canada, les Inuits sont confrontés à une transition nutritionnelle rapide loin de la consommation d'aliments traditionnels (TF) qui coïncide avec une prévalence plus élevée de l'obésité, du diabète de type 2 (DM2) et un statut inférieur de vitamine D. Le statut en vitamine D est inversement associé à des risques de développement du prédiabète, du syndrome métabolique (METS) ainsi que le DM2. La diaphonie entre le métabolisme énergétique et de l'os par l'ostéocalcine est impliquée dans la physiopathologie et l'étiologie de DM2. Les études épidémiologiques démontrent que l'ostéocalcine est associée à une reduction de la résistance à l'insuline ainsi qu'une sécrétion accrue d'insuline du pancréas.

Cette thèse est composée de 4 études utilisant des données des adultes inuits (≥18 ans) qui ont participé à l'enquête transversale de 2007-2008: International Polar Year Inuit Health Survey; menée dans trois régions arctiques: Nunavut, Nunatsiavut et région désignée des Inuvialuit. Les mesures comprenaient la glycémie à jeun (FPG), le sérum de 25-hydroxyvitamine D (25(OH)D), l'insuline, les lipides sériques, la pression artérielle, la leptine, l'adiponectine, l'ostéocalcine, les acides gras des globules rouges (RBC FA) et la densité minérale des os de l'avant-bras (FaBMD). Les données alimentaires ont été obtenues à partir d'un rappel alimentaire de 24 h et un questionnaire de fréquence alimentaire (FFQ).

Étude 1: Le premier objectif (n= 1418) de cette étude était d'examiner si le statut en vitamine D chez les adultes inuits est associée à la glycémie à jeun (FPG) et l'évaluation du modèle homéostatique de résistance à l'insuline (HOMA-IR) après ajustement pour les covariables. Un objectif secondaire (n= 1250) était d'explorer si L'Adipokine outre expliqué FPG et HOMA -IR. Sérum 25(OH)D (par 30 nmol/L) a été associée à une plus faible HOMA-IR (β = -0.058; IC à 95%: -0.111 to -0.005, $P< 0.05$), mais pas FPG (β =-0.032; IC à 95% :-0.14 à 0.080, $P> 0.05$) après ajustement pour l'âge, le sexe, la région arctique, l'indice de masse corporelle (IMC) et les variables représentant les apports de TF et RBC FA. Il y

avait une association positive entre la consommation de mammifères marins et la FPG. La région arctique avait modulé à la fois FPG et HOMA-IR. L'Adiponectine était inversement proportionnelle à HOMA-IR, La leptine a été positivement liée à la fois au FPG et HOMA-IR, et RBC-Omega 3 a été inversement proportionnelle à la FPG.

Étude 2: L'objectif était d'évaluer si l'augmentation des 25(OH)D et de l'apport du TF ont été associés à une réduction de METS (n= 1725) et de ses composants ou bien du DM2 n= 1857) après ajustement pour les covariables. Il a été constaté qu'une plus grande consommation de TF a non associé à un risque plus faible de METS (OR: 0.88; IC à 95% : 0.74 à 1.05, *P* = 0.158), alors que l'association entre le taux sérique de 25(OH)D et METS n'a pas été concluante (OR: 0.86; IC à 95%: 0.71 à 1.03, *P*= 0.107). Un taut élevé du sérum 25(OH)D réduit les chances de taux élevé de triglycérides sériques (TG) et- à faible sérum-des lipoprotéines de haute densité (HDL), alors que des apports plus élevés de poisson abaissent les chances d'un ratio élevé de lipoprotéines de basse densité du sérum (LDL/HDL). Étant originaire de Nunatsiavut par rapport au Nunavut et une plus grande consommation de mammifères marins ont été associés avec des cotes plus élevées pour METS. Ni le sérum 25(OH)D ou TF ont été associés à DM2, mais la région arctique, l'obésité et le RBC-Sat ont modulé les chances de DM2.

Étude 3: L'objectif était d'évaluer l'association entre l'ostéocalcine sérique et HOMA-IR et FPG n= 256). Bien que l'ostéocalcine sérique était inversement corrélée avec HOMA-IR (r= -0.17, *P*= 0.005) et FPG (r= -0.14, *P*= 0.023), l'ostéocalcine n'a pas été associée à HOMA-IR (β = 0.001, IC à 95%: -0.023 à 0.025, *P*= 0.921) ou FPG (β = 0. 008; IC à 95%: -0.146 à 0.162, *P*= 0.917) dans les modèles entièrement corrigés.

Étude 4: L'objectif était d'examiner la santé des os chez les femmes inuites avec altération de la glycémie à jeun (IFG) et DM2. Le FaBMD ne différait pas entre les cas de DM2 et IFG et les contrôles, mais l'absence de l'ostéoporose est plus fréquente dans les cas

de DM2 par rapport aux témoins (OR: 12.8; IC 95%: 1.5 à 109.9, *P*= 0.016). Les apports en poissons et mammifères marins ont été positivement associés à FaBMD.

Conclusions: En résumé, chez les Inuits adultes sériques de 25(OH)D, RBC-Omega3 et total TF sont des élements protecteurs contre la résistance à l'insuline et des anomalies métaboliques. L'ostéocalcine sérique n'a pas été liée la régulation du glucose dans cette étude, alors que le DM2 peut être associé à un FaBMD supérieur. Il semble que des anomalies métaboliques chez les adultes inuits sont liés à la répartition géographique, avec des personnes dans les régions du Nunatsiavut et Inuvialuit ayant des taux plus élevés d'anomalies métaboliques par rapport au personnes du Nunavut. En outre, les différences ont été notées sur la façon dont les facteurs alimentaires modulent les maladies, par exemple le sérum 25(OH)D était inversement associée à la résistance à l'insuline et la dyslipidémie, mais pas le IFG et le DM2, ce qui suggère que le sérum 25(OH)D est un protecteur avant que le DM2 devient evident. Ces résultats indiquent que le poisson est une source de nutriments métaboliquement plus neutre, y compris la vitamine D et que le modes de vie traditionnels sont une protection contre les maladies chroniques.

ADVANCE OF SCHOLARLY KNOWLEDGE

1. Original Contribution to Knowledge

This dissertation is the first in Canada to address the question of the relationship between vitamin D status and outcomes of glucose homeostasis, presence of prediabetes, metabolic syndrome and type 2 diabetes mellitus (DM2) among the adult Inuit population. Also, it is the first study in Inuit in Canada to address the question of the relationship between osteocalcin, leptin, and adiponectin with outcomes of glucose homeostasis. Vitamin D status in relationship to glucose homeostasis indicators was assessed while adjusting markers of nutrition transition, dietary fatty acids and geographical region. It was clear that an inverse association between vitamin D status and insulin resistance as assessed by homeostatic model assessment of insulin resistance existed despite robust adjustment for all markers of obesity including body mass index, waist circumference and fat mass percent. This robust adjustment for obesity indicators was rarely performed in previous studies in the scientific literature. Also, the relationship between serum 25(OH)D and insulin resistance existed even after adjustment for dietary factors including fatty acids in red blood cells and food frequency data about traditional foods. Arctic region is used as covariate and the finding that metabolic outcomes were different with Nunavut showing better health parameters is novel to this thesis. The present thesis for the first time analyzed the association between metabolic syndrome and vitamin D status and other dietary markers such as fatty acids and traditional foods among Inuit. It was shown that metabolic syndrome differs by region. In addition, this thesis presented comprehensive analysis for the factors that could contribute to DM2 in the Canadian arctic and it clearly shows that obesity continues to be the most important factor in predicting DM2. Among Inuit, the association between osteocalcin and insulin resistance

was tested. In addition, the impact of type 2 diabetes mellitus and prediabetes on bone health and fracture in Inuit was addressed.

The following are the papers stemming from this work:

1. Determinants of Insulin Resistance Among Inuit: Role of Diet, Adipokines and 25-Hydroxyvitamin D. Nihal Natour, Kristine Koski, Grace Egeland, Hope A. Weiler. (Not submitted)
2. Determinants of Metabolic Syndrome and Type 2 Diabetes Mellitus among Inuit: Role of Diet, Obesity and 25-Hydroxyvitamin D. Nihal Natour, Kristine Koski, Grace Egeland, Hope A. Weiler. (Not Submitted)
3. Osteocalcin is not Related to Insulin Resistance among Inuit Women: The 2007-2008 International Polar Year Inuit Health Survey. Nihal Natour, Hope A. Weiler. (Not Submitted).
4. The Association of Type two Diabetes Mellitus and Impaired Fasting Glucose with Bone Mineral Density and Fracture among Inuit Women: The 2007-2008 International Polar Year Inuit Health Survey. Nihal Natour, Suzanne Morin, Hope A. Weiler. (Not Submitted).

CONTRIBUTION OF AUTHORS

This thesis was written in manuscript format. The candidate designed, reviewed, wrote and edited all the details of article review. For Manuscript 1-2: the candidate designed the research questions and concepts, analyzed the data, interpreted the results, wrote and edited the content. Drs. Hope Weiler, Dr. Kristine Koski and Dr. Grace Egeland guided the design of concepts of manuscript 1 and 2, provided critical evaluation of data analysis and interpretation. For Manuscript 3, the candidate codesigned the research questions and concepts, performed data analysis, interpreted the results and edited the content of manuscript. Dr. Hope Weiler provided guidance in the design of the analysis along with critical review of the data analysis and edited the content of each chapter. For Manuscript 4, both the candidate and Dr. Hope Weiler designed the research questions and concepts of the manuscript. The candidate performed data analysis, wrote and edited the manuscript. Drs. Hope Weiler and Suzanne Morin provided critical review of the data analysis and concepts of manuscript 4. The candidate did not participate in data collection or measurement of study outcomes.

Data for Inuit Health Survey (IPY-IHS) was collected, entered and cleaned by teams of the students and researchers who made this work possible. Dr. Hope Weiler, the candidate's supervisor and co-applicant on IPY-IHS provided essential laboratory materials and reagents for the assessment of vitamin D status of participants within her laboratory. On the other hand, Dr. Grace Egeland as Principal Investigator on the IPYIHS, designed the survey in collaboration with the community partners.

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LIST OF ABBREVIATIONS

AHA	American Heart Association
AMPK	Adenosine monophosphate protein kinase
AUC	Area under the curve
BMI	Body mass index
C	Control group
CCHS	Canadian Community Health Survey
CHMS	Canadian Health Measures Survey
DBP	Vitamin D binding protein
7DHC	7 Dehydrocholesterol
DM2	Type 2 diabetes mellitus
DXA	Dual-energy X-ray absorptiometry
FA	Fatty acid
FN	First Nations
FGF23	Fibroblast growth factor 23
FFA	Free fatty acid
F	Female
FFQ	Food frequency questionnaire
FPG	Fasting plasma glucose
25(OH)D	25-hydroxyvitamin D
HDL-C	High density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment of insulin resistance
HOMA-B%	Homeostatic model assessment of beta cell function
I	Intervention group

IDF	International Diabetes Federation
IGF	Insulin growth factor
IGT	Impaired glucose tolerance
IFG	Impaired fasting glucose
IFN-λ	Interferon gamma
IPY-IHS	International Polar Year Inuit Health Survey
IOM	Institute of Medicine
ISI	Insulin sensitivity index
ISR	Inuvialuit Settlement Region
IVGT	Intravenous glucose tolerance test
LDL	Low density lipoprotein
METS	Metabolic syndrome
MF	Market food
M	Male
NCEP-ATP III	National Cholesterol Education Program Adult Treatment Panel 111
NHLBI	National Heart Lung and Blood Institute
NHANES	National Health and Nutrition Examination Survey
PTH	Parathyroid hormone
OGTT	Oral glucose tolerance test
OGT	Oral glucose tolerance
PCOS	Polycystic ovarian syndrome
RBC	Red blood cells
RBC-Omega3	Red blood cell content of omega 3 fatty acids
RBC-Sat	Red blood cell content of saturated fatty acids
RBC-Trans	Red blood cell content of trans fatty acids
RDA	Recommended Dietary Allowance

RCT	Randomized controlled trial
SBP	Systolic blood pressure
SRH	Self-reported health
TG	Triglyceride
TF	Traditional food
TNF-α	Tumor necrosis factor alpha
Tx	Treatment group
UL	Tolerable upper intake level
UVB	Ultraviolet beta radiation
VDR	Vitamin D receptor
WC	Waist circumference
WHO	World Health Organization
WHR	Waist to hip ratio

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Inuit and other Aboriginal communities have been challenged by a transition that affects all indicators of health as a result of interaction with Europeans [1,2]. Inuit live across the northern circumpolar region spanning from Alaska to Western Russia passing through Greenland and Northern Canada. Specifically, almost 167,000 Inuit live in Greenland, Denmark, Alaska, other parts of USA, Russia and many parts of Canada [3]. The number of Inuit living in Canada is just over 59,445 and they mostly live in the Nunavut Territory and in the northern parts of the province of Québec [3]. Factors such as change in dietary habits, smoking, pollution, climate change, household crowding, lower income and education are all determinants of compromised health outcomes among Inuit [3].

Health transition among Inuit is a result of interactions among genetic and environmental factors [4]. Particularly, the shift away from a traditional way of living has contributed to the prevalence of chronic diseases including type 2 diabetes mellitus (DM2) and cardiovascular diseases [3]. In the past, high intake of marine mammals and fish, in addition to physical activity associated with hunting protected Inuit people from such chronic diseases [5,6,7]. More recently, the use of a traditional food (TF) system has declined [8], a problem that when combined with food insecurity among Inuit could lead to micronutrient deficiencies and reduced diet quality [9]. For example having lower vitamin D status is a nutrition and health problem that Inuit in Canada were shown to face as a result of consuming less TF that is rich in vitamin D [10]. This is important since insulin resistance and DM2 are now hypothesized to be ascribed in part to low vitamin D status [11]. In addition, new endocrine organ systems have been identified including bone since some bone-derived proteins show endocrine activity. Bone as an endocrine organ relates to pancreatic function, owing to a bone derived protein called osteocalcin [12]. Osteocalcin acts by enhancing insulin

expression and increasing the number of beta-cell islets in cell culture obtained from P7 pups (7 days of age) [13]. Furthermore, osteocalcin improves insulin sensitivity in animal models and also in human studies through various mechanisms that are detailed later in this thesis [14,15]. The complex associations between lower vitamin D status and insulin resistance among Inuit, in the context of obesity and TF intakes will be the focus of this dissertation.

Obesity and DM2 co-associate with lower vitamin D status and thus it is theorized that lower vitamin D status may contribute to deteriorated metabolic conditions [16,17] and increased risk of cardiovascular diseases [18] among Aboriginal people. The prevalence of DM2 among Inuit was comparable to the general Canadian population in 2008/2009, whereas the prevalence of DM2 among some Aboriginal people (First Nations: FN) is three to five times higher than the rest of the Canadian population according to data from Canadian Chronic Disease Surveillance System [19]. Earlier reports from the 1960s indicated the rarity of DM2 among Inuit in Greenland, Canada and Alaska as in some reports no cases of DM2 were found in population level assessments [20]. In a survey among 32,249 Inuit of Greenland, only 10 cases of DM2 were documented in 1962 [20]. On the other hand, among 705 adult Eskimos (American Inuit) in western Alaska, only one person with DM2 was reported in 1967 [21]. In stark contrast to these early studies of Inuit in Greenland and Alaska, the most recent report on the prevalence of DM2 in Northern Labrador, Inuvialuit Settlement Region (ISR), Nunavut and Nunatsiavut indicated a rate as high as 12.2% (95% CI: 8.7% to 15.7%) in Inuit adults older than 50 y [22]. Another recent report from Greenland indicated that the age adjusted prevalence of DM2 was 10.8% among Inuit men aged ≥ 35 y [23]. If not adequately managed, DM2 could lead to further complications. It is worthy to emphasize that higher rates of DM2 complications such as renal disease were reported in communities of Aboriginal ethnicity in Canada and Alaska and other parts of the world according to a study performed on Indigenous Peoples worldwide [24]. Thus, further work regarding the correlates

of prediabetes and established DM2 is required to guide future interventions towards improved health in Inuit populations.

DM2 is well known to be a disease of multiple etiologies, factors such as obesity, sedentary lifestyles, malnutrition, alcohol, aging and genetics all contribute to the development of prediabetes and eventually DM2 [25]. Among the risk factors for DM2, fat mass represents a strong modulator of insulin resistance [26]. In a study that used data from 1990 to 2001 with pooled results from Inuit adults from Russia, Canada and Greenland the prevalence of obesity among Inuit men was 15.8%, whereas the prevalence of obesity among Inuit women was 25.5%. In the same study 36.6% of Inuit men and 32.5% of Inuit women were overweight [27]. In the International Polar Year Inuit Health Survey (IPY-IHS) of 2007-2008, the prevalence of obesity among Inuit adults (body mass index (BMI) more than 30 kg/m^2) was 35.1% (95% CI: 32.5% to 37.7%), whereas 28.3% (95% CI: 25.9% to 30.8%) of the Inuit surveyed were overweight (BMI between 25-30 kg/m^2) [22]. Relatively, in 2007-2009 the prevalence of obesity among adults in Canada was 24.1% [28]. In another report in 2012, 59.9% Canadian men and 40% of Canadian women were classified as either overweight or obese, among them 18.4% were classified as obese [29]. According to the Canadian Health Measures Survey (CHMS) 2012/2013, 62% of Canadian adults aged 18-79 y were considered overweight or obese [30]. These studies suggest that the rates of obesity and DM2 among Inuit relative to rest of Canada are similar and that Inuit have similar concerns in regard to obesity and its metabolic complications as the rest of the Canadian population.

Given the high rates of obesity and DM2 in Inuit, an in depth understanding of the possible causes or modifiable factors in the etiology is important. The transition away from traditional ways of living [31] could contribute to the higher rates of these diseases. TF which marks a traditional lifestyle, is a rich source of nutrients including many micronutrients that could help prevent DM2 [32]. In general, TF consists of wild game, waterfowl, fish, marine

mammals and various plants such as berries [33]. Having low vitamin D status is of concern as was recently illustrated in a study by El Hayek and colleagues and could be a consequence of decreased consumption of TF [10].

Low vitamin D status demarked by a serum 25-hydroxyvitamin D (25(OH)D) concentration below 50 nmol/L is highly prevalent among Aboriginal people. Almost, 42.2% (95% CI: 39.5% to 44.9%) of Inuit adults were observed to have 25(OH)D concentrations below 50 nmol/L at the end of summer or early fall in 2186 Inuit 18 years of age and above in the IPY-IHS [10]. If a higher cut-off for vitamin D status is used in accordance with the Endocrine Society [34], 67.4% (95% CI: 64.7% to 70.0%) of the surveyed adults had 25(OH)D below 75 nmol/L. In another study among Inuit in Nunavut, 47% of the men and 69% of the women had vitamin D intake below 600 IU/d [35]. Such low vitamin D status is not unique to Inuit alone. In a representative population study, urban and rural FN women had significantly lower serum 25(OH)D than urban white woman. In addition, 32% of rural Aboriginal, 30.4% of urban Aboriginal and 18.6% of urban white women were vitamin D deficient, with serum 25(OH)D concentrations <37.5 nmol/L [36]. As to whether low vitamin D status underpins some of the health disparities in Aboriginal Canadians is not clear.

Plasma glucose and insulin regulation is a newly identified extra skeletal function of vitamin D [37]. In particular, the active vitamin D metabolite calcitriol ($1,25(OH)_2D$) is involved in insulin secretion directly through gene transcription [38], or indirectly [39] through calcium homeostasis [39] as a function of parathyroid hormone (PTH) action [40]. Few studies have investigated the thresholds of serum 25(OH)D that are needed to prevent conditions such prediabetes, metabolic syndrome (METS) and DM2. Thus far, growing epidemiological evidence suggests that there is an association between lower vitamin D status and increased risk for prediabetes, METS and DM [37,41]. Suggested targets for the most effective vitamin D status in terms of skeletal health are either 50 nmol/L or 75 nmol/L [42,43] but for insulin

sensitivity the target was suggested to start at 75 nmol/L [17,34]. To date, clinical trials on the relationship between vitamin D and glucose homeostasis have not provided consistent results, probably due to differences in the design of those experiments in terms of populations, length of intervention and vitamin D supplementation regimens [37]. A recent meta-analysis of 35 clinical trials showed no effects of vitamin D_3 supplementation on markers of insulin resistance and secretion and progression to DM2 [44].

Obesity could be an effect modifier in the link between low plasma 25(OH)D and insulin resistance or risk of DM2 among Aboriginal people [45]. Possibly this relates to the observation that adipose tissue is the storage site for vitamin D [46]. Vitamin D is implicated in obesity [46], insulin resistance and DM2 [37], which could in turn collectively impact bone metabolism. There is cross talk between endocrine hormones derived from bone and organs of energy metabolism and adipose tissue [47]. Recently, osteocalcin, a protein derived from bone, was found to be relevant to glucose homeostasis, at least partly, through adipose tissue markers [47]. Osteocalcin stimulates insulin expression and sensitivity [48] and its own production is induced by $1,25(OH)_2D$ [49]. Studies addressing the interrelationships between adipose tissue markers and bone derived endocrine hormones in relation to insulin resistance and secretion among Aboriginal people are very few. To the best of the author's knowledge there is only one study that addresses the relationship between osteocalcin and energy and glucose homeostasis in FN women. In that study, osteocalcin was negatively and significantly related to homeostatic model assessment of insulin resistance (HOMA-IR; β= -0.23; 95% CI: -0.399 to -0.058, P= 0.009) [50]. In addition, adipose tissue is no longer considered an inert tissue since adipokines which are produced from adipose tissue play a role in the regulation of plasma glucose [51].

It is particularly important to address the interrelationships among bone, vitamin D and insulin resistance because Inuit people may have different calcium metabolism regardless

of vitamin D status [52]. However, low serum calcium has been reported among Inuit in Greenland (2.22 ± 0.01 mmol/L) relative to Danes in Denmark (2.29 ± 0.02 mmol/L) which was attributed to a diet lower in calcium [53]. Despite the fact that Greenlanders on traditional Inuit diets had lower plasma 25(OH)D, lower total calcium and higher plasma $1,25(OH)_2D$, they had lower PTH [54]. The data from the study by Rejnmark et al. indicate that Inuit had an inherently lower "set-point" for calcium-regulated PTH release or an enhanced renal $1,25(OH)_2D$ production [54]. However, the markedly low calcium diet combined with low vitamin D status could contribute to lower serum calcium and hence osteoporosis [53]. In the Greenlanders, Inuit men (P=0.004) and women (P=0.01) had lower heel bone mineral density (BMD) relative to Caucasians, whereas distal forearm BMD was not significantly different. In this study, BMI was the most significant predictor of both heel and forearm BMD [55].

Adults with insulin resistance have a higher odds for having osteoporosis or osteopenia [56]. However, adults with DM2 could have higher bone mass than adults without DM2 which could be attributed to long-term hyperinsulinemia [57,58]. Nevertheless, after accounting for obesity and other covariates some studies indicated that increasing insulin concentration was associated with lower total body mineral content (β= -105.2) in men, (β= -32.4) in premenopausal women and (β= -35.0) in postmenopausal women [59]. It is also possible that the feedback loops among these endocrine systems function until a critical point is reached where the direction of the relationship is reversed. For example insulin increases bone mass until insulin resistance and hyperglycemia develops (i.e. DM2), at this point the relationship between insulin and bone becomes inverse [59].

In summary, high rates of DM2 associate with high rates of obesity among Inuit adults. Both of the diseases are thought to be related to shifting away from a traditional way of living to a modern lifestyle. Conditions such as low vitamin D status could be attributed to lower reliance on TF and is possibly linked to the increased burden of DM2. The relationship

between insulin resistance and vitamin D is multifaceted and could be related directly to the active vitamin D metabolite signaling on insulin secretion and sensitivity, or through regulation of intracellular calcium fluxes. On the other hand, the involvement of vitamin D in adipokine metabolism could affect glucose homeostasis. Finally, vitamin D could be related to insulin secretion and resistance through its skeletal function.

1.2 STATEMENT OF PURPOSE

Inuit are currently facing a double burden of nutrition transition and food insecurity which together contribute to limited diet quality. The food choices Inuit face contribute to lower vitamin D status as well as obesity and DM2. There is a critical need to identify the role of vitamin D status in insulin resistance, presence of diseases such as METS and DM2. Also, whether vitamin D status plays a role in the relationships among osteocalcin, bone health and insulin resistance needs further investigation. All of the objectives tested in this thesis are specific to adults 18 y of age and over who participated in the cross-sectional IPY-IHS of 2007-2008.

1.3 SPECIFIC OBJECTIVES and HYPOTHESES

Study 1

Objectives: to examine whether vitamin D status as indicated by 25(OH)D among Inuit adults is associated with fasting plasma glucose (FPG) and HOMA-IR after adjustment for region of the arctic, fat mass, waist circumference (WC) or BMI as well as age, sex, marine mammal and fish intake and fatty acid status (including red blood cells content of (RBC) saturated fatty acids (FA) (RBC-Sat), RBC-Omega 3 FA and RBC- Trans FA). As a secondary goal, the study aimed to identify other factors that contribute to insulin resistance including leptin and adiponectin.

Hypothesis: Serum 25(OH)D will be inversely associated with both HOMA-IR and FPG.

Study 2

Objectives: to assess whether increases in vitamin D status, or intakes of TF, reduce the odds of METS and DM2 in Inuit adults after accounting for obesity as assessed by BMI and fat mass, FA status including RBC-Sat FA, RBC-Omega3 FA, RBC-Trans FA, intakes of fish and marine mammals and region of residence in the Arctic.

Hypothesis: Serum 25(OH)D and TF will be inversely associated with the odds of METS, metabolic risk factors and DM2 after adjustment for covariates**.**

Study 3

Objectives: to test the relationships between osteocalcin and insulin resistance, obesity and adipokines among Inuit women in models that adjust for dietary markers and RBC FA.

Hypothesis**:** Osteocalcin is inversely related to FPG and HOMA-IR.

Study 4

Objectives: *1*) to compare BMD and BMD T-scores between Inuit women with impaired fasting plasma glucose (IFG) and age and BMI matched control women or DM2 to age and BMI matched control women; and 2) to assess whether other factors that accompany IFG and DM2 are related to BMD.

Hypothesis**:** Either DM2 or IFG along with factors associated with them are positively related to BMD.

CHAPTER 2: LITERATURE REVIEW

2.1 Aboriginal People in Canada.

2.1.1 Background Information on Aboriginal People.

In Canada, Aboriginal people consist of 3 distinct populations: First Nations (FN) who are indigenous to North America; Métis of mixed FN and European ancestry; and the Inuit who reside predominantly in remote Arctic communities [60]. Overall, almost one million Aboriginal people live in Canada, of them there are 59,445 Inuit [3] . Almost, 78% of Inuit live in Inuit Nunaat. Inuit Nunaat means the homeland of Inuit and includes four Inuit regions (Nunatsiavut, Nunavik, Nunavut and The Inuvialuit Settlement Region) [61]. Inuit Nunaat comprises one third of the Canadian land mass and stretches from Labrador to the Northwest Territories [62]. On the other hand, FN represent the largest group of Aboriginal people in Canada as represented by 600 different bands. FN mainly live in urban centers or on reserves in almost all provinces [63].

The relationship between land and human lends uniqueness to the Aboriginal peoples' culture [60]. Besides providing family needs, hunting, fishing, harvesting and gathering food from the land is used for social and ceremonial gatherings, for trading and for clothing [64]. Both FN [65] and Inuit [66] communities were enforced to assimilate and relocate under the pressure of colonial laws which represented a traumatic experience of cultural and land dispossession. Accompanied with poverty and lower access to services [33], gaps in education, income, employment and housing standards still exist between Aboriginal and non-Aboriginal Canadians [67]. Consequently, the community wellbeing index for Inuit and FN lags behind other Canadian sectors [68]. Food insecurity, health transition, suboptimal housing conditions [69], climate change, limited access to health care and contamination of natural resources represent major problems that affect the wellbeing of Aboriginal communities and individuals [33].

2. 1.2 Health Transition among Aboriginal People

It is well accepted that socio-cultural transition towards a western lifestyle has changed dietary and other lifestyle and health behaviors among Aboriginal people [70]. Transition in dietary patterns and intakes have been linked to escalating rates of obesity and its associated diseases. Historically food of Aboriginal people in Northern Canada consisted of wild plants and all parts of hunted animals (liver, gonads, gut, brain and bone marrow) [71]. A traditional Inuit diet (TF) is comprised of seal, whale or other sea mammals, caribou, fish and berries [71], which is overall very rich in vitamin D and other micronutrients including omega 3 fatty acids that become reflected in the content of red blood cell (RBC-Omega 3) fatty acids (FA) [72] and has historically been linked to protection from chronic diseases [73]. Despite longstanding health protection ascribed to TF, recent issues related to availability and exposure to chemical toxins, such as persistent organic pollutants and metals, has reduced reliance on TF among Inuit populations [74]. Contemporary dietary intakes include less TF and greater intakes of foods obtained commercially. The high cost and limited availability of healthy market food (MF) options for Inuit communities complicate the nutrition transition.

Only 10-36% of total energy comes from TF among Aboriginal people including Inuit in Canada, with intake of TF more common among in older people > 40 y. Energy intake from TF corresponds with higher vitamin D intake [31]. Higher vitamin D status and higher intake are more often observed in older Inuit people who consume more TF [31,10], whereas lower vitamin D status presents in younger groups who consume less TF [70]. Instead, MF is adopted by the younger generation of Aboriginal people is often comprised of foods with lower nutrient density and of relevance to this thesis less vitamin D content [75]. The location of the community (coastal versus inland) is a significant factor in the nutrition transition. Nutrition transition can be evaluated using the level of omega-3 fatty acid versus saturated or trans-fatty acids in RBC membranes [76].

In a recent report from the International Polar Year Inuit Health Survey (IPY-IHS) 2007-2008, the energy from TF for all respondents (per capita) in the past 24 hours was 16.1% versus 23.4% in 1999 survey ($P \leq 0.05$), and the percent of energy intake from TF declined for all women ($P < 0.05$), but not for men over the 10 years which could explain the higher rates of obesity among women versus men [77,70]. In summary, TF which represented a healthy nutritious diet was used more often by Inuit previously. Currently Inuit are more often consuming MF which is reflected in fatty acid status. This shift in reliance on TF likely affects vitamin D consumption and possibly other aspects of metabolism. Although men were more likely to use TF, their use of MF is also increased which may explain the high prevalence of obesity among Inuit nowadays.

2.2 Vitamin D Physiology

2.2.1 Vitamin D Functions and Forms

Vitamin D is considered a nutrient or prehormone. Vitamin D is acquired endogenously following cutaneous exposure to ultraviolet beta radiation (UVB) or obtained exogenously from foods such as TF or supplements. Regardless of source, all vitamin D is converted into a metabolically stable metabolite, 25-hydroxyvitamin D (25(OH)D), that upon further 1-alpha hydroxylation becomes the active metabolite called calcitriol ($1,25(OH)_2D$). Calcitriol is best known for its function in calcium homeostasis in an endocrine manner as has been extensively reported [78,79]. On the other hand, active vitamin D has many other autocrine and/or paracrine functions such as pancreatic synthesis and release of insulin. It also has paracrine/autocrine actions in the adipose tissue [80] and muscle, pancreas and bone [81-83].

Vitamin D exists naturally in two forms: ergocalciferol or (vitamin D_2) from fungal sources, and cholecalciferol, or (vitamin D_3) from animal source foods or endogenous synthesis. Ergocalciferol, is derived from UV irradiation of ergosterol in yeast or fungi, and is argued to be almost two to nine times less potent than cholecalciferol in humans [84]. It is

likely that most of TF which represents a key source of vitamin D for Inuit people contain vitamin D in the form of vitamin D_3, and so it could be speculated that Inuit who consume TF are likely to have higher 25(OH)D_3 than 25(OH)D_2.

2.2.2 Solar Dermal Formation of Vitamin D

The main source of vitamin D_3 in most people is thought to be obtained from the conversion of 7-dehydrocholesterol in the plasma membrane of dermal keratinocytes and epidermal fibroblasts upon exposure to UVB (290-315 nm) [85,86]. Upon exposure to UVB, the B ring of cutanuous 7-dehydrocholesterol is broken and double bonds are rearranged to form pre-vitamin D_3. Previtamin D_3 stays in the plasma membranes of cells until it is thermally modified to form thermo-stable vitamin D_3 that is released into extracellular spaces. Afterwards, vitamin D_3 is bound by vitamin D binding protein in the capillary bed of the dermal layer [85,87]. The formation of pre-vitamin D_3 is rapid and depends on the intensity of UVB. Excess pre-vitamin D_3 and vitamin D_3 formed from exposure to UVB are not toxic because they are converted to less toxic metabolites such as lumisterol, tachysterol and 5,6 transvitamin D suprasterol that have little activity on calcium metabolism [88].

Melanin in the skin has the capacity to absorb UVB and reduce the amount of UVB directed to the formation of pre-vitamin D_3 [88]. Consequently, formation of vitamin D_3 by exposure to sun depends on skin pigmentation, surface area exposed, time of the year and day, and location in reference to the equator, which represents the area with most intense UVB. Also, any factor that blocks solar radiation penetrating the skin such as using sun screen creams or concealing clothing could partially or almost fully block vitamin D production [88]. Although speculative due to limited data on the topic, the use of sunscreen are possibly not an issue among Inuit living in the Arctic.

The impact of various skin color types on vitamin D status has not been found consistent in white and non-white populations [89,90]. Skin type can range from type I (white

skin that burns and never tans) to VI (which is a pigmented black skin) [91]. The skin type of Canadian Inuit may be similar to American Indian skin type IV [92]. The darker the skin pigmentation, the longer it takes to synthesize vitamin D following exposure to UVB and thus Inuit may be predisposed for lower endogenous synthesis. In addition, latitude and solar zenith angel could be important factors that affect dermal formation of vitamin D [93] . Inuit live in higher latitudes (more than 70^{o}N) with limited UVB throughout the year which could lead to limited endogenous formation of vitamin D. Although thick ozone reduces dermal formation of vitamin D [94], it is well known that the ozone layer is getting thinner in the arctic [95]. Also, covering the body due to cooler weather even during the summer months could interfere with UVB exposure. However, the ample amount of snow in the area could lead to high intensity of UVB radiation in days of sunshine [91]. In fact, a recent study among Greenland Inuit relative to non-Inuit suggested that season contributed to vitamin D status even after adjustment for diet, ethnicity and vitamin D intake, which could indicate that UVB exposure could play a role in vitamin D biosynthesis among this group. Having serum 25(OH)D >50 nmol/L was more common in summer-autumn versus winter-spring (OR: 2.6; 95% CI 1.6 to 4.3) in model adjusted for age, sex, weight, residence, origin and diet [96]. In conclusion, it is hard to determine if sun exposure contributes to vitamin D synthesis among Inuit living in the Canadian Arctic and more studies are needed in this regard.

2.2.3 Other Sources of Vitamin D

In addition to endogenous formation of vitamin D, animal based foods and supplements represent the other source of vitamin D. The sources of vitamin D in the human diet are usually very limited. Some sources of vitamin D include fatty fish, fish oil, organ meats, egg yolk and some irradiated mushrooms. In addition, fortified food such as milk, margarine and orange juice are considered important sources of vitamin D in Canada [97,98,98,99]. It was demonstrated previously that Aboriginal people have low intakes of milk [100] due to a high prevalence of lactose intolerance [101,102]. Understandably, TF of Inuit contribute

to most of the vitamin D (Table 2) intake despite the increasing reliance on MF [71,31]. Data from a previous study indicated low intake of supplements among Aboriginal adults [103]. Thus the contribution of MF and supplements to vitamin D status is relatively low and typically below 300 IU/d [104] . Various sources of vitamin D in market foods in Canada are summarized in Table 1, although many are not highly available in the northern regions of Canada.

2.2.4 Absorption and Transport of Exogenous Vitamin D

Previous studies of absorption of vitamin D is hampered by the lack of clinical tests for vitamin D_3 [105]. It has been long assumed that vitamin D is absorbed by passive diffusion through apical cells of the intestine [106]. A recent study has shown that cholecalciferol, at physiological concentrations, is transported by plasma membrane carriers such as scavenger receptor class B type 1, cluster determinant 36 (CD36), and Niemann-Pick C1 Like *in vitro* using Caco-2 cells, a model system for intestinal transport mechanistic studies [107]. Upon absorption into the enterocyte, vitamin D is incorporated into chylomicrons and almost 80% is transported through the lymphatic system to liver [34]. In blood, under physiological conditions, cholecalciferol and its metabolites are mainly carried by vitamin D binding protein, and only, 12-15% are bound to albumin [108]. Circulating 25(OH)D that is bound to vitamin D binding protein gets filtered at the glomerulus of the kidney, and then is reabsorbed by megalin mediated endocytosis in the proximal renal tubule, where 25(OH)D can be metabolized to $1,25(OH)_2D$ [109].

2.4 Storage of Vitamin D in Adipose Tissues

On the basis of vitamin D being a fat soluble vitamin, it is thought to be sequestered in adipose tissues [46,110]. High amounts of vitamin D or its metabolites were found in adipose tissue in both humans and rodents after ingesting radiolabeled vitamin D [111]. Vitamin D is rapidly taken up by adipose tissues after pharmacological oral dosages and is slowly released into the circulation [112]. Upon weight loss, plasma 25(OH)D concentration is increased [113]. In

a study by Lin et al., plasma concentration of 25(OH)D was significantly increased 1 month after bariatric surgery among 20 women who were morbidly obese (63.8 ± 9.1 vs. 76.9 ± 11.1 nmol/L, P=0.02) [113]. Since there is a high prevalence of obesity among Aboriginal people, addressing the impact of obesity on vitamin D status is of relevance to this thesis that deals with the relationship between vitamin D and insulin secretion and resistance in Aboriginal people.

2.2.5 Enzymatic Activation of Vitamin D

Vitamin D is converted into the active form in two successive steps. The first of which involves 25-hydroxylation in the liver to produce 25(OH)D which is the main circulating form of vitamin D. The second step which happens in the kidney and other tissues involves 1-α hydroxylation of 25(OH)D into $1,25(OH)_2D$, the third step involves 24-hydroxylation of vitamin D and leads to inactivation of vitamin D [114]. The 25-hydroxylation step is exhibited by 6 cytochrome P450 enzymes, but the most important are CYP27A1, CYP2R1, CYP3A4. On the other hand, 1-α hydroxylation is exhibited by CYP27B1, whereas 24-hydroxylation is exhibited by CYP24A [114].

The second hydroxylation towards synthesis of the active form of vitamin D, $1,25(OH)_2D$, is made in kidney in the mitochondrial proximal renal convoluted tubule (PTH regulated), or in the mitochondrial proximal straight tubule [114]. This is the pathway of the well-known endocrine action of vitamin D metabolism. Studies in knockout mice indicate CYP27B1 is the sole source for 1-α hydroxylation, at least for mice. At the endocrine level, ionized calcium and phosphate regulate the enzyme CYP27B1 through the hormones PTH, calcitonin [115]. Through a negative feedback loop, $1,25(OH)_2D$ down-regulates its own expression by vitamin D receptor (VDR) suppression of the promoter of CYP27B1 [114].
It is worthy to mention that although circulating $1,25(OH)_2D$ is mainly derived from the kidney [116], numerous other extra-renal sites are capable of synthesizing $1,25(OH)_2D$ in an

Table 2.1 Vitamin D content of selected commercially available foods in Canada.

Natural and Fortified Foods	**Vitamin D (IU/100 g)**
Natural Sources	
Shiitake mushrooms, irradiated, cooked	28
Egg yolk, two large	57-88*
Salmon	
Humpback/pink, raw or cooked	588
Coho, wild, raw or cooked	436-452
Tuna	
Tuna, albacore, raw or cooked	110-140
White tuna, canned with water	80
Cheese, mozzarella, parmesan, cheddar and others	0-20
Fortified Sources	
Cows' Milk, 2%, 1%, skim, chocolate	40
Orange juice	38
Margarine	530

Source [117]. * per two eggs.

Table 2.2 Vitamin D content of selected traditional food in the Inuit diet.

Inuit Traditional Food Examples	**(Vitamin D IU/100 g)**
Arctic char, flesh raw	1032
Cisco, egg raw	260
Lake trout, flesh raw	788
Loche, egg raw	608
Loche, liver raw	12716
Sculpin, flesh and bones, boiled	80
Sculpin, flesh and bones raw	564
Sculpin, white Fish, flesh raw	176
Beluga, blubber boiled	532
Beluga, muktuk, raw	72
Beluga, oil, aged	1068
Narwhal, blubber, raw	400
Narwhal, oil, aged	160
Ringed seal, blubber, raw	60
Ringed seal, brain, raw	60
Ringed seal, eyes, raw	104
Ringed seal, liver, raw	428

Source [118].

autocrine/paracrine manner including liver which is a major site of insulin action [119], and bone which is another site that interacts with insulin function and resistance [120] and the pancreas which all express CYP27B1 [121]. Extra-renal CYP27B1 is controlled by signaling molecules such as cytokines and inflammation markers [122, 123]. In summary, insulin secreting and target tissues are capable of activation of vitamin D in an autocrine manner. According to author's knowledge, the way vitamin D reaches to CYB27B1 action in insulin secreting and target tissue is not known.

A third step in the metabolism of vitamin D is catabolism and performed by the enzyme CYP24A1, which is a multifunctional inner mitochondrial cytochrome P450 enzyme present in many tissues. The main substrates for the enzyme CYP24A1 are 25(OH)D and $1,25(OH)_2D$ which are hydroxylated to $24,25(OH)_2D$ and $1,24,25(OH)_3D$ [124]. These metabolites are further catabolized to the final product known as calcitroic acid [124] which is conjugated to several metabolites such as glucuronides, carboxylic acids and others [125] and excreted through the bile.

2.2.6 Vitamin D Receptor (VDR)

The fundamental endocrine role of $1,25(OH)_2D$ is to regulate calcium homeostasis. This is accomplished by the action of $1,25(OH)_2D$ in bone, intestine and kidney by suppression of PTH and induction of fibroblast growth factor 23 (FGF23) in bone cells. Free $1,25(OH)_2D$ acts by binding nuclear vitamin D receptor (VDR) which regulate many cellular functions through genomic VDR (slow action that may take hours or days), or non-genomic (rapid which may take from 1-2 or 15-20 minutes) signaling [126]. Genomic VDR acts by regulation of transcription of many genes: first upon ligand binding VDR binds to a vitamin D response element (VDRE) upstream of the promoter region of a target gene [127]. Ligand bound VDR heterodimerizes with RXR which induces conformational changes in VDR that leads to recruitment of coactivators such as receptor coactivator 1 or vitamin D receptor interacting protein [127]. Variant VDRE exist with consequent recruitment of various gene

modulators and hence actions [127]. On the other hand, the repression of gene transcription involves recruitment of co-repressors which change chromatin to heterochromatin by enzymes such as histone deacetylase and demethylase after binding to VDRE [128]. Tissues involved in the action and secretion of insulin such as the pancreas [129] and skeletal muscles [130] were found to express VDR. VDRE was found on the insulin receptor gene promoter [131], osteocalcin gene[132] and in insulin like growth factor binding protein 3 [133], all of which are involved in glucose metabolism as described in this thesis.

2.2.7 Vitamin D Deficiency Definition and Dietary Reference Intakes

There is currently no consensus on what constitutes an optimal serum or plasma 25(OH)D or optimal dietary intake of vitamin D. Usually, the thresholds of vitamin D intake and status are based on maximal suppression of PTH, and acquisition of maximum bone mineral density (BMD) in healthy people [134,135]. According to the 2011 report of the Institute of Medicine (IOM), a plasma 25(OH)D concentration less than 30 nmol/L is considered at risk of deficiency. For people over 1 y of age the plasma concentration of 40 nmol/L corresponds to median intakes of the population, which corresponds to the Estimated Average Requirement (EAR; 400 IU/d)[42]. The concentration which is considered the target for skeletal health is at least 50 nmol/L 25(OH)D [42] and corresponds with the Recommended Dietary Allowance (RDA) value of 600 IU/d for adults 18 to 70 y and 800 IU/d for adults over 71 years of age. Dietary Reference Intake values for vitamin D are summarized in Table 2.3. There is debate on the recent IOM decision to use 50 nmol/L as a goal for skeletal health [43]. The endocrine society suggested 75 nmol/L as a target for serum 25(OH)D [136]. Heaney challenged the IOM conclusion regarding using the plasma 25(OH)D of 50 nmol/L as a goal for skeletal health, in that at least three rigorous studies indicated that plasma 25(OH)D concentrations above 50 is needed for optimal bone health [43]. The first study was a UK based large scale randomized control trial (RCT) which showed that plasma 25(OH)D concentrations of 52.5-72.5 nmol/L are needed to reduce all osteoporotic fractures by 33%

[137]. Also, a meta-analysis by Bischoff-Ferrari et al. showed that fracture reduction consistently occurs at a plasma 25(OH)D concentration of 75 to 80 nmol/L [138]. Furthermore, a large German autopsy study indicated that osteoid seam width did not reach a fully normal value until plasma 25(OH)D is > 75 nmol/L [139]. High doses of vitamin D intakes (for example 50,000 IU/d) that are taken over a long duration may cause toxicity, mainly, hypercalcemia and calcification of soft tissues [42]. Nonetheless, the Tolerable upper intake Level (UL) for vitamin D intake for age groups 9 to > 71 y is set at 4000 IU/d by the IOM as indicated in Table 2.3.

2.2.8 Vitamin D Deficiency and Variables Associated with Vitamin D Status among Canadians and Aboriginal People

Having a plasma 25(OH)D concentration lower than 50 or 75 nmol/L is common in the Canadian population despite mandated fortification of milk and margarine with vitamin D since the 1970s [140,141]. In the Canadian Health Measures Survey (CHMS) in 2007-2009 of 5306 Canadians, 6-79 y old, and living in or within 50 km of a metropolitan area, a plasma concentration of 25(OH)D below 27.5 nmol/L, below 37.5% nmol/L, below 75 nmol/L was present in 4.1 % (95% CI: 2.9% to 5.8%), 10.6% (95% CI: 8.2% to 13.6%) and 64.6% (95% CI: 61.1% to 68%), respectively [140].

A more recent data-set from CHMS (2012-2013) indicated that 65% of Canadians have vitamin D status ≥ 50 nmol/L, 25% had plasma 25(OH)D between 30 to 50 nmol/L, and only 10% had plasma 25(OH)D less than 30 nmol/L. Canadians who reported being of ethnicity other than white were more likely to have inadequate vitamin D status [142]. Among the general Canadian population, ethnicity, season and milk intake were found to be the most important predictors of vitamin D status [143]. Also supplement use could be a factor that affects serum 25(OH)D among Canadians [144]. Of those who use supplements (400 - 1000 IU/d of vitamin D), 82.7% had plasma 25(OH)D ≥ 50 nmol/L relative to 69.6% among non-supplement users [145].

Table 2.3. Dietary reference intakes for total exogenous vitamin D

Life Stage (Sex)	**RDA**	**UL**
1-3 y (M+F)	600	2500
4-8 y (M+F)	600	3000
9-13 y (M+F)	600	4000
14-18 y (M+F)	600	4000
19-30 y (M+F)	600	4000
31-50 y (M+F)	600	4000
51-70 y (M+F)	600	4000
More than 71 y (M+F)	800	4000
Pregnant and lactating	600	4000

[42]The Estimated Average Requirement for vitamin D is 400 IU/day and corresponding plasma 25(OH)D is 40 nmol/L. Corresponding plasma 25(OH)D value for RDA is 50 nmol/L. M corresponds to male. F corresponds to female. RDA: Recommended Dietary Allowance. UL: Tolerable upper intake level. y: year.

The data that was shown for Canadians regarding plasma 25(OH)D concentration in the CHMS did not capture information based on Aboriginal ethnicity or those residing in remote regions such as the Arctic. A large proportion of Aboriginal people live in remote, subarctic and arctic regions of Canada well beyond 42ºN such that endogenous synthesis of vitamin D may be limited to certain seasons. In the past, Aboriginal people obtained 100% of their diet from land and marine animals and other TF. Some TF types contain very high amount of vitamin D as outlined in Table 2.2.

Vitamin D status of Inuit and FN is arguably lower than white Canadians. Almost, 67.4% (95% CI: 64.7% to 70.0%) and 42.2% (95% CI: 39.5 to 44.9) of Inuit adults were found to have 25(OH)D concentrations below 75 nmol/L and 50 nmol/L, respectively, at the end of summer and early fall in a study of n=2186 Inuits ≥ 18 years the Canadian Arctic [10]. Furthermore, 27.2% (95% CI: 24.9% to 29.4%) had 25(OH)D values < 37.5 nmol/L. Similar trends of vitamin D deficiency and insufficiency were found in males and females. However, serum 25(OH)D concentration and higher vitamin D intake were positively associated with age. While a healthy waist circumference (WC) was found to be an important positive correlate of plasma 25(OH)D in this study, having a hunter (as a proxy for access to TF sources of vitamin D) in the home did not reach statistical significance [10]. Although Inuit are speculated to get most of their intakes of vitamin D from TF which represents a rich source for vitamin D, many are no longer consuming TF in sufficient quantities to provide for vitamin D needs [70]. Many risk factors for vitamin D deficiency aggregate in the Inuit including: higher adiposity, poor dietary habits as related to the nutrition transition, lower socioeconomic status and limited use of vitamin D supplements [10] in addition to ethnicity and residence in a geographical location with diminished UVB [146].

2.3 The Role of Vitamin D in Glucose Homeostasis

2.3.1 Physiological Pathways of the Associations of Vitamin D with Insulin Secretion and Resistance.

Of relevance to this thesis and extraskeletal functions of vitamin D metabolites, VDR is expressed in pancreatic [129], skeletal muscle and adipose tissue [130]. The fact that the enzyme CYP27B1 is expressed in pancreatic β-cells implies an autocrine capacity for activation of vitamin D [38]. Also, vitamin D stimulates the peroxisome proliferator activator receptor σ [147] which implies involvement of vitamin D in sensitizing cells for glucose and in lipid metabolism via this receptor. Animal models provide strong evidence of the involvement of vitamin D in glucose metabolism. Vitamin D deficient mice and rabbits had impaired insulin function and secretion; and treatment with vitamin D reversed insulin dysfunction [148,149]. Results from a cell culture study confirm the relationship between vitamin D and insulin secretion and function. In cell culture, $1,25(OH)_2D$ stimulates the expression and release of insulin protein [150], whereas it decreased fatty acid induced insulin resistance in cell culture of C2C12 cells [151]. On the other hand, and at least partially, $1,25(OH)_2D$ could play an anti-inflammatory role due to its involvement in immune function which could be partially relevant for DM2 [152].

The β-cells of the pancreas also express the vitamin D sensitive-calcium protein transporter calbindin-D28k [129] which is controlled by a positive VDRE as was shown in a mouse gene [153]. Researchers speculate that regulation of calcium influx into β-cells could affect the depolarization of β-cells, and consequently insulin exocytosis [154]. The same mechanism of calcium regulation by calbindin-D28K was shown to protect β-cells from destruction by pro-inflammatory cytokines [155]. The regulation of calcium fluxes in β-cells of the pancreas has been shown to be induced via genomic and non-genomic vitamin D (via putative VDR) effects [156]. In addition to the direct effects of $1,25(OH)_2D$ on insulin synthesis

and release, insulin sensitivity which reflects insulin action, is indirectly associated with vitamin D through calcium homeostasis.

Indeed, PTH has been linked to insulin resistance through mechanisms that may involve modulation of intracellular calcium [157]. Insulin sensitivity reflects the positive response of tissues to actions of insulin, while insulin resistance reflects the resistances of target tissues to the action of insulin. PTH is independently associated with insulin sensitivity [40]. Among 52 normotensive adults, PTH was inversely related to insulin sensitivity index (r^2=0.104, *P*= 0.02), whereas first phase insulin response was positively associated with PTH (r^2= 0.098, *P*= 0.023) [158]. The possible effect of PTH on insulin sensitivity could be ascribed to interference with insulin signaling as was demonstrated in adipose tissue cell culture [159]. Whether the relationship between PTH and insulin sensitivity has any connection to calcium homeostasis needs further investigation.

High intracellular calcium interferes with dephosphorylation of glycogen synthase and insulin regulated glucose transporter (GLUT-4), a process that follows insulin receptor binding [160,161] . Also, insulin induced phosphorylation of tyrosine kinase and activation of phosphatidyl inositide kinase (PI3-K) is interrupted by calmodulin, a calcium activated protein that can bind to insulin receptor substrate 1 (IRS-1) [162,163,164].

Variations in vitamin D binding protein are linked to variation in glucose metabolism among Japanese [165] and Pima Indian ethnic groups [166]. However, similar results could not be reproduced in European and American populations [167]. Variations in VDR were linked to DM2, glucose intolerance and HOMA-IR [168]. Finally, vitamin D could be linked to glucose metabolism through its link to insulin-like growth factor-1 (IGF-1) and IGF binding proteins (IGFBP). IGF-1 regulates the endocrine activation of vitamin D, and calcitriol regulates IGFBP and both may play an important role in glucose homeostasis [169]. It is worthy to

mention that the skeleton is the site for FGF23 production and the second major site for the production of IGF-I. In addition, both vitamin D and IGF-1 are related to BMD [170].

The phosphaturic hormone, FGF23, is a part of the endocrine pathway that regulates the production of $1,25(OH)_2D$. Recently, FGF23 has been implicated in energy and glucose metabolism. In fact, insulin and homeostatic model assessment of insulin resistance (HOMA-IR) correlated negatively with FGF23 in a study among adolescents [171]. In animal studies, Klotho which is a receptor for FGF23 could mediate oxidative stress and thus has implications in DM2 [172]. A study by Anour indicated that mice with deleted Klotho gene had exaggerated insulin sensitivity which was restored to normal upon ablation of vitamin D receptor [173].

In conclusion, vitamin D is involved in glucose metabolism through VDR which is expressed in many tissues that are applicable to insulin action and resistance, and indirectly through calcium/phosphate homeostasis. Finally, the implications of VDR in glucose homeostasis is further supported by the existence of various polymorphisms that interfere with glucose metabolism.

2.4 Adipose Tissue Endocrine Role as Related to Insulin Resistance and Plasma 25(OH)D

Adipose tissue is currently considered an endocrine organ that produces many inflammatory cytokines [174]. Besides production of cytokines from adipose tissue, obesity is associated with chronic inflammatory processes that could be manifested by production of acute-phase reactants such as C-reactive protein (CRP) [175]. Inflammation is manifested in insulin resistance as inflammatory cells such as activated macrophages could play a significant role in inducing insulin resistance [176]. Besides being a source of inflammatory mediators, there are many other factors that are produced from adipose tissues including adipokines such as adiponectin, leptin, visfatin and resistin [177]. Plasma 25(OH)D correlated

positively with adiponectin in adult females in an epidemiological study [178], whereas $1,25(OH)_2D$ suppressed the secretion of leptin from an adipose tissue cell line [179].

Regarding adiponectin, it can be related to insulin resistance through suppression of inflammation cytokines such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-λ), and suppression of activated macrophages [180]. Adiponectin knockout mice have less insulin sensitivity [181]. Also, adiponectin is involved in phosphorylation and activation of adenosine monophosphate protein kinase (AMPK) [182] and improves the sensitivity of the cells for insulin and thiazolidinedione [182,183].

Leptin is another adipocytokine that is involved in glucose metabolism indirectly through regulation of energy intake and weight control [184]. The plasma concentration of leptin is proportional to fat content in humans and mice [185,186]. Leptin can regulate glucose homeostasis by changing the expression of key enzymes that are involved in gluconeogenesis and glycogenolysis [187]. In addition, leptin regulates glucose uptake through central pathways that involves signaling through the hypothalamus. Leptin is involved in the regulation of energy molecules such as AMPK [188,189]. Serum concentration of leptin was positively associated with plasma insulin and glucose tolerance among Aboriginal people, whereas data on the relationship between adiponectin and glucose metabolism among Aboriginal are lacking [190]. Treatment with $1,25(OH)_2D$ was shown to suppress the production of leptin from an adipose tissue cell line [179]. In summary, both leptin and adiponectin are related to energy, obesity and glucose metabolism, and both of them are related to vitamin D metabolism and thus vitamin D could be indirectly modulating glucose homeostasis via adiponectin and/or leptin.

2.5 Diabetes, Prediabetes, Metabolic Syndrome

Although DM2 was previously rare among Inuit people, currently, Inuit are facing escalating rates of DM2 [22]. The World Health Organization (WHO) definition of DM2 is

based on hyperglycemia at thresholds that could lead to impaired fat metabolism and microvascular complications. Mainly, pancreatic insulin secretion, insulin action and endogenous hepatic glucose biosynthesis are impaired in DM2 [191]. The Public Health Agency of Canada defines DM2 as having either fasting plasma glucose higher than 7 mmol/L, or two hour after 75 g oral glucose tolerance test (OGTT) a plasma glucose value that is higher than 11.1 mmol/L. Impaired fasting glucose (IFG) is defined as having a plasma glucose level that is higher than 6.1 mmol/L and post-OGTT challenge less than or equal to 7.8 mmol/L. On the other hand, impaired glucose tolerance (IGT), is defined as having OGTT plasma glucose concentration between 7.8-11.0 mmol/L and fasting glucose < 6.1 mmol/L [192]. In 2003, the American Diabetes Association lowered the FPG cut-point to define IFG from 6.1 mmol/L to 5.6 mmol/L. However, WHO and many other diabetes organizations did not adopt this change [193]. Another tool that is used in diagnosis of DM2, IFG and IGT is glycated haemoglobin (HBA1C). Having either IGT or IFG is now defined as prediabetes, a condition that has been recently defined and is considered as a target for early intervention before frank DM2 is established; both confer higher risk for development of DM2 [194]. Various criteria for definitions of DM2, IFG and IGT including HBA1C are summarized in Table 2.4.

Around 5-10% of adults with prediabetes develop DM2 every year. The annualized incidence rates of progression to diabetes among adults of both IFG and IGT was 15-19% and is higher than adults with isolated IGT (4–6%) or isolated IFG (6–9%) [195]. Thus it is important in populations at high risk for DM2 to also monitor for IFG and IGT. The prevalence of IFG (FPG > 5.6 mmol/L) among Inuit was estimated to be 15.6% (95% CI: 13.9% to17.7%) [196]. Both IGT and combined IFG plus IGT were related to intima-media thickness which is an indicator of atherosclerosis [197]. It is worthy to emphasize that the physiological impairment in IFG is different from IGT which is manifested by the shape of the curve after ingestion of oral glucose challenge. In adults, IFG is characterized by

elevated FPG and exaggerated early rise in plasma glucose following glucose ingestion, while adults with IGT have normal FPG, but experience rapid and continuous rise in plasma glucose and lack of decline in plasma glucose two hours after the OGTT challenge [198]. It seems that adults with IFG have marked hepatic insulin resistance, whereas adults with IGT have marked increase in skeletal muscle insulin resistance [198]. Insulin action depends on signaling through GLUT-4 which is a cellular transporter that depends on phosphorylation of IRS-1 and IRS-1-associated phosphatidylinositol 3 phosphate kinase (PIK3) [199,200]. In obese adults there is an increase in the concentration of free fatty acids (FFA). The higher concentration of FFA leads to accumulation of harmful metabolites, such as diacylglycerol, which accumulate in organs of insulin action such as skeletal muscle and liver causing a condition of resistance to insulin action. Harmful lipid metabolites could interfere with GLUT-4 function through interference with enzymes such as PIK3 [201]. Feutin A could mediate lipid induced insulin resistance [202] through interference with phosphorylation of GLUT-4 resulting in a condition of resistance to insulin action [203].

Insulin resistance could be defined as a decrease in the response of target organs to insulin which eventually leads to deterioration in insulin secretion. Insulin resistance varies between individuals and could exist in adults with normal glucose tolerance IGT [204]. DM2 manifests in patients when the pancreas is no longer able to compensate for insulin resistance leading to hyperglycemia [204]. Studies using an euglycemic hyperinsulinemic clamp indicate that humans with IGT have marked insulin resistance in skeletal muscle, whereas humans with IFG have high hepatic resistance to insulin-induced suppression of glucose production [198].

It is worthy to highlight the fact that insulin resistance exists despite normoglycemia which could increase the risk for cardiovascular diseases (CVD) [205]. It is estimated that 25% of individuals with normal glucose tolerance have insulin resistance [204]. Insulin resistance is

accompanied by obesity, hypertension and dyslipidemia, all of which are important risk factors for CVD [206]. Adults who have insulin resistance also have increased risk for metabolic syndrome (METS) [207].

Although a controversial term, METS is a cluster of risk factors that has the ability to predict both DM2 and CVD [208]. METS (with variable definitions as summarized in Table 2.5) is widely debated, not only on its utility, but also on its definition and etiology [209]. For consistency, the National Cholesterol Education Program - Adult Treatment Panel (NCEP-ATPIII) definition of METS will be used throughout this thesis. Similar criteria for WC and TG risk groups were used to study Adults from IPY-IHS previously [22]. In a multiethnic study that included Caucasians, FN Cree and Inuit, the prevalence of METS according to NCEP-ATPIII definition was 7.7% among Inuit, 21.2% among FN and 12.5% among white [209].

2.5.1 Data from Epidemiological Studies

Numerous epidemiological studies on the associations between vitamin D status and the prevalence of DM2 [210], METS [211] and markers of insulin resistance and secretion [212] now exist. However, the interpretations appear inconsistent. Various studies used different populations and variable methods including measures of insulin concentration insulin resistance and plasma 25(OH)D (Table 2.6). In terms of plasma 25(OH)D and incidence of DM2 or METS, Liu and colleagues first established the predicted 25(OH)D (the predicted values were derived from multiple regression of serum 25(OH)D based on age, BMI, month, dietary intakes and smoking). Then they showed that adults in the highest tertile of predicted plasma 25(OH)D had 40% lower risk of developing DM2 (hazard ratio: 0.60; 95% CI: 0.37 to 0.97; P= 0.03) compared to the lowest tertile of plasma 25(OH)D in a group of 1972 adults after adjustment for covariates [213]. Similarly, the OR for having METS in 834 men and 820 women over 20 years was 0.27 (95% CI: 0.15 to 0.46, P= 0.001) in the highest serum

Table 2.4 Clinical criteria for the diagnosis of impaired fasting glucose, impaired glucose tolerance and Diabetes Public Health Agency of Canada and American Diabetic Association [192,193,214].

Condition	Definition
Impaired fasting glucose	Having fasting plasma glucose between 6.1-6.9 mmol/L and 2 h post 75 OGTT < 7.8 mmol/L or having HBA1C between 5.7-6.4% [192,193]
	Having fasting plasma glucose between 5.6 – 6.9 mmol/L and 2 h post 75 OGTT < 7.8 mmol/L or having HBA1C between 5.7-6.4% [214]
Impaired glucose tolerance	Having fasting plasma glucose < 6.1 mmol/L and 2 h post 75 OGTT between 7.8-11.0 mmol/L or having HBA1C between 5.7-6.4% [192,193]
Type 2 diabetes mellitus	Having fasting plasma glucose ≥ 7.0 mmol/L or 2 h post 75 OGTT ≥11.1 mmol/L or random blood glucose‡ ≥ 11.1 mmol/L with symptoms of diabetes (polyuria, polydipsia, unexplained weight loss); or having HBA1C ≥ 6.5% [192,193]

† Fasting based on no caloric intake for at least eight hours.
‡ Plasma glucose measured at any time of the day without regard to the interval since the last meal.
Adapted from the following sources [192,193, 214].

25(OH)D quintile relative to the lowest serum 25(OH)D quintiles after adjustment for covariates [215].

Another study that investigated the relationship between METS and plasma 25(OH)D among 1818 Canadian adults from the CHMS 2007-2009 found that having plasma 25(OH)D lower than a 50 nmol/L was associated with a 50% increase in the odds for developing METS (OR= 1.54; 95% CI: 1.00 to 2.38). An inverse linear dose dependent relationship was found between vitamin D and the prevalence of METS. In addition, plasma 25(OH)D was significantly lower among people with METS in comparison to people without it (60.2 vs. 70.8 nmol/L) [216].

Regarding insulin resistance as an outcome in Caucasian adults > 65 y, higher quintiles of 25(OH)D concentration was significantly associated with a reduction in insulin resistance indicators such as HOMA-IR, fasting insulin and glucose, HBA1C, two hour OGTT glucose in a group of 3206 adults who are older than 20 years without DM2. The relationships remained significant after adjustments for covariates, except for 2 h OGTT results [217].

Moreover, the OR for having hyperglycemia as measured by HBA1C > 6.5% was 2.3 if plasma 25(OH)D was less than 25 nmol/L and 2.09 if 25(OH)D was between 25 and 50 nmol/L relative to adults with plasma 25(OH)D >75 nmol/L [218]. In the NHANES study, plasma 25(OH)D as high as 81.0 nmol/L was considered the goal at which vitamin D exerts a beneficial function regarding lower insulin resistance [219].

In other studies, the association between 25(OH)D concentration and outcomes of glucose intolerance became weak after adjustments for covariates, especially adiposity [16,17]. Similarly, in the Framingham Heart Study, 3890 adults were included, the correlation between plasma 25(OH)D concentration and indicators of insulin resistance were weakened

Table 2.5. A Comparison of various definitions of METS [220].

	WHO	EGIR	NCEP/ATPIII	AACE	AHA/NHLBI/ADA	IDF	Harmonized [a]
Year	1999	1999	2001	2003	2004	2005	2009
Number of risk factors	IFG/IGT/T2DM or insulin resistance [b] and 2 of the following	Insulin resistance [c] and 3 or more of the following	Three or more of the following criteria	IGT/IFG with any of the following criteria	Three or more of following criteria	Obesity and 2 of the following criteria	Three or more of following criteria
Obesity	WC/hip ratio M>0.9, F>0.85 Or BMI>30 kg/m^2	WC M≥94 cm F≥80 cm	WC M≥102 cm F≥88 cm	BMI ≥>25kg/m^2	WC M≥102 cm F 88 cm	WC M≥94 cm M≥90 (Asian M) F≥80 cm	WC Geographic and ethnic specific
Dyslipidemia	HDL-C M<0.91 mmol/L F<1.0 mmol/L TG≥1.7 mmol/L	HDL-C<1.0 mmol/L TG≥2.0 mmol/L or treated	HDL-C M<1.0 mmol/L F<1.3 mmol/L TG≥1.69 mmol/L	HDL-C M<1.0 mmol/L F<1.3 mmol/L TG≥1.69 mmol/L	HDL-C M<1.0 mmol/L F<1.3 mmol/L TG≥1.69 mmol/L or treated	HDL-C M<1.0 mmol/L F<1.3 mmol/L TG≥1.7 mmol/L or treated	HDL-C M<1.0 mmol/L F<1.3 mmol/L TG≥1.7 mmol/L or treated
Hyperglycemia	DM2 FPG≥6.1 mmol/L 2 h OGTT>7.7 mmol/L	Not DM2 FPG>6.1 mmol/L	DM2 FPG≥6.1 mmol/L	Not DM2 FPG≥ 6.1 mmol/L 2 h OGTT>7.7 mmol/L)	DM2 FPG ≥5.6 mmol/L	DM2 FPG≥5.6 mmol/L	FPG≥5.6 mmol/L or treated
Hypertension (mm Hg)	SBP≥140 DBP≥90	SBP≥140 DBP≥90 or treated	SBP≥130 DBP≥85	SBP≥130 DBP≥85	SBP≥130 DBP≥85 or treated	SBP≥130 DBP≥85 or treated	SBP≥130 DBP≥85 or treated
Additional Components	Microalbuminuria 20 mg/min Albumin/creatinine 30 mg/g			Insulin resistance (family history DM2, age, PCOS ethnicity, inactive)			

Abbreviations: AHA, American Heart Association; BMI, body mass index; DBP, diastolic blood pressure in mm Hg; F, female; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; M, male; NHLBI, National Heart, Lung and Blood Institute; OGT, oral glucose tolerance test; PCOS, polycystic ovarian syndrome; SBP, systolic blood pressure in mm Hg; TG, triglyceride.

[a] Harmonized Definition from the Joint statement from the IDF, AHA/NHLBI, the World Heart Federation, the International Atherosclerosis Society, and the International Association for the Study of Obesity.

[b] If fasting glucose < 6.1 mmol/L, insulin resistance measured by hyperinsulinemic-euglycemic clamp with lowest quartile for glucose uptake.

[c] Modification to WHO definition to use upper quartile of fasting insulin levels.

This table was revised and based upon Samson et al. [220].

yet remained significant even after adjustment for BMI: HOMA-IR (*P*= 0.003); log insulin (*P*= 0.003); and fasting glucose (*P*= 0.047). After adjusting for waist hip ratio (WHR), the correlation of plasma 25(OH)D with log insulin remained significant (*P*= 0.006); and after adjustment for subcutaneous adipose tissue, the correlation of plasma 25(OH)D was significant for HOMA-IR (*P*= 0.014), log insulin (*P*= 0.024) and glucose (*P*= 0.036). Finally, visceral adipose tissue attenuated the relationship between 25(OH)D concentration and insulin sensitivity indicators to non-significant levels [221]. It appears that the inverse association between serum 25(OH)D and insulin resistance is partially mediated by obesity since adipose tissue can store fat soluble vitamins such as vitamin D and hence mask their effect.

The masking effect of adiposity on the relationship between 25(OH)D and insulin secretion and sensitivity was further confirmed in other studies, that specifically targeted morbidly obese adults; rather PTH was found to predict METS [222]. Contrarily, in a study that targeted lean adults 18-30 y, there was a robust significant inverse association between plasma 25(OH)D and HOMA-IR, fasting and post-challenge insulin and glucose regardless of BMI and WC [223].

The results from multiethnic populations vary, for example in the NHANES III the negative association between insulin resistance (HOMA-IR) and vitamin D status was present in Caucasian and Mexican Americans, but not African American men [210]. Among Cree adult men and women there was weak association between vitamin D status and both HOMA-IR (β= -0.005, SE= 0.002, *P*= 0.006) and HOMA-B% (β=-0.004, SE= 0.002, *P*= 0.006), but the relationships were not persistent upon including BMI as covariate in the final statistical model.

In the study by Del Gobbo and colleagues there was very high prevalence of high body weight and low vitamin D status which may have limited the interpretation of results [17].

However, in another study among FN women [50] and white women, the relationship of insulin resistance (HOMA-IR) as related to 25(OH)D was significant and inverse. Ethnicity has a significant interaction with quartiles of plasma 25(OH)D as related to insulin resistance ($P<$ 0.05) [50]. In conclusion, cross- sectional studies provide some evidence for the relationship between higher plasma 25(OH)D and healthier glucose homeostasis outcomes. However, obesity obscured this relationship in a few studies and this relationship was not significant in some ethnic groups. Tables 2.6 and 2.7 summarize the epidemiological evidence related to the relationship between plasma 25(OH)D and insulin secretion and resistance or the relationship between plasma 25(OH)D and incidence of prediabetes and metabolic syndrome and DM2.

2.5.2 Data from Randomized Clinical Trials

Clinical trials provide the highest level evidence on the effect of treatment on clinical endpoints. The effect of vitamin D supplementation on outcomes of insulin resistance likely needs to be extended and is inconsistent. Of the trials registered, at least 50 studies were performed on the effect of vitamin D supplementation on glucose homeostasis (see Table 2.6 to 2.7 for most recent studies).

In a meta-analysis of RCT, supplementation with vitamin D was not associated with a decrease in fasting plasma glucose in those with normal glucose tolerance (0.01 mmol/L; 95% CI: -0.06 to 0.09, $P>$ 0.05), whereas in adults with abnormal glucose tolerance there was significant reduction in plasma glucose (-0.30 mmol/L; 95% CI: −0.57 to −0.07, $P=$ 0.01) [224]. On the other hand, studies that used euglycemic or intravenous glucose tolerance to measure insulin sensitivity failed to find any effect of vitamin D supplementation on insulin sensitivity [225,226,227], probably because vitamin D in the studied groups was sufficient, or because $1,25(OH)_2D$ was used in the trial [37]. Finally, the RCT regarding vitamin D supplementation effects on insulin secretion and resistance are inconsistent in terms of study design, duration of the trial, form and dose of vitamin D used.

Table 2.6. Relationship between plasma 25(OH)D and insulin resistance and secretion outcomes.

Author/year/ Design	**Characteristics (age in y; BMI in kg/m^2)**	**Mean plasma 25(OH)D (SE) nmol/L**	**Insulin outcome**	**Association of 25(OH)D with insulin outcome**	**Covariates**	**Limitation**
M Chonchol et al., 2006 [228] Cross-sectional	American adults>20 y NHANES, multiethnic	78.0(0.7)M 71.6(0.7)F	Insulin HOMA-IR	Inverse (*P*= 0.002) Inverse (*P*= 0.002)	Age, sex, ethnicity, BMI	No adjustment for physical activity, dietary intake, or others.
Forouhi et al**.,** 2008 [229] Prospective (10 y)	524 nondiabetic men and women Age (40-69 y), UK European Origin	60.2(25.3) Sex, age Adjusted	 FPG 2h OGTT PG insulin HOMA-IR Metabolic syndrome	Per 25 nmol/L there was a a decrease of 0.05 mmol/L (*P*= 0.074) 0.25 mmol/L (*P*= 0.013) 4.2 pmol/L (*P*= 0.012) 0.16 unit (*P*= 0.009) 0.05 z score (*P*= 0.06)	Age, sex, smoking, BMI, season, IGF-1, PTH, physical Activity, social class	No adjustment for supplement intake, central obesity, plasma lipids. Small sample size.
Liu et al., 2009 [178] Cross-sectional	808 nondiabetic Caucasian, Framingham, USA Age 59.6	47.4(0.6)	 FPG insulin HOMA-IR $ISI_{0,120}$ 2h OGTT PG	β: -0.003 (*P*= 0.007) -0.003 (*P*= 0.001) -0.004 (*P*< 0.001) 0.023 (*P*= 0.31) -0.005 (*P*= 0.49)	Age, sex, BMI, waist, and smoking and physical activity	No adjustment for PTH. Small sample size for some of analysis.
Kayaniyil et al., 2010[230] Cross-sectional	712 Canadian free of DM2, ≥ 30 y	55.8(22.9)	IS-OGTT HOMA-IR IGI-IR ISSI-2	0.004 (*P*= 0.0003) -0.003 (*P*= 0.007) 0.004 (*P*= 0.03) 0.003 (*P*= 0.001)	Age, sex, season, ethnicity, supplements, PTH, physical activity, BMI	No adjustment for central obesity.
Pinelli et al., 2010 [231] Cross-sectional	542 Arab American (20-75 y), USA	45.2(16.0)M 35.2(17.7)F	In men HOMA-IR FPG HBA1C	Negative correlation -0.19 (*P*= 0.004) -0.15 (*P*= 0.027) -0.14(*P*= 0.038)	No adjustments	This study presented simple statistics with no adjustments.

Fraser et al., 2010 [232] Cross-sectional	3958, NHANES (2001-2006) Multiethnic, USA	59.6(1.0)	 IFG 2h OGTT PG insulin HBA1C	β -0.05 (*P*= 0.03) -0.00 (*P*= 0.89) -0.06 (*P*= 0.009) -0.02 (*P*= 0.12)	Age, sex, ethnicity, smoking, waist, PTH, and calcium	Not adjusted for physical activity, dietary intake, and BMI.
Alvarez et al., 2011 [40] Cross-sectional	25 African American and 25 Caucasian American females Age 38.2 (13.1) BMI 26.4 (4.7) USA	55.7(34.2)	FPI HOMA-IR IVGTT (ISI)	NS NS β= 0.28 (*P*= 0.04)	Race, age, PTH, intra-abdominal adipose tissue	Not adjusted for BMI, physical activity and others. Small sample size.
Del Gobbo et al., 2011 [17] Cross-sectional	510 Cree Canadians Age 36 y BMI 32.5 (6.4)	52.4(16.4)	HOMA-IR HOMA-B	NS NS	Age, gender, physical activity, alcohol, education, BMI.	No adjustment for dietary intake, and PTH. Small sample size.
Park et al., 2012 [233] Cross-sectional	301 Korean adults Age 70.4 (5.2) y BMI 24.7 (2.9) Asians	41.7(19.4)	 FPG insulin HOMA-IR	β -0.60 (*P*= 0.57) -1.35 (*P*= 0.004) -0.424 (*P*= 0.007)	Age, sex, smoking, alcohol, exercise, diabetes, hypertension, dyslipidemia, sunshine	Not adjusted for dietary intake, and PTH. Small sample size.
Hurskainen et al., 2012 **[234]** Cross-sectional	1756 adults Finland Age 62.9 (6.5) BMI > 25 Ethnicity: NA	43.4(17.6) Tertiles	FPG insulin 2h OGTT PG	NS NS Negative association (*P*= 0.002)	BMI, waist to hip ratio, smoking, physical activity, fruit intake, month, DM2 history	Not adjusted for PTH. Insulin resistance was not assessed.
Karnchanasorn et al., 2012 [235] Cross- sectional	150 healthy American adults Age 26 (1) BMI 25.4 (0.6) Multiethnic	76(5)	Hyperglyc emic clamp ISI β-Cell Function	β 0.065 (*P*= 0.005) 105.6 (*P*= 0.02)	Age, sex, BMI, ethnicity, physical activity, season	No adjustment for dietary intake or PTH, or central obesity.

Weiler et al., 2013 [50] Cross-sectional	368 urban Aboriginal and White Canadian females Age 45.3 (13.6) BMI 29.3 (6.5)	62.6(29.3)	FPG HBA1C HOMA-IR C-peptide	β -0.05 (*P*= 0.032) -0.04 (*P*= 0.028) -0.16 (*P*= 0.04) -0.11 (*P*= 0.026)	Age, ethnicity, osteocalcin, sometimes BMI, fat	No adjusted for many covariates including PTH, physical activity, central obesity, and others.
Manickam et al., 2013 [236] Cross-sectional	1074 African and Caucasian American Men Age> 25 Mean BMI > 25,USA	Normal 20.4(6.4) Prediabetes 17.6(5.4) DM2 18.0(6.7)	HBA1C	β -0.002 (*P*= 0.009)	Age, BMI, BP,GFR,TG,HDL, dietary calcium, education, alcohol use, exercise, marital status, health perception	Not adjusted for PTH, central obesity.
Tepper et al. 2014 [237] Cross-sectional	358 Israeli Age 48.8(10.2) BMI 27(3.8) Ethnicity: NA	22.1(7.9)	insulin HOMA-IR HOMA-B	Plasma 25(OH)D explained 2% of variation in FPI and HOMA-IR	Age, sun exposure, Physical activity, season	Not adjusted for dietary intake, PTH, or central obesity.
Kramer et al., 2014 [238] Prospective	494 Women of high risk of prediabetes and DM2 at postpartum, Canada Age 34.5 (4.2) BMI 27.8 (3.9) Multiethnic	35.7(10.2)	FPG and OGTT including 2h PG Mastuda Index ISI after 2 h	25(OH)D was inversely and significantly related to plasma glucose, and positively related to Mastuda Index and ISI after 12 month postpartum	age, ethnicity, family history of T2DM, previous GDM, BMI, 2-h glucose at 3 months duration of breast-feeding, physical activity and season	No consideration of dietary intake, multiple categorization of data.

Plasma 25(OH)D unit was nmol/L, age by y and BMI by kg/m^2. 25(OH)D, 25 hydroxyvitamin D; SE, standard error; NHANES, National Health and Nutrition Examination Survey; M: male; F, female; BMI, body mass index; UK, United Kingdom; FPG, fasting plasma glucose; 2 h OGTT, two hour post oral glucose tolerance test; PG, plasma glucose; IGF-1, insulin like growth factor; PTH, parathyroid hormone; $ISI_{0,120}$, two hour insulin sensitivity index; USA, United States of America; HBA1C, glycated hemoglobin C; IVGT, intravenous glucose tolerance; BP, blood pressure; GFR, glomerular filtration rate; IGI, Insulinogenic index; IR, insulin resistance; ISS-2, Insulin secretion sensitivity index; TG, triglyceride; HDL, high density lipoprotein; T2D,type 2 diabetes mellitus; GDM, gestational diabetes mellitus.

Table 2.7. Relationship between plasma 25(OH)D and diabetes, prediabetes, and metabolic Syndrome.

Author/year	Baseline characteristics (age in y; BMI in kg/m^2)	Plasma 25(OH)D nmol/L Mean (SD) or median (IQR)	Outcome	Association of 25(OH)D with insulin outcome	Covariates
Kayaniyil et al., 2011 [239] Prospective 3 years	N= 489 Canadian, Age 50 (10) y	58.01(23.26)	DM2, IFG, IGT development	No significant association OR (0.78 [0.59-1.02])	For age, sex, ethnicity, season, and both baseline and change in both physical activity and vitamin D supplement use, change in BMI.
Forouhi et al., 2012 [240] Retrospective 9-13 y	n= 1852 European, Epic Norfolk mean BMI> 25 Age 58(9.4) y	Cases 61.6(22.4) Non-case 65.3 (23.9)	DM2 Incidence	50% lower hazards for DM2 in Highest to lowest tertile of 25(OH)D	Age, sex, season, BP and lipids.
Husemoen et al., 2012 [241] Prospective 5 y	n= 6405, Denmark Age 46.3 y Northern European Race	46.3 range (29.7-61.3)	Incidence of DM2	OR per 10 nmol/L was 0.92, *P*= 0.08	Season, sex, age, family history of diabetes, BMI, and change in weight during follow-up; physical activity, dietary habits, alcohol, consumption, smoking status, total energy intake, and social class; randomization group and self-reported changes in dietary habits, physical activity, smoking status, and alcohol consumption during follow-up.
Gupta et al., 2012 [242] Cross-sectional	NHANES 2001-2006 621 African American	35.9(0.9)	OR for Prediabetes	1.51 in adults with 25(OH)D ≤ 45.4 relative to ones with > 45.4	Age groups, gender, BMI groups.
Afzal et al., 2013 [243] Prospective 29 y	9841 adults, Denmark Median 57-59 y Median BMI 24.2-25.5 Ethnicity: NA	41	Incidence of DM2	Hazards ratio 1.35 lowest versus highest quartiles (*P*= 0.002)	Sex, age, smoking status (never/ever), BMI, income, and duration and intensity of leisure time physical activities, season.

Tsur et al., 2013 [244] Prospective 2 y	117,960, Israeli Age 56.6(7.9) y BMI 27.0(5.1) Ethnicity: NA	50(22.9)	Progression to IFG DM2 (from normal) DM2 (from IFG)	OR 1.13 (P< 0.05) 1.77 (P< 0.05) 1.43 (P< 0.05) in 25(OH)D < 25 vs. 25(OH)D ≥ 75 nmol/L	Age, sex, population group, immigrant status, BMI, season of vitamin D measurement, LDL and HDL cholesterol, triglycerides, estimated glomerular filtration rate, history of hypertension or cardiovascular disease, Charlson comorbidity index, smoking, and socioeconomic status.
O'Hartaigh et al., 2013 Cross-sectional [245]	3316, Germany Age 62.7 (10.6) y Mean BMI 27.0-27.7. Caucasian of German ancestry	Not provided	DM2 (both fasting and postload	OR 0.61 (P= 0.001) in 25(OH)D < 25 vs. 25(OH)D ≥ 75 nmol/L	Adjusted for age, gender and BMI, smoking, alcohol, physical activity, waist circumference, cystatin C, interleukin-6, high-sensitivity C-reactive protein and seasonal change.
Vujosevic et al., 2014 Prospective 2 y [246]	97 Caucasian women with osteoporosis Age 51.6(5.9) y Mean BMI 25-30 Montenegro	Group with DM2 44.4 (19.0) Group without DM2 57.8(27.6)	Presence of DM2	OR for DM=0.958 (P< 0.05).	BMI, total-C and TG.
Kayaniyil et al., 2011 [247] Cross-sectional	654, Toronto, Multiethnic Age 49.1(9.8) y BMI 31.1(6.3)	56.4(23.1)	Presence of METS (Harmonized definition)	OR= 0.76 (95% CI 0.62 to 0.93)	Age, sex, season, ethnicity, supplement use, physical activity, and PTH.
Brenner et al., 2011 [248] Cross-sectional	1818, Canada CHMS BMI 26.6 Ethnicity: NA	67.8	Presence of METS NCEP-ATP III	14% lower OR for having METS for each 10 nmol/L 25(OH)D	Physical activity, smoking status, month of interview, age, sex and ethnicity.
Barchetta et al., 2013 [249] Cross-sectional	107 obese adults Europeans, Rome. Age 45.3(13.3) y BMI 43.1(8.3)	Cases 33.7(25.5) Non-Cases 43.4(30.7)	Presence of METS NCEP-ATPIII	OR 4.1 (P= 0.02) For lowest quartile vs. highest quartile 25(OH)D	Gender, age, serum PTH and body fat mass.

Mitri et al., 2014 [250] Cross-sectional and prospective 2 y	2161, American Multiethnic Age> 25 y BMI> 24	53.9(24.2)	Presence of METS Modified NCEP-ATPIII (Traditional) Incident metabolic syndrome	OR= 0.62 in the highest vs. lowest tertile 25(OH)D NS	Adjusted for recruitment location, month , age, gender, race, ultraviolet radiation index at participant's recruitment location, smoking status alcohol consumption (g/day), C-reactive protein (mg/l), physical activity and total energy intake, BMI.
Bea et al., 2015 [251] Cross-sectional	1084 American Mainly Caucasian. Mean Age 64.8-65.9 y Mean BMI 26.5-30.8	Not provided	Presence of METS Modified NCEP-ATPIII	OR 0.55(P< 0.05) in 25(OH)D< 50 vs. 25(OH)D≥ 75	Age, race/ethnicity, supplemental calcium, and WHR, sex.

Plasma 25(OH)D unit was nmol/L, age was y and BMI was kg/m^2**.** SD, standard deviation; IQR, interquartile range; BMI, body mass index; DM2, type 2 diabetes mellitus; 25(OH)D, 25 hydroxyvitamin D; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; NA, not available; Y, year; TG, triglyceride; C, cholesterol; TG, triglyceride; CHMS, Canadian Health Measures Survey; NCEP-ATP III, National Cholesterol Education Program Adult Treatment Panel III; WHR, waist to hip ratio.

Also, many studies were weakened by the small number of the sample, lack of randomized placebo control design and most importantly, short term supplementation [252,253]. In addition, some studies used calcium with vitamin D supplementation, and hence one cannot differentiate whether the study results were due to vitamin D or calcium [37]. Some of the most recent RCT that tested the effect of vitamin D on insulin secretion and resistance are summarized in Table 2.8; and the quality of these studies are summarized in Table 2.9. Previous trials were reviewed elsewhere [254].

2.6 Relationships among Bone Mineral Density, Energy Metabolism

2.6.1 Osteocalcin and Glucose Homeostasis

Osteocalcin, an osteoblast specific protein, represents as promising endocrine factor that links bone to the regulation of fat and glucose metabolism [255] and interestingly this protein has a VDRE [256]. Osteocalcin plays a major role in the coordinated metabolism of insulin and glucose, while the insulin receptors on osteoblasts play an important role in the regulation of bone turnover and circulating osteocalcin [257].

Total osteocalcin represents both carboxylated and uncarboxylated osteocalcin. The role of each form of osteocalcin needs further investigation. The enhanced signaling of insulin receptor on the osteoblast is associated with enhanced resorptive acidification of bone cavities which leads to decarboxylation and release of uncarboxylated osteocalcin. The uncarboxylated osteocalcin has been primarily linked to increased insulin concentration and β-cell number and function [258]. Adult osteocalcin-deficient mice are hyperglycemic and hypoinsulinemic while showing decreased insulin sensitivity, increased fat mass and decreased energy expenditure [48].

The mechanism(s) by which osteocalcin affects glucose metabolism involves insulin expression, secretion and insulin sensitivity. Gene expression analysis of pancreatic cells treated with osteocalcin should increase in the expression of insulin genes (Ins1 and Ins2).

Table 2.8. Recent clinical trials on the effect of vitamin D supplementation on insulin resistance and secretion.

Author/year	Characteristics (age in y; BMI in kg/m^2)	Placebo	Supplement	Duration of Supplement	Change in 25(OH)D (nmol/L)	Results
Mitri et al, 2011 [259]	92 adults, age 57 y, BMI ≥ 25 with glucose intolerance or early DM2. From USA, multiethnic.	Placebo	2-by-2 factorial design 2000 IU/day D_3 and/or calcium carbonate 400 twice a day	16 weeks	25(OH)D was higher in the I group vs. no C group 76.4 vs. 45.9 nmol/L, *P*< 0.001	Using IVGTT, B-cell function as measured by disposition index was significantly enhanced (*P*= 0.011), which was associated with increase in insulin secretion (*P*=0.046) in vitamin vs. no vitamin group.
Nazarian et al., 2011 [260]	8 American adults with vitamin D deficiency (< 75 nmol/L) and with IFG, age 18-65 y. USA, multiethnic	NA	10.000 IU/day	4 weeks		Using IVGTT; acute insulin response to glucose decreased (*P*= 0.011), and insulin sensitivity increased (*P*=0.012)
Harris et al. 2012 [261]	89 overweight or obese African American with prediabetes or early DM2 ≥40 y	Placebo	Oral 4000 IU/day D_3	12 weeks	+ from 40 to 81 in I group	After 2h OGTT insulin sensitivity decreased by 4% in I group, but increased by 12% in the C group (*P*=0.024), but no effect on disposition index or glycemia.
Simha et al., 2012 [262]	12 healthy American adults with 25(OH)D < 50 mnol/L Ethnicity: NA	Placebo	Oral 50000/week IU D_2	8 weeks	In I group changed from 33.2 to 46.9, whereas in the C group changed from 39.2 to 31.2 nmol/L of 25(OH)D	Insulin-stimulated glucose infusion rate during the last 20 minutes of a hyperinsulinemic-euglycemic glucose clamp did not change significantly with treatment.
Davidson et al., 2013 [263]	109 adults with prediabetes and 25(OH)D < 75 nmol/L, Latino and African Americans	Placebo	Oral based on weight and baseline 25(OH)D	12 months	25(OH)D increased from 55 to 174.7 nmol/L	No change in FPG, 2 h OGTT PG, insulin sensitivity and secretion, or % progressed to DM2 or returned to normal.

Belenchia et al., 2013 [264]	35 obese adolescent American Age 14.1 y BMI 39.8 kg/m^2 Multiethnic	Placebo	Oral 4000 IU/day D_3	6 months	Increase in serum 25(OH)D by 48.7 nmol/L in I group vs. increase by 7 nmol/L in C group (*P*< 0.001).	FPI (-6.5 in I group compared with +1.2 µU/mL for C group; *P* = 0.026) HOMA-R (-1.363 in I group compared with +0.27 for C group; *P* = 0.033)
Hung et al., 2013 [265]	10 African American patients with chronic hemodialysis, age 49 y	Cinacalcet	Paracalcitol	8 weeks	-	No change in insulin resistance or glucose disposal rate as measured by hyperinsulinemic euglycemic clamp
Wongwiwatthananukit et al. , 2013 [266]	90 patients with metabolic syndrome, Thailand, Age 63.7 (11.7) y, with 25(OH)D < 50 nmol/L Asian	Placebo	20,000 IU D_2/week 40,000 IU D_2/week	8 week	In 20,000 IU D_2, there was + by 29.2. In 40,000 IU D_2, there was + by 39.2 relative to 7.00 in C group, *P*<0.001.	HOMA-IR, FPG, FPI were not different
Salehpour et al., 2013 [267]	77 healthy women with BMI > 25 kg/m^2, age 38 (8) y Iranian	Placebo	Oral 1000 IU D_3 daily	12 weeks	+ by 38.2 in I group, 4.6 in C group	FPG - by 0.65(0.4) mmol/L in I group relative to -0.28(0.4),*P*<0.001 in C group HbA1C – by 19 (17)% in I group vs. 13(18)% in C group.
Strobel, 2014 [268]	86 patients DM2 Germany, age 18-80 y, with no vitamin D supplement Ethnicity: NA	Placebo	1904 IU daily	6 months	After 6 month 78% achieved 50	Patients with 25(OH)D> 50 had lower HBA1C at baseline (0.008) and after tx (*P*= 0.009), but no change in C-peptide, insulin, or HOMA-index
Jehle, 2014 [269]	55 patients with 10 y DM2, Switzerland Ethnicity: NA	Placebo	Intramuscular 300000 IU/3 months	6 months	+ by 6.9% in I group vs. 2.9% in C group	HBA1C + 2.9% in tx group vs. 6.9% in I group. HOMA-IR – 12.8% in I group and + 10% in C group.

Kampmann, 2014 [270]	16 Danish patients with DM2 and hypo-vitamin D Ethnicity: NA	Placebo	Oral Daily 11200 IU D_3 for 2 weeks and daily 5600 for 10 weeks	12 weeks	I group from + 31 to 104.9	-borderline significant increase (P =0.08) in the insulin secretory burst mass (non-stimulated). -IVGTT incremental AUC of insulin increased significantly after 12 weeks within the I group (P = 0.05). -Insulin resistance measured by hyperinsulinemic euglycemic clamp was not significantly different.
Madar et al., 2014 [271]	215 immigrants in Norway, healthy 18-50 y Multiethnic	Placebo	Oral 400 IU or 1000 IU	16 weeks	I group + from 29 to 49	HBA1C was not significantly different between I group and C group.
Elkassaby et al.,2014 [272]	50 adults with DM2 diagnosed in last 12 month, with normal 25(OH)D Melbourne Australia Caucasian	Placebo	Oral 6000 IU D/day	6 months	I group + from 59 to 128 in 6 month, no change in C group	At 3 month there was significant decrease in FPG and postprandial PG in I group vs. C group, but not in C-peptide or HBA1C. There was no significant change in 6 months.
Javed et al. , 2015 [273]	47 Obese, healthy adolescents 12-18y Caucasian American	400IU D_3	Oral 2000 IU D_3/day	12 weeks	I group (+7.7 (16.2), p = 0.04), but not in C group (P= 0.39).	NS change in 3h OGTT ISI and insulin disposition index

Plasma 25(OH)D is expressed by nmol/L. I, intervention; C, control; BMI, body mass index; DM2, type 2 diabetes mellitus; USA, United States of America; IVGTT, intravenous glucose tolerance test; tx, treatment; 25(OH)D, 25 hydroxyvitamin D; HOMA-IR, homeostatic model assessment of insulin resistance; IV, intravenous; FPG, fasting plasma glucose; IU, international unit; FPI, fasting plasma insulin; -, decreased; +, increased; NA, not available; AUC, area under the curve; PG, plasma glucose; OGTT ISI, oral glucose tolerance test insulin sensitivity index; NS, not significant; HBA1C, glycated hemoglobin C.

Table 2.9. Quality assessment of clinical trials performed on the effect of vitamin D supplementation on insulin resistance and secretion.

Author/year	Quality of allocation concealment	Potential for selection bias	Intention to treat	Blinding patients	Blinding healthcare professionals and investigators	Comparability of groups	Registration
Mitri et al., 2011 [259]	random	present	present	present	present	comparable	registered
Nazarian et al., 2011 [260]	NA	minimal	absent	minimal	absent	NA	registered
Harris et al. 2012 [261]	random	minimal	absent	minimal	absent	comparable	registered
Simha et al., 2012 [262]	random	minimal	absent	present	present	comparable	registered
Davidson et al., 2013 [263]	random	present	present	present	present	comparable	registered
Belenchia et al., 2013 [264]	random	minimal	absent	present	present	comparable	registered
Hung et al., 2013 [265]	random	present	absent	present	present	some discrepancies	registered
Wongwiwatthananukit et al. , 2013 [266]	random	present	absent	present	present	comparable	NR
Salehpour et al., 2013 [267]	random	minimal	absent	present	present	comparable	registered
Strobel et al., 2014 [268]	random	minimal	absent	present	present	comparable	NR
Jehle et al., 2014 [269]	random	present	present	present	present	some differences in medications	registered
Kampmann et al., 2014 [270]	random	present	absent	present	present	comparable	registered
Gagnon et al., 2014 [274]	random	present	absent	present	present	comparable	registered
Elkassaby et al.,2014 [272]	random	present	unknown	present	present	higher FPG in I group	unknown
Javed et al., 2015 [273]	random	present	unknown	present	present	comparable	registered

FPG, fasting plasma glucose; I, intervention; NA, not available; NR, not registered.

Also, osteocalcin enhances the expression of cyclin dependent kinase, cyclin D1 and cyclin D2 which suggests that osteocalcin could regulate β-cell proliferation [275]. The action of osteocalcin on pancreatic cells is mediated by G-protein coupled receptor family C group 6 member (Gprc6a) [13]. In addition, uncarboxylated osteocalcin acts through Gprc6a receptors in epithelial and STC-1 enteroendocrine cells to stimulate the production of glucagon-like peptide-1 which is an insulin secretagogue [276].

Osteocalcin could enhance the insulin sensitivity in obesity by reducing endoplasmic reticulum stress based on animal studies and cell culture [14] of adipose tissue, L6 muscle (skeletal muscle) and liver cell lines [14]. The reduction of endoplasmic reticulum stress is associated with enhanced IRS-I phosphorylation, and is mediated via NF-KB which leads to improved insulin signaling. Also, osteocalcin could be associated with increases in mitochondrial function and mass [14]. Finally, osteocalcin could affect insulin sensitivity indirectly by increasing the secretion of adiponectin [15]. VDR is expressed in osteoblasts and $1,25(OH)_2D_3$ was found to stimulate the expression of osteocalcin [277]. Consequently, vitamin D action on glucose metabolism could be mediated indirectly via osteocalcin.

The relationship between total osteocalcin and hepatic insulin resistance (as measured by HOMA-IR) in elderly men, postmenopausal women and overweight adults is inconsistent [15,278]. For example, there was a borderline significant positive relationship between total osteocalcin and insulin sensitivity as measured by an euglycemia-hyperinsulinemia clamp among young adults (slope= 1.6, P= 0.056), however, this relationship was significant only before adjustment for BMI. In this work, carboxylated osteocalcin correlated significantly with total osteocalcin [279]. In addition, total osteocalcin was associated with insulin sensitivity measured after an intravenous glucose tolerance test in a multiple linear regression model (β= 0.45; 95% CI: 0.27 to 0.63, P= 0.006) [278]. Other studies found inverse relationship between carboxylated osteocalcin

and HOMA-IR [15] and insulin [15]. Furthermore, both carboxylated osteocalcin and uncarboxylated osteocalcin were positively associated with insulin response in overweight adults with impaired fasting glucose tolerance [278]. Thus contrary to early studies, it is possible that both carboxylated osteocalcin and uncarboxylated osteocalcin have a role in the endocrine regulation of glucose metabolism.

Given the possible importance of osteocalcin in glucose metabolism, Iglesias et al. studied 64 obese adults (20 with NGT, 20 with IGT, and 24 with DM2) for correlates of osteocalcin. In this study, total osteocalcin concentration was significantly different between the three groups ($P< 0.01$) [280]. Similarly, the relationship between osteocalcin, plasma 25(OH)D and outcomes of glucose metabolism was investigated among 368 Aboriginal and white women over 25 y of age in Manitoba; there was a significant relationship between osteocalcin and the aforementioned outcomes; glucose (β=−0.108; 95% CI: -0.055- to -0.161, *P*-value < 0.0001), HBA1C (β=-0.101; 95% CI: -0.059 to - 0.143, $P< 0.0001$), and HOMA-IR (β=-0.229; 95% CI: -0.058 to -0.399, $P= 0.009$) [281].

Finally, osteocalcin is significantly associated with METS components such as elevated WC, serum TG, blood glucose concentration [282]. In a cohort study of 2765 adults older than 70 y of which 28.8% had METS, the OR for having METS was 2.39 (95% CI: 1.71 to 3.35) if total osteocalcin is was < 13.25 μg/L and 1.51 (95% CI: 1.07 to 2.13) if total osteocalcin is was between 13.25 to 16.55 μg/L relative to osteocalcin higher than 30 μg/L [282]. In summary osteocalcin, which is a bone derived protein, has relationships to insulin resistance and secretion. This relationship implies a role for bone as an endocrine organ in regulating glucose metabolism.

2.6.2 The Relationship between Glucose Metabolism and BMD

Following on the cross talk between energy and bone metabolism that was described with regard to osteocalcin, there is an inconsistent relationship of BMD to prediabetes, metabolic

syndrome and DM2. It can be summarized from previous sections that prior to any health condition, insulin resistance exists even in otherwise healthy adults. When insulin resistance increases to the degree that the pancreas cannot compensate for impaired glucose handling by increases in insulin, conditions of impaired glucose regulation are evident such as prediabetes and the METS which together predict the development of frank DM2. It is not known how stages of glucose impairment will affect BMD.

Insulin resistance which precedes any pathological conditions was itself linked to BMD, but the studies in this regard are also inconsistent. Insulin resistance and the associated hyperinsulinemia has been shown to be accompanied with increases in BMD [283, 284, 285]. Insulin resistance causes a refractory increase in insulin secretion and insulin is an anabolic factor for bone [286,287]. Also, hyperinsulinemia may have a negative impact on hormone binding globulin, thus increasing the bioavailability of sex hormones, which may protect against bone loss [288,289]. Positive relationships between insulin secretion and resistance and BMD have been observed, yet are attenuated after adjustment for BMI [288]. In addition, another study that assessed insulin sensitivity using an intravenous glucose tolerance test (IVGT) found a negative association between insulin sensitivity (log transformed) and whole body BMD ($r=0.29$, $P< 0.01$) and femoral neck BMD ($r=0.35$, $P< 0.001$); which means that insulin resistance is correlated positively with BMD [284]. However, other studies have shown inverse association of HOMA-IR with bone strength (a composite measure of femoral neck axis width and length, hip BMD and body weight) and BMD [290]. The inverse association between BMD and HOMA-IR was also seen in Korean adults [291] and in adolescents with non-alcoholic fatty liver disease [292]. It is thus possible that any elevations in BMD owing to insulin resistance do not necessarily associate with enhanced bone strength. At certain concentrations of fasting plasma glucose and post OGTT glucose, a condition of prediabetes is denoted when fasting glucose exceeds 5.6 mmol/L; this

condition has been scarcely studied in relationship to BMD and fracture. A study among 802 men with prediabetes showed that there was no significant difference between BMD T-score among DM2 cases and men with prediabetes [293]. Another study in Baltimore of men and women >55 y, showed that only in women was IGT associated with reduced bone mineralization (β= -0.076) and hip strength (β= -0.097, $P< 0.05$), whereas no associations were found with BMD [294]. On the other hand, Australian women with prediabetes had reduced risk of fracture (OR: 0.70; 95% CI: 0.52 to 0.95) [295] which was consistent with another study [296]. Further studies on the effect of prediabetes on bone health outcomes are needed to understand the impact of pathophysiological disturbances in this stage on BMD, bone strength and fracture.

METS is a more advanced stage of insulin resistance that needs to be addressed. According to the Camargo cohort study, Caucasian postmenopausal women with METS had higher BMD at the lumbar spine, femoral neck and total hip than postmenopausal women without METS after adjusting for age, this difference became insignificant after adjustment for BMI, but there were no significant differences in BMD in men with and without METS [297]. In American NHANES III, which enrolled 8,197 Americans, adults with METS had higher femoral neck BMD than those without METS [298]. However, in the Rancho Bernardo Study, which included Caucasian men and postmenopausal women, men with METS showed distinctly lower BMD than men without METS after adjustment for covariates [299]. It is well known that METS includes having high WC which was associated with low BMD [300]. Adipose tissue could be a source of many proinflammatory cytokines that could contribute to bone resorption [300].

DM2 represents the frank end of the impaired glucose regulation. Fracture is related to DM2 as some studies indicated that people with DM2 have higher risk for hip [301,302], proximal humerus [302, 303], foot and all non-vertebral fracture [302,303,304]. One factor that could be related to fracture risk is BMD. In a meta-analysis and review by Vestergaard, DM2 was associated with

increased BMD [57], which appears counter-intuitive. However, BMI which is generally increased in patients with DM2 could in part explain the higher BMD [305]. Regardless, for a given BMD, adults with DM2 seem to be more prone to fracture [305]. It seems that despite the association of DM2 with increases in BMD, the bone structure and quality are prone to damage. For example, advanced glycation end product in bone collagen could contribute to increased fracture risk associated with DM2 [306]. In addition, porosity is increased in cortical bone in patients with DM2 [307], however it is speculated the time to observed altered porosity is not clear and influenced not only by duration of DM2 but also by adherence to treatments and management of hyperglycemia.

One study that showed a positive link between DM2 and BMD is the Hertfordshire Cohort Study that included 465 men and 444 women aged 59 to 71 years. Men with newly diagnosed DM2 had significantly higher BMD at the lumbar spine even after adjusting for covariates including BMI and age relative to adults without DM2 (1.16 ± 0.12 versus 1.07 ± 0.16 g/cm^2, $P = 0.05$). BMD at total femur and femoral neck tended to be higher in adults with DM2 relative to adults with normal glucose tolerance. On the contrary, some cross-sectional studies found normal, or lower BMD in adults with DM2 [308]. One factor to consider is the association of DM2 with higher weight which itself is associated with higher BMD and less fracture. For each one standard deviation of less BMI there was 19% increase in the risk of fracture (95% CI :1.01 to 1.35) [309]. In summary, there seems to be positive association between BMD and conditions of insulin resistance, including insulin resistance itself, METS and DM2, but this yet is not well studied in prediabetes states. The positive association with BMD comes with simultaneous deterioration of bone quality that needs to be further assessed as it causes a net increase in fracture risk [310].

One of the challenges in measuring BMD in overweight and obese people is that the two dimensional dual-energy x-ray absorptiometry (DXA) tests most commonly used may

overestimate BMD based on tissue thickness [311]. Upon using phantom simulations of BMD and varying tissue thickness, errors as large as 20% were reported in BMD measured using central DXA [312]. In comparison, quantitative computed tomography (QCT) which measures volumetric BMD can accurately identify soft tissue compartment while calculating BMD and seems to be more precise in cases of fat layering [313]. In some anatomical sites, for example the forearm, the error in measuring *in vivo* BMD was as low as 3% which could be attributed to lower soft tissue mass surrounding the bone at this site [314,315]. Thus the methods of assessment of BMD should be considered when interpreting the relationship between glucose handling and BMD. Methods of measuring BMD in most of the studies done so far were done at locations which could be affected by fat layering and to this author's knowledge, very few studies were done using sites such as forearm BMD that would be less affected by adiposity.

In summary, the relationship between DM2 or METS and BMD is not conclusive. Many studies show that DM2 is related to higher BMD which could be justified by higher BMI and insulin concentrations among patients with METS and DM2. However, in the scientific literature there is some evidence to support the association between insulin resistance as measured by HOMA-IR and deteriorated BMD. Studies on the association between insulin resistance and BMD using other rigorous methods to assess insulin resistance such as the hyperinsulinemic euglycrmic clamp are lacking. Thus conclusions regarding the relationship between BMD and insulin resistance, DM2, or METS remain elusive. However, understanding the association between insulin resistance, IFG and DM2 among Inuit with bone is important to help understand the fracture risk among this ethnic group as fracture is associated with increased cost [316] and mortality [317].

2.7 Summary

This review has summarized existing knowledge regarding Inuit health and the relationship of vitamin D status and TF to glucose homeostasis from one side and the association of adipokines and both osteocalcin and BMD with glucose homeostasis. Inuit continue to experience the nutrition transition [1] that is accompanied with food insecurity [318]. Both of these problems impact the nutritional status of Inuit people causing multiple micronutrient deficiencies combined with obesity such as low vitamin D status [10]. Recently vitamin D status has been linked to glucose homeostasis [37]. Plasma 25(OH)D has been found to be associated with lower incidence of prediabetes [319], METS [216] and DM2 [210]. The link between vitamin D and glucose metabolism could be direct [129], mediated by calcium and PTH metabolism [40], mediated by dyslipidemia [320] and adipokines modification [179,321] and mediated by the relationship of vitamin D to the skeletal system [288].

It has been shown that VDR is expressed in pancreatic cells [129], in addition to cells that represent the target of insulin action such as skeletal muscle cells [130]. Moreover, both PTH and calcium were found to play a role in insulin secretion and insulin resistance. In addition, adiponectin [321], leptin [179] and plasma TG [320] which are implicated in glucose metabolism are affected by the active vitamin D metabolite. Finally, plasma 25(OH)D could have an effect on glucose metabolism through the skeleton [288]. Osteocalcin, which is a bone derived protein, links to lower insulin resistance and enhanced insulin expression representing a connecting factor between energy metabolism and the skeleton [12]. However, the relationship between dysglycemia or insulin resistance and BMD is inconclusive in the literature. All the aforementioned relationships were not previously studied in the Inuit population given that TF is the main source of vitamin D and it is considered a source of other active compounds including nutrients and harmful contaminants. Thus, these existing gaps in the literature need to be addressed.

Bridge 1

As was shown in the previous chapter, Inuit are a unique Indigenous group who live at remote arctic lands in countries including USA, Canada, Greenland and Russia [3]. Inuit have passed through a transition that affected the way of living and dietary habits. Mainly, the Inuit intake of food became more dissociated form full dependence on TF to more intake of MF which is more energy dense and of lower nutrient value [8]. As consequence of this transition, there is both an increase in the rate of DM2 and obesity among Inuit according to IPY-IHS [22].

The IPY-IHS speaks to the needs of researching the basic health aspects of Inuit people [322] who live in remote arctic regions that are difficult to reach and hence are not covered adequately by other Canadian health surveys [140]. This thesis attempts to understand how vitamin D status is related to insulin resistance at various pathological stages including adults who have normal FPG, then adults with IFG (prediabetes) and METS, and finally adults with DM2. Vitamin D actions on insulin resistance could be mediated by leptin, adiponectin and osteocalcin and could be reflected on the association between BMD and insulin resistance. Understanding of such complex relationships in Inuit who use TF as source of vitamin D could be different than other ethnicities. Hence, it was important to understand various physiological, sociodemographic factors and dietary factors in relation to Inuit health in the most recent work from the IPY-IHS which is covered in the next chapter and represents the foundation for this thesis.

CHAPTER 3: The International Polar Year Inuit Health Survey

The International Polar Year Inuit Health Survey (IPY-IHS) of 2007-2008 is the first large-scale comprehensive evaluation of the health of Inuit in Canada. It is a culturally appropriate and acceptable survey which responded to Inuit needs and priorities. Adults ≥ 18 y from randomly selected households completed initial parts of the survey in their communities. Then the second part of the survey was done on the Canadian Coast Guard Ship called the Amundsen, where participants had clinical appointments. In 2007, Inuit adults in 18 coastal communities in the Baffin and Kivalliq regions of Nunavut were involved [323]. In 2008, the IPY-IHS included in-land communities of the Inuvialuit Settlement Region, the remainder of Nunavut and Nunatsiavut.

The communities involved in the survey are all located between 54° 10' and 74° 43' north. Stratified random sampling was used to select households where communities were strata and where homes were randomized using either a computer random generation of numbers or a random digit table. The household participation rate was 68% and total 2595 male and female adults were studied [10].

During the survey, Inuit adults were asked questions about household crowding, nutrition, country/traditional food (TF), eating habits, mental health, community wellness and a medical history. The clinical tests that were performed included: blood glucose using one touch, ultra 2 TM glucometer, heart health such as carotid artery health, nutrition status such as vitamin A, vitamin D, iron, selenium, and omega 3 fatty acids. Also, the clinical tests included oral glucose tolerance test (OGTT) in a subgroup of adults, exposure to contaminants, exposure to infection and bone health in a subgroup of women 40 y of age and over [323].

The health and conditions of Inuit were studied from many perspectives by many researchers. In the area of obesity which represents the foundation for this thesis, the prevalence

of type 2 diabetes mellitus (DM2) was studied in relation to an at risk waist circumference phenotype combined with hypertriglyceridemia. In this report it was found that Inuit are no longer protected from the consequences of obesity. Also, DM2 occurs at rate as high as 12.2 % (95% CI: 8.7 to 15.7%) among adults who ≥ 50 y [22].

In addition, on the basis of body mass index (BMI), the prevalence of obesity among Inuit from IPY-IHS was found to be 36%, whereas the prevalence of overweight was 28%. Adults with at risk BMI (> 25 kg/m^2) consumed a high percent their total daily energy intake from sugar sweetened beverages (> 15.5% of total energy intake), whereas consumption of total carbohydrate was associated inversely with at risk BMI (P< 0.05) [324]. In analyses of sociodemographic data, variables including age, gender and geographic region of the Canadian arctic, higher education, any employment, personal income and private housing were all significantly positively correlated with an at-risk BMI (> 25 kg/m^2) (P≤ 0.001). Smoking, Inuit language as primary language spoken at home and time spent walking were inversely associated with overweight and obesity [325]. Comparatively, in the Canadian Community Health Survey (CCHS) of 2009-2010 for women, lower household income and education level were associated with higher BMI, whereas in men higher income and education were associated with higher BMI [326].

Furthermore, the study by El Hayek and colleagues demonstrates the pressing need for examining the relationships among obesity and vitamin D status. In this report, 67.4% (95% CI: 64.7% to 70.0%) and 42.2% (95% CI: 39.5% to 44.9%) of Inuit adults with available data on plasma 25(OH)D had values below 75 nmol/L and 50 nmol/L respectively, whereas 27.2% (95% CI: 24.9% to 29.4%) had plasma 25(OH)D below 37.5 nmol/L. The strongest positive predictor of plasma 25(OH)D was older age and having a healthy waist circumference (WC). This is important since those who are younger had lower vitamin D status and if combined with lower

WC could have important ramifications for health. Having higher plasma 25-hydroxyvitamin D (25(OH)D) concentrations did not predict forearm bone mineral density (BMD) in pre and postmenopausal females. Both BMI and fat mass were positively related to BMD in both female groups and age and osteocalcin were negatively associated with BMD in postmenopausal women [10].

High sitting height ratio (SHR) is associated with Inuit morphology. Inuit are described as having shorter leg length and high trunk-to-stature proportions such that cut offs for obesity derived from European populations may not be appropriate for Inuit populations. The high SHR may provide an explanation for the protection of Inuit from deteriorated metabolic profile that accompanies obesity. However, a recent analysis performed by Galloway et al. did not find any consistent relationship between BMI and SHR among Inuit [327]. The lack of significance of SHR in explaining body morphology is relatively new and contradicts what was previously reported in 1982 [328].

Food insecurity was different across the studied Inuit regions with Nunavut having the highest food insecurity (68.8%; 95% CI: 66.1 to 71.4%) significantly higher than that observed in Inuvialuit Settlement Region (43.3%; 95% CI: 37.2 to 49.3%) and Nunatsiavut Region (45.7%; 95% CI: 39.7 to 51.7%) ($P \leq 0.01$) [329]. The condition of food insecurity seemed to affect the quality of nutrient intake in combination with the nutrition transition. It was shown that food insecurity was associated with lower intake of energy and energy-adjusted fiber, vitamin C, iron, zinc and magnesium. In women, food insecurity was associated with a higher intake of carbohydrates and lower intake of fiber, dietary folate equivalent, vitamin C, iron, magnesium, calcium and vitamin D [70]. In fact, vitamin D intake was significantly lower in the food insecure group relative to the food secure group ($P < 0.001$); whether they were or were not consuming TF

as measured by food frequency questionnaire (FFQ) making vitamin D a good dietary biomarker of food insecurity [69].

In addition food insecurity associated with higher red blood cell content of trans fatty acid (RBC-Trans FA) and lower hemoglobin levels and serum ferritin, whereas higher or more frequent TF consumption was associated with higher serum 25(OH)D, red blood cell content of omega-3 fatty acids (RBC-Omega-3 FA) and serum ferritin ($P \leq 0.05$) [70]. BMI, WC and percent body fat were lower among individuals from food insecure households compared to food secure households ($P \leq 0.001$). Adults from food insecure households had a significantly lower Healthy Eating Index score and consumed fewer vegetables and fruit, grains and dairy products; and consumed a greater percent of energy from high-sugar foods than adults from food secure households ($P \leq 0.05$) [9].

Regarding TF intake, one study indicated that the contribution of TF to energy intake over the last 10 years has decreased ($P < 0.05$) with a simultaneous increase in the intake of energy from market food (MF). The overall percentage of energy from TF for all respondents (per capita) in the past 24 hours in the 2007-2008 survey was 16.1% versus 23.4% in 1999 survey ($P \leq 0.05$). The intake of sweetened beverages, potato chips and pasta all increased as represented by percentages of energy. Older adults consumed more TF than younger adults. Besides beluga, of which consumption was increased over 10 years, the consumption of other TF items such as ringed seal, narwhal and caribou have declined significantly, for example % energy caribou was 32.2% in 1999 relative to 24.1 in 2008 [70].

TF intake carries the risk for exposing Inuit to contaminants. Higher concentrations of mercury exist in marine and humans of the arctic happens due to bioaccumulation and biomagnification [330]. Markers of TF intake such as eicosapentaenoic acid (EPA: 20:5 omega-3) and docosahexaenoic (DHA: 22:6 omega-3) and selenium correlated significantly with mercury

(Hg) intake. The estimated intakes of each of the nutrients were strongly correlated (Se: r= 0.92, P< 0.001; EPA: r = 0.82, P< 0.001; DHA: r= 0.81, P< 0.001) with estimated Hg intake and the mean estimated Hg intake was 7.9 $\mu g \cdot kg^{-1} \cdot wk^{-1}$ which is higher than 5 $\mu g \cdot kg^{-1} \cdot wk^{-1}$ the toxicological reference value used as an upper accepted limit for Hg exposure [330].

TF intake can be assessed by concentration of omega-3 fatty acids in red blood cells (RBC-Omega-3). There can be a relationship between fatty acid status of the Inuit and iron deficiency that could be reflected on the activity of desaturase 5 (Δ5), which is crucial in the biosynthesis of highly unsaturated omega fatty acids in humans. The results from 1511 Inuit Canadian adults indicate that serum ferritin positively and significantly correlated with RBC-Omega-3 fatty acid status (r = 0.172, P< 0.0001) after adjustment for age, WC and C-reactive protein. Also, serum ferritin positively correlated with Δ5 (r = 0.126, P< 0.0001) after adjustment for omega-3 fatty acids [331]. This observation is physiologically sound since iron is a cofactor for the desaturase enzyme [332].

Jamieson et al. found that anemia was moderately prevalent (16.1%; 95% CI: 12.5 to 20.6%) among Inuit men. For men with CRP ≤ 10 mg/L, 6.5% (95% CI: 4.8 to 8.7%) had depleted, 19.8% (95% CI: 16.7 to 23.2%) had low and 10.3% (95% CI: 7.5 to 13.9) had elevated iron stores. Adiposity, TF intake, long-chain polyunsaturated FA content in RBC status and inflammation were positively associated with serum ferritin and food insecurity, smoking and *H. pylori* seropositivity were negatively associated with serum ferritin. Food insecurity and not having an active hunter in the home were associated with having depleted iron stores [333].

Adequate shelter is a basic human need. A study published from IPY-IHS points to hidden homelessness among Inuit as 19.1% (95% CI: 17.0 to 21.3%) of homes provided shelter for homeless people, and 40.4% (95% CI 38.3 to 42.5%) of the homes needed a major repair,

whereas the prevalence of crowding varied according to Inuit region with Nunavut having a prevalence of crowding as high as 29.7% (95% CI: 27.5 to 31.9 %) [334].

In the area of self-reported health (SRH), Saudny et al. documented that 27.8% of Inuit from IPY-IHS reported having poor SRH. Older age was significantly associated with poor SRH. The relative risk ratios for poor SRH was 2.0 (95% CI: 1.3 to 3.1) for men aged 50 years or older and 2.3 (95% CI: 1.7 to 3.0) for women aged 50 years or older, compared with men and women 29 years or younger. In addition, poor SRH was significantly and positively associated with smoking status, at risk fasting glucose, elevated C- reactive protein and hypertriglyceridemia WC phenotype (including high or low WC and/or high or low triglyceride) [335].

Finally, oxidative stress was measured by isoprostanes and isofurans. Plasma F2 isoprostane correlated positively with CRP (r= 0.132, *P*= 0.048), systolic blood pressure (r= 0.157, *P*= 0.024) after adjustment for age, sex and BMI. On the other hand, isofurans correlated significantly and positively with WC (r= 0.190, *P*= 0.005) and with systolic blood pressure (r= 0.137, *P*= 0.048) [336]. In addition, both F2 isoprostanes and isofuran correlated significantly and negatively with blood and toenail selenium which marks TF intake, whereas only isofuran was correlated with plasma Hg level [337].

In summary, the reports discussed above detail important findings regarding the health of Inuit adults, at least according to the 2007-2008 IPY-IHS results. According to these findings, chronic diseases such as obesity, DM2 and anemia are being observed at increasing rates among Inuit living in Northern Canada. The higher prevalence of chronic diseases is often accompanied with deteriorated socioeconomic status of the Inuit. Food insecurity has been found at a high rate among Inuit communities and is associated with unhealthy eating patterns. Studies indicate that the rate of consumption of TF, rich in nutrients and anti-oxidant properties among Inuit is

declining. As a result of decreased intake of TF, low vitamin D status are present at a high rate among Inuit people. Plasma 25(OH)D was not related to BMD among Inuit in the IPY-IHS. Whether vitamin D and its relationship to extraskeletal health conditions, particularly insulin sensitivity, is present among Inuit will be the topic of this dissertation.

Bridge 2

Epidemiological data that was presented indicated the presence of a relationship between glucose homeostasis outcomes such as insulin resistance as measured by plasma glucose and HOMA-IR and serum 25(OH)D [217]. Also, some clinical trial showed impact of vitamin D supplementation on insulin resistance markers.

Ethnic groups tend to have different associations in terms of the relationship between vitamin D and glucose metabolism [210]. This relationship has not yet been studied among Inuit groups residing in Canada considering their unique dietary habits and geographical distribution across the arctic. Thus the next chapter will address the relationship between vitamin D status and fasting plasma glucose, prediabetes (impaired fasting glucose), and insulin resistance (HOMA-IR) in Inuit adults of the Canadian arctic for the total sample. This relationship will be adjusted for dietary markers of TF and market food including red blood cell content of FA. Arctic region, obesity in addition to age and sex, and in subgroup adiponectin and leptin will be included in model. Adiponectin and leptin are both adipokines that are produced from adipose tissue and are related to both plasma glucose and insulin resistance through various mechanisms.

CHAPTER 4: Determinants of Insulin Resistance Among Inuit: Role of Diet, Adipokines and 25-Hydroxyvitamin D

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Key words, 25-hydroxyvitamin D, glucose, insulin resistance, Inuit, leptin, adiponectin.

ABSTRACT

Background: The association between commonly low vitamin D status and increasingly prevalent insulin resistance among Inuit adults has not been explored.

Objective: The primary aim of this study was to examine whether vitamin D status among Inuit adults is associated with fasting plasma glucose (FPG) and homeostatic model of insulin resistance (HOMA-IR) after adjustment for arctic region, obesity as well as age, sex, marine mammal (MM) and fish intakes and fatty acid status. A secondary aim was to explore if adipokines further explain FPG and HOMA-IR in a subgroup analysis.

Design: The present analysis involves 1418 Inuit adults from 3 geographical regions of the Canadian arctic: Nunavut (n=1032), Inuvialuit Settlement Region (n=194) and Nunatsiavut (n=192) without a diagnosis of DM2. Primary outcomes were FPG and HOMA-IR. Independent variables included 25-hydroxyvitamin D (25(OH)D), adiponectin, leptin and fatty acids in red blood cells (RBC) and MM and fish intakes. Covariates included age, sex, anthropometry and arctic region. Data was analyzed using multiple linear regression.

Results: Serum 25(OH)D (per 30 nmol/L) was not associated with FPG (β= -0.031; 95% CI: -0.140 to 0.080, $P> 0.05$) whereas it was inversely associated with HOMA-IR (β= -0.058; 95% CI: -0.111 to -0.005, $P< 0.05$) after adjustment for age, sex, arctic region, BMI and variables representing MM and fish intakes and fatty acids in RBC. Frequency of consuming marine mammals was positively associated with FPG and HOMA-IR. Arctic region was associated with HOMA-IR and FPG. Adiponectin was inversely associated with HOMA-IR, but not FPG. Conversely, leptin was positively associated with both HOMA-IR and FPG. In the subgroup model, total omega-3 fatty acids in red blood cells was inversely associated with FPG.

Conclusions: These data suggest that vitamin D and omega-3 fatty acids, as commonly obtained from traditional food, may be protective against insulin resistance in Inuit adults.

Introduction

Healthy vitamin D status has been linked to lower insulin resistance and lower fasting plasma glucose (FPG) in epidemiological studies [11]. The association between serum 25-hydroxyvitamin D (25(OH)D) and insulin resistance is highly relevant to the Inuit Aboriginal population of Canada where there is a high prevalence of low vitamin D status [10] and a growing concern for risk of obesity, insulin resistance and type 2 diabetes mellitus (DM2) [22]. Elevated visceral adipose tissue [338] and red blood cell (RBC) membrane saturated fatty acids [339] have been associated with increases in homeostatic model of insulin resistance (HOMA-IR) in Inuit. Interestingly, adiponectin [340] and some forms of omega-3 fatty acids in RBC such as C20:5 n-3 and C22:3 n-3 inversely associate with HOMA-IR in various populations of Inuit [341]. Traditional food (TF), such as marine mammals and fish, is a rich source of vitamin D and omega-3 fatty acids. Further research regarding the inter-relationships among vitamin D, omega-3 fatty acids, TF, adipokines and insulin resistance in Inuit is required to guide strategies for the prevention of insulin resistance.

Insulin resistance involves peripheral tissues such as muscle, liver and adipose tissue that have become unresponsive to the action of insulin [342]. Insulin resistance has been shown to be linked to impairments in glucose regulation [343] where small increments in plasma glucose over time, even within the normal range [344] (< 5.6 mmol/L), predict future DM2. For large epidemiological studies, the assessment of insulin resistance is usually performed by proxy measures of insulin resistance such as HOMA-IR [345]. Impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) now define prediabetes, a predictor of DM2 and other chronic diseases [198,346]. Prediabetes is significantly and positively linked to visceral or central adiposity [347]. Adipose tissue is a source of adipokines, such as leptin and adiponectin, that play important

roles in the regulation of glucose metabolism and insulin signaling [348]. Leptin partially explained variance in HOMA-IR and insulin in a family study of women from Finland, whereas adiponectin associated inversely with HOMA-IR [349].

The active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), suppresses leptin production from adipose tissue [179], whereas enhancing the precursor pool through vitamin D supplementation elevates plasma adiponectin concentration after 12 months [350]. Thus a higher vitamin D status would be anticipated to relate to lower insulin resistance as a function of reduced leptin and elevated adiponectin. Conversely, having low vitamin D status as defined by a serum 25(OH)D below 44 nmol/L has been associated with a 47% increase in risk for prediabetes relative to concentrations of 81 nmol/L or more in the third U.S. National Health and Nutrition Examination Survey [351].

Vitamin D is involved in insulin secretion and action in an autocrine manner [37]. The 1α-hydroxylase enzyme and vitamin D receptors are expressed in pancreatic β-cells, muscle and adipose tissue [38], reinforcing that 25(OH)D is the ideal biomarker to examine relationships among vitamin D and insulin resistance. Therefore, this study aims to examine whether vitamin D status among Inuit adults is associated with FPG and HOMA-IR after adjustment for geographical region of the arctic, fat mass, waist circumference (WC) or BMI as well as age, sex, marine mammal and fish intakes, and fatty acid status (including RBC saturated fatty acids, RBC omega-3 fatty acids and RBC trans fatty acids). As a secondary goal, the study aimed to identify other factors that contribute to insulin resistance including leptin and adiponectin.

Subjects and Methods

Participants and data collection

Data was obtained from a cross-sectional survey of Inuit residing in 36 Arctic communities in the late summer and early fall of 2007 (August-September) and 2008 (August-

October). The survey included all communities in three jurisdictions: Inuvialuit Settlement Region (ISR), Nunavut and Nunatsiavut. The communities recruited for the study were located between 54° 10' and 74° 43' north. Details of the IPY-IHS survey are published in full elsewhere [22]. In brief, stratified random sampling was used to select households where communities were strata and where homes were randomly selected using either a computer random generation of numbers or a random digit table [10]. The household participation rate was 68% with 2595 male and non-pregnant female adults included in the main survey.

Study Design

The primary outcomes were FPG and HOMA-IR. Independent variables for both primary outcomes included serum 25(OH)D, RBC fatty acids, both marine mammal and fish intakes, and in subgroup adiponectin and leptin. Covariates for analysis included obesity markers, age, sex and region. Inclusion criteria in this analysis involved male or female adults without DM2 and with data available for all studied variables. In-line with the objective to study prediabetes, participants with DM2 (n= 210) were excluded from this analysis. Specific exclusion criteria on the basis of DM2 were: 1) if a participant had a prior diagnosis of DM2; 2) if the survey results for FPG exceeded 7 mmol/L; or 3) in a sub-group of the population undergoing oral glucose tolerance test (OGTT) if 2-h plasma glucose exceeded 11.1 mmol/L [214]. Of the 2002 adults without prior or newly diagnosed DM2, data for the present analysis was limited to 1418 Inuit adults 18 years of age or older with a complete set of study variables. A subsample (n=1250) was included on the basis of values being available for leptin and adiponectin. Ethics for the study was approved from McGill University, Faculty of Medicine Research Ethics Board, the Nunavut Research Institute and the Aurora Research Institute. Signed informed consent was obtained from each participant [10].

Dietary assessment

Data on dietary habits were collected from one 24-h recall and a food frequency questionnaire (FFQ) [352] by trained bilingual (English and Inuit dialects) interviewers. The 24-h dietary data was collected using the 5-stage multiple-pass technique [69]. Portion sizes were estimated by food model kits (Santé Quebec). The dietary information was entered into CANDAT Software (Godin London) and nutrient intake generated according to the 2007b Canadian Nutrient File [353]. Nutrient content of food items that were not located in the Canadian Nutrient File were imported from an additional School of Dietetics and Human Nutrition in-house food file. Food labels, recipes and other resources such as nutrient values from the U.S. Department of Agriculture were used to estimate nutrient composition that was not otherwise available. The FFQ was customized to capture information within the past year about Inuit traditional food (TF) items that are abundant in regions of ISR, Nunavut and Nunatsiavut [352]. Inuit adults were asked about how often in the last year they consumed (in season or off season) TF items from a list of 47 items, or how often they consumed in the last month from a list of 7 store bought items. Participants were given pictures to quantify the usual serving size. Data for frequency of TF intakes was expressed as total TF, total marine mammals or total fish per day.

Clinical assessment

A portable stadiometer (Road Rod 214 Portable stadiometer, Seca, Maryland, USA) was used to measure height to the nearest 0.1 cm. Body weight was recorded to the nearest 0.1 kg and 0.4 kg was subtracted to account for clothes; body fat % was measured using the same scale (Tanita TBF-300GS with goal setter, Tanita Corporation of America Inc. Arlington Height, Illinois). Body mass index (BMI) was then calculated. WC was measured to the nearest 0.1 cm using a cloth retractable measuring tape (ERP, Laval, Quebec) [353].

Biochemical analysis

Samples for FPG and serum insulin were kept at 4ºC. For FPG, plasma was separated and frozen at -80ºC, then assayed for glucose by Glucose Hexokinase II method [354]. The glucose assay was conducted by Nutrasource Diagnostics, Guelph, ON. Normal FPG was defined as < 5.6 mmol/L and IFG was defined by values ≥ 5.6 mmol/L [198]. Analysis for serum insulin was performed at Institut Universitaire de Cardiologie et de Pneumologie de Québec (Québec City, QC) using a Roche Modular Analytics E170 platform and electrochemiluminescent immuno assay. The interassay CV of control samples was 2.4% and 2.7% at 31.5 and 80.1 μIU/mL levels, respectively. Then HOMA-IR was calculated as a proxy measure of insulin resistance during fasting [355,356]: HOMA-IR = fasting serum insulin (μIU/mL) * fasting plasma glucose (mmol/L) /22.5.

Serum 25(OH)D was measured as it reflects both endogenous and exogenous vitamin D sources [357]. Serum total 25(OH)D was measured using the LIAISON total 25(OH)D chemiluminescent assay (Diasorin Inc, Stillwater, MN, USA) at McGill University [10]. The inter-assay and intra-assay coefficient of variation (CV) % were 4.5 and 11.1% for the low 25(OH)D control (38.2 nmol/L) and 6.2 and 5.3% for high control (127.2 nmol/L), respectively. The laboratory that performed the measurement for 25(OH)D was certified by the Vitamin D External Quality Assessment Scheme for the year 2009-2010 which reflects that at least 80% of the results in this report are within 30% of all laboratory trimmed mean [357].

Serum adiponectin was measured using a Meso Scale Discovery Multi-Array Assay (MSD cat#K151BXC) System with a detection limit of 0.005 ng/mL. Leptin was measured in serum using a manual sandwich ELISA assay (Linco cat# EZHL-80SJK, Linco Research, St Charles, Missouri) with a detection limit of 0.5 ng/mL. For adiponectin, the intra-assay and inter-assay CV were 5% and 15%, respectively, whereas for leptin, the intra-assay and inter-

assay CV were < 10%. All of these analyses were conducted by Nutrasource Diagnostics, Guelph, ON [353].

Fatty acid (FA) status was examined using RBC membrane FA expressed as percent of total FA [353] using the methodology of Folch et al. [358]. Fatty acid methyl esters were prepared using standard techniques [359] with boron trichloride-methanol to reduce artifact formation [360] and separated on a Varian 3400 GLC (Palo Alto, CA) with a 60-m DB-23 capillary column (0.32 mm internal diameter). Red blood cell content of saturated fatty acid (RBC-Sat) represents fatty acids from 14:0 to 24:0. RBC-Omega-3 represents all of the n-3 FA from 18:3 n-3 to 22:6 n-3. Trans fatty acid of RBC (RBC-Trans FA) were separated with a 100-m Supelco SP-2560 capillary column (0.23-mm internal diameter) [69]. Fasting serum total cholesterol, low density lipoprotein (LDL) and triglyceride (TG) concentrations were determined using enzymatic colorimetric tests (Nutrasource Diagnostics, Guelph, ON) [361]. LDL cholesterol was then calculated [336].

Statistical analysis

Continuous variables were checked for normality using Kolmogorov–Smirnov, Shapiro-Wilk and Anderson Darling tests. Descriptive statistics were calculated as means and proportions for the data, or medians (inter-quartile range; IQR) if not normally distributed. Medians were compared using Wilcoxon two-sample tests. Mixed model ANOVA was used to calculate adjusted least square means for study outcomes in different regions with differences among arctic regions identified using Bonferroni post hoc tests. Throughout the analyses, HOMA-IR was log transformed. The hypothesis that both serum 25(OH)D and adiponectin are inversely associated with insulin resistance and FPG, whereas leptin is positively associated with insulin resistance and FPG was tested using multiple linear regression to identify correlates of diabetes risk factors; model 1, was adjusted for age, sex, BMI, arctic region (Nunavut, ISR and

Nunatsiavut), marine mammal intake, fish intake, RBC-Sat, RBC-Trans FA and RBC-Omega-3 and the interaction between serum 25(OH)D with BMI. The same model was repeated using WC instead of BMI (model 2) and then using fat mass percent (FM%) instead of BMI (model 3). The analysis was repeated for FPG using the three models. Serum 25(OH)D was evaluated per 30 nmol/L increments which was selected on the basis of being almost equal to one standard deviation [362]. Total TF was explored, but not included. In the subsample (n=1250), both adiponectin and leptin were entered in the studied regression models for both FPG and HOMA-IR. When interaction terms were significant, associations between FPG and HOMA-IR in relation to serum 25(OH)D were checked in subgroups of BMI, WC and FM%. Final regression models were checked for residual distribution and normality, influential points, and co-linearity using tolerance which is 1/variance inflation factor. Data was analyzed using statistical analysis software (SAS 9.4, SAS Institute Inc., Cary, NC., USA). Relationships were considered significant if the *P*-value was < 0.05, after adjustment for multiple comparisons where appropriate.

Results

The present analysis involves 1418 Inuit adults from 3 arctic regions: Nunavut (n=1032), Inuvialuit Settlement Region (n=194) and Nunatsiavut (n=192) without a diagnosis of DM2 and a subgroup (n=1250) with adiponectin and leptin measurements. Median age was 39 y (IQR: 29, 49), BMI 27.8 kg/m^2 (IQR: 23.9, 32.3), 25(OH)D 50.1 nmol/L (IQR: 31.2, 73.4), FPG 4.9 mmol/L (IQR: 4.6, 5.2) and HOMA-IR 1.64 (IQR: 1.08, 2.59). Distributions and variation across age for FPG, HOMA-IR and serum 25(OH)D are shown in Figure 4.1.

Regional differences were noted (Table 4.1). With Nunavut as the reference, in Inuvialuit Settlement Region all three measures of obesity (BMI, WC, FM%) were higher and on

average participants consumed more dietary vitamin D, Ca and total energy. Lower intakes of TF, including marine mammals, was observed with no difference in fish intake. Moreover, higher FPG and HOMA-IR were noted. In addition, higher RBC-Sat and RBC-Trans FA, TG, cholesterol and leptin, but lower RBC-Omega-3, HDL and adiponectin were observed. In Nunatsiavut, WC was higher than Nunavut. In Nunatsiavut, no differences in dietary vitamin D, Ca, total energy, carbohydrate and fat were observed as compared to Nunavut. However, lower total intakes of TF and marine mammals and higher fish and protein intakes were observed in Nunatsiavut relative to Nunavut.

With regard to biomarkers (Table 4.1), RBC-Omega-3, TG and cholesterol were higher in Nunatsiavut than Nunavut. Both RBC-Sat and RBC-Trans FA were lower in Nunatsiavut than Nunavut. FPG, HOMA-IR, HDL, LDL, leptin and adiponectin were not different between Nunatsiavut and Nunavut. On the other hand, relative to Nunatsiavut, Inuvialuit Settlement Region had higher WC, FPG, HOMA-IR, serum TG and lower HDL. With regard to dietary markers vitamin D intake, marine mammal, RBC-Sat and RBC-Trans were higher in Inuvialuit Settlement Region, whereas RBC-Omega-3 was lower than Nunatsiavut. Regional differences were not observed in 25(OH)D with average values above 50 nmol/L in all three regions.

Within the sample of 1418, 10.9% (95% CI: 9.3, 12.6) adults had IFG. Adults with IFG $\geq$ 5.6 mmol/L (Table 4.2) were compared to those below this cut-point. The percent of males with IFG was higher than the percent of males with normal FPG. Those with IFG were older, had higher BMI, WC, FM% and higher intakes of dietary vitamin D, percent of energy as protein and carbohydrate, consumed more marine mammals corresponding with higher serum 25(OH)D. With regard to biomarkers, HOMA-IR was higher with IFG, serum lipids, RBC FA except RBC-Trans FA and adipokines levels were different between adults with and without IFG.

In a multiple regression model that controlled for BMI, region and the expected interaction term BMI* 25(OH)D, serum 25(OH)D and RBC-Trans FA were inversely associated with HOMA-IR. Using WC in place of BMI and controlling for sex and the interaction between WC and 25(OH)D, 25(OH)D and RBC-Trans FA were also inversely associated with HOMA-IR. When FM% was explored while controlling for age, sex and the interaction between FM% and 25(OH)D, the same two factors (25(OH)D and RBC-Trans FA) emerged as inversely associated with HOMA-IR. On the other hand, marine mammal was positively related to HOMA-IR in all models. The following did not enter in any of the models as contributing to explaining the variance: RBC-Sat, RBC-Omega-3, fish intakes (Table 4.3). In the subgroup analysis that included leptin and adiponectin, leptin positively associated and adiponectin negatively associated with HOMA-IR in all three models. Interestingly, RBC-Trans FA and 25(OH)D remained inversely associated with HOMA-IR, in all three models. Marine mammal was positively related to HOMA-IR in models adjusted for WC and FM% (Table 4.4).
When data was categorized by BMI groups, serum 25(OH)D was inversely related to HOMA-IR only when BMI $\geq$ 25 kg/m^2 (β= -0.153,95% CI: -0.241 to -0.065, *P*= 0.0006), but was not significantly related to insulin resistance in groups with BMI < 25 kg/m^2 (β= -0.169,95% CI: -0.285 to -0.053, *P*= 0.122). There was no significant associations between HOMA-IR and serum 25(OH)D in various subgroups of WC and FM%.

With FPG as the dependent variable (Table 4.5) in a model that adjusted for BMI, region, age and sex, neither 25(OH)D or any RBC-FA were associated with FPG, whereas marine mammal intake was positively associated with FPG. The same observations were noted when using WC or FM% in place of BMI. Upon inclusion of adiponectin and leptin in the model (Table 4.6), RBC-Omega-3 emerged as inversely associated with FPG whereas leptin and marine

mammals entered as positively associated with FPG. Similar observations were noted when using WC and FM% as the indicators of adiposity. There were no significant associations between FPG and serum 25(OH)D in various subgroups of BMI, WC and FM%.

Discussion

The inverse association between insulin resistance and vitamin D status stimulated the present investigation of the dietary and socio-physiological determinants of insulin resistance and FPG among Inuit. Reflecting the diversity among Inuit communities, we observed differences in dietary markers of TF and market food and insulin resistance in three distinct regions in the Canadian arctic which may indicate differences in access to food both market and TF. The noted regional differences could be related to varying access to both TF and market food among the Inuit groups included in this study. The prevalence of IFG was modest (10.9%) and coincident with older age, dyslipidemia and evidence of a more traditional lifestyle marked by greater intakes of TF and higher omega-3 FA status. However, after adjusting for age and indicators of obesity, higher omega-3 FA status was protective against higher FPG, suggesting that maintenance of a traditional lifestyle is beneficial to health. Higher vitamin D status was protective against elevated HOMA-IR, especially after adjusting for overweight and obese BMI.

Apart from being a collective marker of fish and marine food intake [118], serum 25(OH)D may have an inverse association with HOMA-IR as evidenced in this study. To the best of the authors' knowledge, this relationship has not been reported in any Inuit population. The results are in contrast with a report on First Nations in eastern Canada, a demographic group similar to American Indians, where the relationship between serum 25(OH)D and HOMA-IR in adults with IFG or normal glucose tolerance was not significant, even when the model was adjusted for obesity [17]. However, another report on First Nations women in western Canada found a

significant relationship between HOMA-IR and 25(OH)D [50]. In addition, the significant association between insulin resistance and secretion with serum 25(OH)D was further confirmed in another study among First Nation adults, but unlike our study there was an inverse association between each standard deviation increment in serum 25(OH)D and dysglycemia, probably because the study did not adjust for BMI [363]. Although the study by del Gobbo et al [17] adjusted for alcohol use, education and smoking, they did not include other measures of traditional diets, or other dietary factors that could interfere with insulin resistance such as trans-FA or saturated FA. The study by Weiler et al [50] accounted for age and adiposity and included both First Nations and Caucasian women, whereas the study by Mansuri et al. did not adjust for TF intake of First Nations [363]. Hence, this study is the first study among Aboriginal people that clarifies the role of vitamin D status taking into consideration the unique dietary and regional aspects of Inuit life. This highlights the fact that Inuit are not a homogenous group given all the differences in metabolic and dietary markers in the arctic regions studied.

Obesity is a strong modulator of the 25(OH)D-insulin resistance relationship among both First Nations and Inuit ethnicities [17]. It has been shown previously that in obese people vitamin D is sequestered in adipose tissue [364], limiting its availability to exert its biological function. In this study, adjustment for FM%, WC, or BMI did not eliminate the inverse association between insulin resistance and vitamin D status. In obese African American females, serum 25(OH)D concentration of 37.5 nmol/L or greater was needed to see a positive association with insulin sensitivity [365], whereas a level as high as 81 nmol/L was associated with improved insulin resistance even after adjustment for BMI in the U.S. Third National Health and Nutrition Examination Survey [228]. Collectively, these studies imply that achieving healthy vitamin D status, even in the context of overweight status or obesity, modulates insulin resistance.

The etiology and the onset of insulin resistance is complex and highly influenced by diet and obesity. In this study, RBC-Trans FA which mainly comes from hydrogenated vegetable oil and is also found in ruminant animals [366], was inversely associated with insulin resistance, but not with FPG. Of the TF variables tested, only intake of marine mammals associated with 0.2 mmol/L increments in FPG, with no relationship to insulin resistance observed in the present dataset. In accordance with this finding, a study among Inuit found that higher TF was associated with higher IFG [367]. In another study, dietary trans fat in Alaskan Inuit was not associated with insulin concentrations [368] whereas HOMA-IR was positively associated with intake of trans fat (β= 1.42, P= 0.032) [369]. Furthermore, there is data to support that dietary trans fats impair insulin sensitivity in adults with DM2 [370]. Insulin resistance could be accompanied by β-cell dysfunction which could involve β-cell dedifferentiation or transdifferentiation [371]. Whether vitamin D status or trans fats are implicated in β-cell dysfunction in Inuit requires further study.

Insulin resistance manifests eventually as elevated FPG. Many studies have observed an inverse relationship between 25(OH)D and FPG [351,372,373], however, the present results did not reach significance. In part, this could be ascribed to exclusion of Inuit with DM2. Similar to our finding, the Canadian Health Measures Survey did not find any association between FPG and serum 25(OH)D in adults (16-79 y) after adjustment for WC [374]. Previously, in Inuit adults it was shown that having high WC and TG were very strongly associated with elevated FPG (OR 4.3; 95% CI: 2.4 to 7.5, P< 0.05) [22] and that WC was inversely associated with 25(OH)D [10]. Thus the lack of association between FPG and vitamin D status could be related to the fact that the association between obesity and FPG could have overwhelmed the relationship between FPG and serum 25(OH)D.

Vitamin D is associated with increases in adiponectin in epidemiological studies [375] and suppresses leptin in cell culture [179], suggesting that this could be a possible mechanism of action of vitamin D on insulin resistance. Leptin relates to glucose homeostasis through regulation of food intake [376], energy metabolism [377] and glucose metabolism [378]. Adiponectin is also involved in glucose metabolism through modulation of inflammation [379], stimulation of β-oxidation, a reduction in free FA in the plasma and down regulation of expression of proteins that are involved in lipid biosynthesis [182,380]. In Canada, people of Aboriginal ethnicity seem to have elevated concentrations of both leptin and adiponectin [381]. For example, the increase in HOMA-IR for each unit decrease in adiponectin was higher ($P< 0.01$) among First Nations (American Indian) relative to the white Chinese Canadians, indicates a possible ethnic divergence in the role of adipokines in insulin resistance [381]. Regarding leptin, similar to our study, elevated insulin resistance was associated with leptin regardless of body fat in American men 25-40 y with normoglycemia [51]. In fact, the relationship between insulin and leptin seems to be reciprocal as was shown in animal studies and human studies with insulin concentration correlating positively with leptin [382]. In another study, leptin was found to be significantly lower in lean adults who were insulin sensitive relative to those with insulin resistance (1.9 ± 0.4 versus 4.35 ± 1.21 μg/L, $P< 0.05$) [51]. Consequently, the adipokine profile that is reflective of obesity contributes to insulin resistance.

This study provides insight regarding vitamin D status and biomarkers associated with glucose metabolism and TF. It is, however, not without limitations including under-representation of males and its cross-sectional design which does not allow identification of true causal relationships. In addition, the 24-h dietary recall was conducted only once due to logistics of the large epidemiological study, hence the intake of food may not represent usual intakes.

This was overcome in part by using biomarkers to reflect longer term intakes of vitamin D and TF, however, blood samples were restricted to only one period (summer/fall) and thus variation in status throughout the year cannot be concluded from the present data. In addition, no data in physical activity were presented in this analysis.

In summary, serum 25(OH)D was inversely associated with HOMA-IR regardless of age and obesity, suggesting that increasing vitamin D status is protective against insulin resistance. At the same time there was no significant association between FPG and serum 25(OH)D. Both adipokines, including adiponectin (inversely) and leptin (positively), associated with HOMA-IR highlighting the role of obesity in insulin resistance. Other components of TF could be protective from high plasma glucose such as omega-3 FA. In conclusion, intake of TF among Inuit should be promoted as rich sources of vitamin D and omega-3 FA. Higher vitamin D status is protective from insulin resistance and greater omega-3 FA status appears to be protective from high plasma glucose.

TABLE 4.1 A comparison of the study variables among geographical regions.

Variable	Nunavut (n=1032)	Inuvialuit Settlement Region (n=194)	Nunatsiavut (n=192)
Age (y)	39.3 ± 13.8[a]	42.1 ± 14.1[b]	42.5 ± 13.5[b]
Male %	37.6%	28.9%	39.6%*
Female %	62.4%	71.1%	60.4%
Obesity			
BMI (kg/m^2)	28.1 ± 6.3[a]	30.7 ± 6.6[b]	29.2 ± 5.5[ab]
Waist Circumference (cm)	92.0 ± 15.4[a]	102.5 ± 16.1[c]	96.6 ± 13.7[b]
FM %	29.9 ± 10.8[a]	35.5 ± 10.2[b]	33.0 ± 9.8[ab]
24-h Dietary Recall (intake/d)			
Vitamin D (µg)	5.8 ± 7.8[a]	7.5 ± 10.1[b]	4.9 ± 5.7[a]
Calcium (mg)	491.0 ± 427.5[a]	608.6 ± 363.0[b]	538.1 ± 332.9[ab]
Total Energy (kcal)	2133.9 ± 1186.7[a]	2435.0 ±1295.8[b]	2158.5 ± 1086.0[ab]
% Energy as Protein	20.8 ± 11.5[b]	19.9 ± 9.1[ab]	18.7 ± 9.2[a]
% Energy as CHO	47.2 ± 17.5	45.7 ± 14.6	47.8 ± 12.2
% Energy as fat	31.0 ± 11.8	32.9 ± 10.2	31.9 ± 9.4
FFQ (frequency/d)			
Total TF	1.09 ± 0.91[b]	0.88 ± 0.79[a]	0.90 ± 0.77[a]
MM	0.29 ± 0.34[c]	0.19 ± 0.27[b]	0.09 ± 0.19[a]
Fish	0.21 ± 0.26[a]	0.24 ± 0.28[ab]	0.28 ± 0.27[b]
Biomarkers			
FPG (mmol/L)	4.9 ± 0.5[a]	5.1 ± 0.5[b]	4.9 ± 0.5[a]
HOMA-IR	2.01 ± 1.50[a]	2.68 ± 2.02[b]	2.19 ± 1.50[a]
RBC-Omega-3 (%)	5.8 ± 3.2[b]	2.5 ± 2.6[a]	6.8 ± 2.2[c]
RBC-Sat (%)	43.1 ± 4.9[b]	50.1 ± 5.6[c]	40.8 ± 3.4[a]
RBC-Trans FA (%)	1.4 ± 0.7[b]	1.6 ± 0.6[c]	1.2 ± 0.6[a]
25(OH)D (nmol/L)	55.7 ± 33.2	53.8 ± 22.6	58.4 ± 26.8
TG (mmol/L)	1.3 ± 0.7[a]	1.8 ± 1.3[c]	1.5 ± 0.9[b]
HDL (mmol/L)	1.5 ± 0.4[b]	1.4 ± 0.4[a]	1.50 ± 0.5[b]
LDL (mmol/L)	2.8 ± 1.0	3.0 ± 0.9	3.0 ± 0.9
Cholesterol (mmol/L)	4.9 ± 1.1[a]	5.1 ± 1.0[b]	5.2 ± 1.0[b]
Adiponectin (µg/L)	11.5 ± 8.5[b]	9.8 ± 5.7[a]	11.6 ± 5.4[ab]
Leptin (µg/L)	18.4 ± 19.5[a]	25.9 ± 22.9[b]	17.9 ± 15.7[a]

* $P < 0.05$, values with different letters are statistically significant; a<b<c. Data are expressed as means± SD. .
BMI, body mass index; CHO, carbohydrate; FM, fat mass; FPG, fasting plasma glucose; 25(OH)D, 25-hydroxyvitamin D; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low density lipoprotein; MM, marine mammal; RBC-Omega-3, red blood cell content of omega-3 fatty acids; RBC-trans FA, Trans fatty acids in red blood cells; RBC-Sat, red blood cell content of saturated fatty acids; TG, triglycerides; WC, waist circumference.

TABLE 4.2 Descriptive characteristics of the study participants according to impaired fasting glucose status

	Impaired Fasting Glucose	
Variable	**No (n=1263)**	**Yes (n=155)**
Male %	35.5 (32.8, 38.2)	46.4 (38.6, 54.3)
Female %	64.5 (61.8, 67.2)	53.6 (45.8, 61.5)**
	Median (IQR)	
Age (y)	38 (28, 47)	50 (41, 59)***
Anthropometry		
BMI (kg/m^2)	27.5 (23.6, 31.9)	30.9 (25.7, 35.2)***
WC (cm)	92.0 (81.0, 104.0)	102.8 (87.0, 111.0)***
FM (%)	30.7 (22.3, 39.2)	35.0 (25.9, 43.3)***
24-recall (intake/d)		
Vitamin D (µg)	2.96 (1.30, 7.20)	3.98 (2.07, 8.67)**
Calcium (mg)	406.0 (239.7, 654.4)	413.0 (258.9, 636.1)
Total Energy (kcal)	1971.0 (1404.9, 2722.1)	1898.0 (1218.3, 2613.9)
% Energy as Protein	17.5 (12.5, 25.4)	20.6 (15.6, 29.5)***
% Energy as CHO	48.3 (36.6, 58.8)	44.1 (30.4, 54.0)**
% Energy as fat	30.9 (24.1, 37.5)	30.6 (23.4, 38.5)
FFQ (frequency/d)		
Total Traditional Food	0.79 (0.31, 1.50)	0.89 (0.42, 1.50)
MM	0.08 (0.02, 0.35)	0.21 (0.04, 0.43)**
Total Fish	0.10 (0.02, 0.29)	0.14 (0.03, 0.32)
Biomarkers		
FPG (mmol/L)	4.9 (4.6, 5.1)	5.9 (5.7, 6.1)***
HOMA-IR	1.5 (1.0, 2.4)	3.1 (2.0, 4.8)**
RBC-Sat (%)	42.2 (39.8, 45.7)	43.4 (41.0, 47.1)**
RBC-Omega-3 (%)	4.9 (3.2, 7.2)	6.1 (2.9, 8.8)**
RBC-Trans (%)	1.3 (0.9, 1.7)	1.2 (0.8, 1.7)
25(OH)D (nmol/L)	48.2 (30.2, 71.1)	61.9 (45.2, 88.6)***
TG (mmol/L)	1.2 (0.8, 1.6)	1.5 (1.0, 2.1)***
LDL (mmol/L)	2.7 (2.2, 3.4)	3.2 (2.6, 4.0)***
HDL (mmol/L)	1.4 (1.2, 1.7)	1.3 (1.1, 1.6)**
Cholesterol (mmol/L)	4.9 (4.2, 5.5)	5.4 (4.6, 6.2)***
Adiponectin (µg/L)	9.9 (6.4, 14.8)	8.8 (5.4, 14.1)
Leptin (µg/L)	13.1 (4.7, 27.0)	15.6 (5.6, 33.8)*

* * $P< 0.05$, ** $P< 0.01$, *** $P< 0.0001$. IQR, interquartile range; BMI, body mass index; CHO, carbohydrate; FM, fat mass; FPG, fasting plasma glucose; 25(OH)D, 25-hydroxyvitamin D; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low density lipoprotein; MM, marine mammal; RBC-Omega-3, red blood cell content of omega-3 fatty acids; RBC-Trans FA, Trans fatty acids in red blood cells; RBC-Sat, red blood cell content of saturated fatty acids; TG, triglycerides; WC, waist circumference.

TABLE 4.3 Multiple linear regression association between anthropometric, demographic, fatty acids and markers of TF variables and HOMA-IR

HOMA-IR (n=1418)			
	Model 1 (BMI) **β (95% CI)**	**Model 2 (WC)** **β (95% CI)**	**Model 3 (FM%)** **β (95% CI)**
BMI (kg/m^2)	0.023 (0.019, 0.027)***		
WC (cm)		0.009 (0.008, 0.010)***	
FM%			0.016 (0.014, 0.018)***
Inuvialuit Settlement Region	0.041 (0.008, 0.074)*	-0.001 (-0.038, 0.036)	0.028 (-0.009, 0.065)
Nunatsiavut	0.032 (-0.001, 0.065)	0.013 (-0.022, 0.048)	0.005 (-0.030, 0.040)
Nunavut	reference	reference	reference
Age (y)	0.0002 (-0.0008, 0.0012)	-0.001 (-0.002, 0.001)	-0.002 (-0.003, -0.001)**
Sex (M vs. F)	0.006 (-0.017, 0.029)	-0.024 (-0.048, -0.0005)*	0.180 (0.150, 0.210)***
25(OH)D (per 30 nmol/L)	-0.058 (-0.111, -0.005)*	-0.079 (-0.15, -0.008)*	-0.040 (-0.073, -0.007)*
RBC-Sat (%)	0.002 (0.0001, 0.004)	0.002 (0.00004, 0.004)	0.001 (-0.002, 0.004)
RBC-Omega-3 FA (%)	-0.002 (-0.008, 0.004)	-0.002 (-0.008, 0.004)	-0.003 (-0.009, 0.003)
RBC-Trans FA (%)	-0.023 (-0.039, -0.007)**	-0.019 (-0.037, -0.001)*	-0.020 (-0.038, -0.002)*
FFQ (frequency/day)			
MM	0.044 (0.007, 0.081)*	0.046 (0.009, 0.083)*	0.051 (0.014, 0.088)**
Fish	-0.026 (-0.069, 0.017)	-0.023 (-0.066, 0.020)	-0.020 (-0.065, 0.025)
BMI*25(OH)D	0.002 (0.0002, 0.004)*		
WC*25(OH)D		0.001 (0.0002, 0.002)*	
FM%*25(OH)D			0.001 (0.0001, 0.002)*
R-squared	0.409	0.395	0.389

Data is expressed in form of β (95% CI). * * P< 0.05, ** P < 0.01, *** P < 0.0001. BMI, body mass index; FFQ, food frequency questionnaire; F, female; FM, fat mass; HOMA-IR, homeostatic model of insulin resistance; 25(OH)D, 25-hydroxyvitamin D; M, male; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference. HOMA-IR was log transformed prior to analysis.

TABLE 4.4 Multiple linear regression association between anthropometric, demographic, fatty acids and markers of TF, adipokines variables and HOMA-IR.

HOMA-IR (n=1250)			
	Model 1(BMI) **β (95% CI)**	**Model 2(WC)** **β (95% CI)**	**Model 3(FM%)** **β (95% CI)**
BMI (kg/m^2)	0.014 (0.010, 0.018)***		
WC (cm)		0.005 (0.003, 0.007)***	
FM%			0.009 (0.007, 0.011)***
Inuvialuit Settlement Region	0.037 (-0.0002, 0.074)	0.010 (-0.027, 0.047)	0.027 (-0.010, 0.064)
Nunatsiavut	0.041 (0.006, 0.076)*	0.030 (-0.005, 0.065)	0.024 (-0.011, 0.059)
Nunavut	reference	reference	reference
Age (y)	0.001 (-0.0001, 0.002)	0.001 (-0.0002, 0.002)	-0.0002 (-0.001, 0.001)
Sex (M vs. F)	0.063 (0.032, 0.094)***	0.054 (0.023, 0.085)**	0.190 (0.160, 0.220)***
25(OH)D(per 30 nmol/L)	-0.061 (-0.117, -0.005)*	-0.087 (-0.163, -0.011)*	-0.042 (-0.079, -0.005)*
RBC-Sat (%)	0.001 (-0.002, 0.004)	0.001 (-0.003, 0.005)	0.001 (-0.003, 0.005)
RBC-Omega-3 FA (%)	-0.003 (-0.009, 0.003)	-0.003 (-0.007, 0.001)	-0.004 (-0.010, 0.002)
RBC-Trans FA (%)	-0.022 (-0.040, -0.004)*	-0.019 (-0.037, -0.001)*	-0.019 (-0.037, -0.001)*
FFQ (frequency/day)			
MM	0.038 (-0.001, 0.077)	0.041 (0.002, 0.080)*	0.044 (0.005, 0.083)*
Fish	-0.009 (-0.056, 0.038)	-0.006 (-0.053, 0.041)	-0.006 (-0.053, 0.041)
Adiponectin (µg/L)	-0.003 (-0.0050, -0.001)***	-0.003 (-0.005, -0.001)**	-0.004 (-0.006, -0.002)***
Leptin (µg/L)	0.003 (0.001, 0.005)***	0.004 (0.003, 0.005)***	0.004 (0.003, 0.005)***
BMI*25(OH)D	0.002 (0.00004, 0.004)*		
WC*25(OH)D		0.001 (0.0002, 0.002)*	
FM%*25(OH)D			0.001 (-0.0001, 0.002)*
R-squared	0.419	0.419	0.417

Data is expressed in form of β (95% CI). * * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$. BMI, body mass index; FFQ, food frequency questionnaire; F, female; FM, fat mass; HOMA-IR, homeostatic model of insulin resistance; 25(OH)D, 25-hydroxyvitamin D; M, male; MM, marine mammal; RBC-Omega3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference. HOMA-IR was log transformed prior to analysis.

TABLE 4.5 Multiple linear regression association between anthropometric, demographic, fatty acids and markers of TF variables and FPG

FPG (n=1418)			
	Model 1(BMI) **β (95% CI)**	**Model 2(WC)** **β (95% CI)**	**Model 3(FM%)** **β (95% CI)**
BMI (kg/m^2)	0.014 (0.012, 0.016)***		
WC(cm)		0.006 (0.002, 0.010)**	
FM%			0.009 (0.005, 0.013)**
Inuvialuit Settlement Region	0.11 (0.035, 0.19)**	0.087 (0.009, 0.165)*	0.11 (0.03, 0.19)*
Nunatsiavut	-0.023 (-0.095, 0.049)	-0.036 (-0.108, 0.036)	-0.041 (-0.113, 0.031)
Nunavut	reference	reference	reference
Age (y)	0.013 (0.011, 0.015)***	0.012 (0.010, 0.014)***	0.011 (0.009, 0.013)***
Sex (M vs. F)	0.140 (0.090, 0.190)***	0.120 (0.070, 0. 170)***	0.250 (0.190, 0.310)***
25(OH)D (per 30 nmol/L)	-0.031 (-0.14, 0.08)	-0.032 (-0.18, 0.12)	-0.040 (-0.110, 0.030)
RBC-Sat (%)	0.002 (-0.004, 0.008)	0.002 (-0.004, 0.008)	0.001 (-0.005, 0.007)
RBC-Omega-3 FA (%)	-0.008 (-0.020, 0.004)	-0.008 (-0.020, 0.004)	-0.009 (-0.021, 0.003)
RBC-Trans FA (%)	-0.022 (-0.057, 0.013)	-0.020 (-0.055, 0.015)	-0.019 (-0.054, 0.016)
FFQ (frequency/day)			
MM	0.130 (0.048, 0.210)**	0.130 (0.047, 0.210)**	0.130 (0.050, 0.210)**
Fish	-0.071 (-0.17, 0.025)	-0.069 (-0.165, 0.027)	-0.067 (-0.16, 0.29)
BMI*25(OH)D	0.002 (-0.002, 0.004)		
WC*25(OH)D		0.0006 (-0.001, 0.002)	
FM%*25(OH)D			0.002 (0.0001, 0.004)
R-squared	0.220	0.220	0.220

Data is expressed in form of β (95% CI). * * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$. BMI, body mass index; FFQ, food frequency questionnaire; F, female; FM, fat mass; HOMA-IR, homeostatic model of insulin resistance; 25(OH)D, 25-hydroxyvitamin D; M, male; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA; red blood cell content of trans-fatty acids. TF, traditional food; WC, waist circumference.

TABLE 4.6 Multiple linear regression association between anthropometric, demographic, fatty acids, markers of TF and adipokines variables and FPG (n=1250).

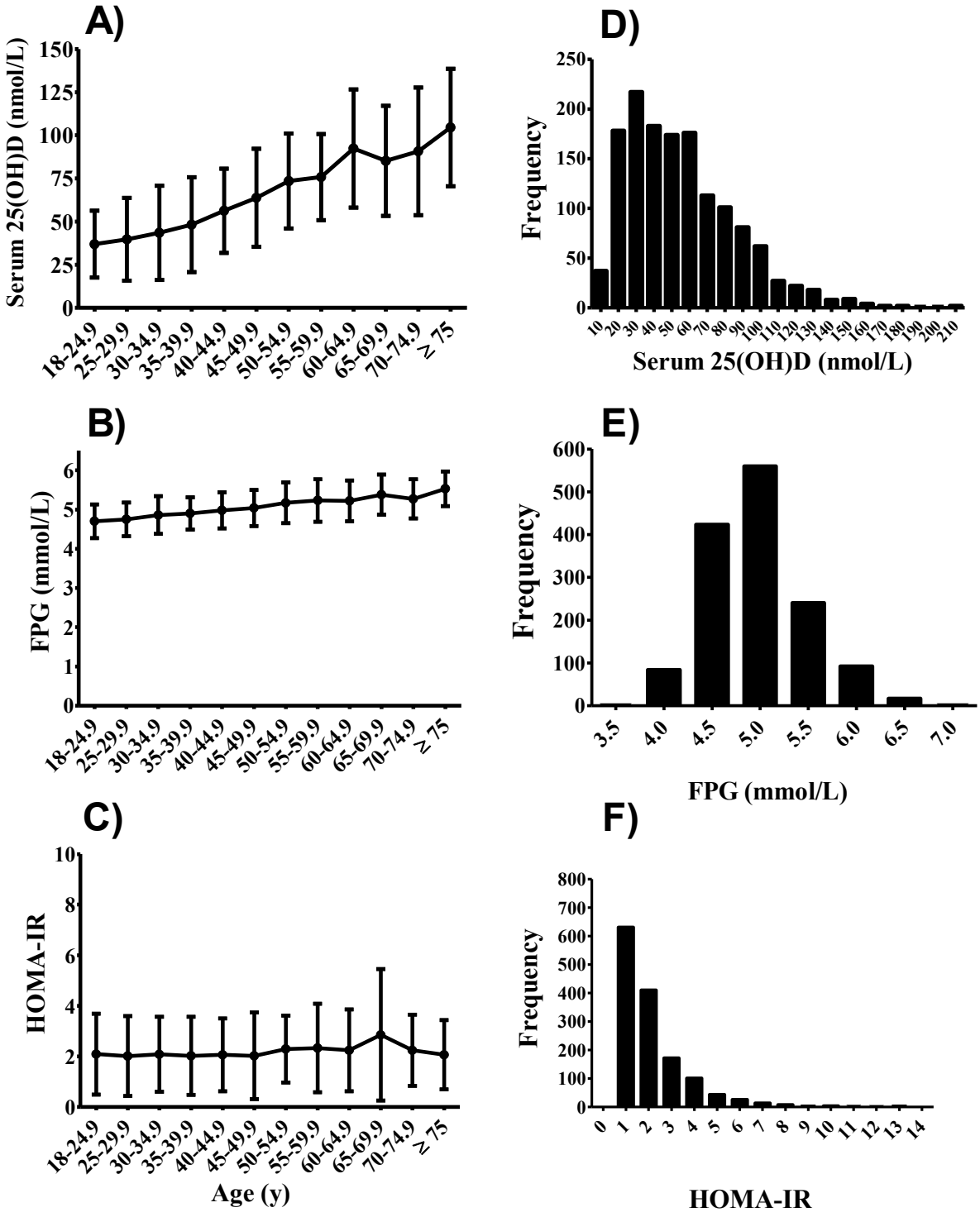
FPG (n=1250)			
	Model 1(BMI) **β (95% CI)**	**Model 2(WC)** **β (95% CI)**	**Model 3(FM%)** **β (95% CI)**
BMI (kg/m^2)	0.007 (-0.003, 0.017)		
Waist (cm)		0.004 (0.0001, 0.008)	
FM%			0.005 (-0.001, 0.011)
Inuvialuit Settlement Region	0.110 (0.032, 0.188)**	0.087 (0.007, 0.167)*	0.097 (0.017, 0.177)*
Nunatsiavut	-0.003 (-0.076, 0.070)	-0.012 (-0.085, 0.061)	-0.015 (-0.089, 0.059)
Nunavut	reference	reference	reference
Age (y)	0.013 (0.011, 0.015)***	0.013 (0.011, 0.015)***	0.012 (0.010, 0.014)***
Sex (M vs. F)	0.190 (0.130, 0.250)***	0.180 (0.120, 0.240)***	0.260 (0.200, 0.320)***
25(OH)D (per 30 nmol/L)	0.010 (-0.11, 0.13)	0.014 (-0.144, 0.172)	-0.006 (-0.084, 0.072)
RBC-Sat (%)	0.0003 (-0.006, 0.006)	0.0001 (-0.006, 0.006)	-0.0002 (-0.006, 0.006)
RBC-Omega-3 FA (%)	-0.013 (-0.025, -0.001)*	-0.013 (-0.025, -0.001)*	-0.013 (-0.025, -0.001)*
RBC-Trans FA (%)	-0.007 (-0.044, 0.030)	-0.005 (-0.042, 0.032)	-0.004 (-0.042, 0.034)
FFQ (frequency/day)			
MM	0.120 (0.037, 0.210)**	0.120 (0.04, 0.200)**	0.130 (0.046, 0.210)**
Fish	-0.060 (-0.158, 0.038)	-0.059 (-0.157, 0.039)	-0.06 (-0.16, 0.004)
Adiponectin (µg/L)	-0.003 (-0.007, 0.001)	-0.003 (-0.007, 0.001)	-0.003 (-0.007, 0.001)
Leptin (µg/L)	0.003 (0.001, 0.005)**	0.003 (0.001, 0.005)**	0.003 (0.001, 0.005)**
BMI*25(OH)D	0.0008 (-0.003, 0.005)		
Waist*25(OH)D		0.0002 (-0.002, 0.002)	
FM%*25(OH)D			0.001 (0.00, 0.002)
R-squared	0.248	0.250	0.251

Data is expressed in form of β (95% CI). * * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$. BMI, body mass index; FFQ, food frequency questionnaire; F, female; FM, fat mass; HOMA-IR, homeostatic model of insulin resistance; 25(OH)D, 25-hydroxyvitamin D; M, male; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference.

Figure Legends

FIGURE 4.1 Key study variables across age A) serum 25(OH)D nmol/L, B) FPG (mmol/L), C) HOMA-IR and histogram distribution of D) serum 25(OH)D nmol/L, E) FPG (mmol/L), f) HOMA-IR. Abbreviations: FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment of insulin resistance; 25(OH)D, 25-hydroxyvitamin D.

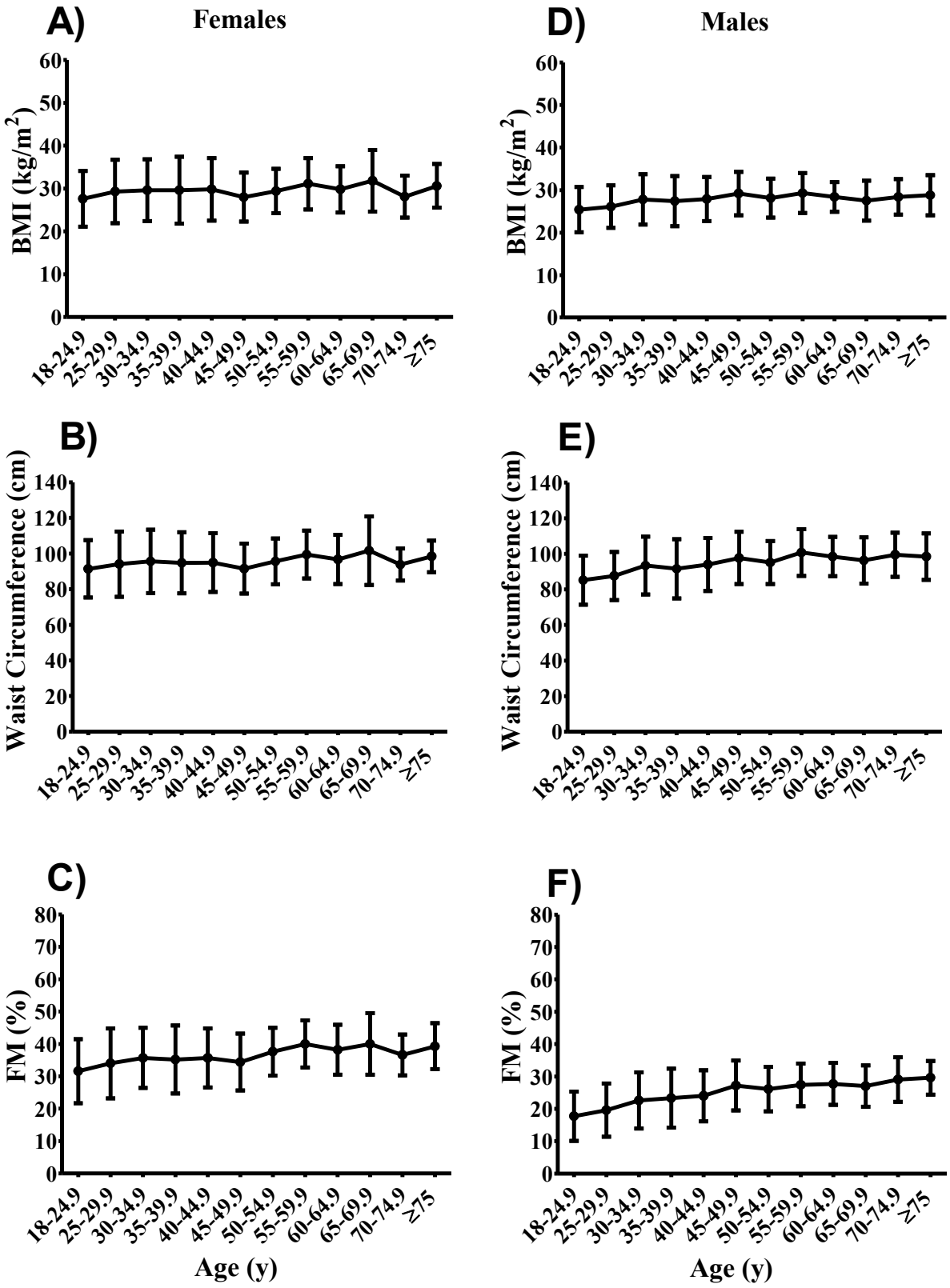
Figure 4.1.


A)
Serum 25(OH)D (nmol/L)
150
125
100
75
50
25
0
18-24.9
25-29.9
30-34.9
35-39.9
40-44.9
45-49.9
50-54.9
55-59.9
60-64.9
65-69.9
70-74.9
≥ 75
D)
Frequency
250
200
150
100
50
0
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210
Serum 25(OH)D (nmol/L)
B)
FPG (mmol/L)
6
5
4
3
2
1
0
18-24.9
25-29.9
30-34.9
35-39.9
40-44.9
45-49.9
50-54.9
55-59.9
60-64.9
65-69.9
70-74.9
≥ 75
E)
Frequency
600
500
400
300
200
100
0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
FPG (mmol/L)
C)
HOMA-IR
10
8
6
4
2
0
18-24.9
25-29.9
30-34.9
35-39.9
40-44.9
45-49.9
50-54.9
55-59.9
60-64.9
65-69.9
70-74.9
≥ 75
Age (y)
F)
Frequency
800
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
HOMA-IR

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE 4.1 Anthropometric variables across age for females, A) BMI (kg/m^2), B) waist circumference (cm), C) FM%. For males, D) BMI (kg/m^2) E) waist circumference (cm), F) FM%. Abbreviations, BMI, body mass index; FM%, fat mass percent.

Supplemental Figure 4.1.



Acknowledgements

Conflicts of interest, none.

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Bridge 3

In the previous chapter it was observed that vitamin D status is inversely associated with HOMA-IR. This relationship remained significant after adjustment for obesity as assessed by BMI, WC and fat mass percent. Both insulin resistance and WC are suggested mechanisms that could contribute to METS [383,384]. Previously, Inuit were protected from the metabolic consequences of obesity [385,386]. While controversial, METS is commonly believed to predict advancement to DM2 and cardiovascular disease [387,388]. Hence, the second study in this thesis attempts to address the association between vitamin D status and traditional food with METS and DM2 in detail including exploration of the association for each individual component of the METS.

Higher Vitamin D status was associated with lower METS [250,389] in various cross sectional studies and contributes to lower risk of DM2 in various prospective epidemiological studies [244,390-392]. However, there is a gap in literature in regard to studies on the relationship between vitamin D status and both METS and DM2 in other ethnic groups besides those of white skin. To the author's knowledge, there were two studies that were performed on the relationship between vitamin D status and METS in Canada, but both of them did not address Aboriginal ethnicity. Moreover, TF intake was associated with favorable TG and HDL [392], but studies on the association between other metabolic outcomes and TF are lacking especially those considering covariates such as fatty acid and regional differences among Inuit. Hence comes the second study in this thesis to address the gap in the literature regarding the association between vitamin D status, traditional food and both METS and DM2 among Inuit living in the Arctic.

CHAPTER 5: Determinants of Metabolic Syndrome and Type 2 Diabetes Mellitus among Inuit: Role of Diet, Obesity and 25-Hydroxyvitamin D.

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Key words, 25-hydroxyvitamin D, metabolic syndrome, traditional food, type 2 diabetes mellitus, Inuit.

ABSTRACT

Background: Higher vitamin D status lowers the risk of metabolic syndrome (METS) and type 2 diabetes mellitus (DM2).

Objective: To test whether increases in vitamin D status, or intake of TF, reduce the odds of METS and DM2 after accounting for covariates.

Methods: Participants (≥18 y) from the cross-sectional 2007-2008 International Polar Year Inuit Health Survey (IPY-IHS) were evaluated. Primary outcomes included METS and DM2 with the independent variables being 25-hydroxyvitamin D (25(OH)D) and TF. Red blood cell fatty acids and demographic variables were explored as covariates. Multivariate logistic regression models were used for estimating the risk of METS and DM2.

Results: Of 1725 adults, 317 (18.4%; 95% CI: 16.6% to 20.2%) had METS with a median age of 39 (IQR 29, 49) y and body mass index (BMI) of 27.1 (23.3, 31.8) kg/m^2. The proportion with METS was higher in both Nunatsiavut (29.8%) and Inuvialuit Settlement Region (32.5%) relative to Nunavut (13.9%). Serum 25(OH)D, (per 30 nmol/L), inversely associated with METS (OR: 0.81; 95% CI: 0.69 to 0.96, *P*= 0.012) as did TF intake (OR: 0.82; 95% CI: 0.69 to 0.97, *P*= 0.020) in a model adjusted for age, sex and BMI kg/m^2. Further adjustment of the models eliminated these relationships. Greater intakes of fish was associated with lower odds of having an elevated LDL/HDL ratio in the fully adjusted model. Higher serum 25(OH)D associated with lower waist circumference, triglycerides and greater high density lipoprotein concentrations. DM2 was present in 8.7% (95 % CI 7.4% to 10.0%, n=1857) with no relationships to serum 25(OH)D or TF; yet age, BMI, waist circumference, FM%, saturated fatty acids and geographical region were important determinants of DM2.

Interpretation: Dyslipidemia was reduced with increasing vitamin D status, but neither 25(OH)D nor TF were associated with DM2 among Inuit. Consumption of TF, especially fish, as a source of vitamin D may offer a culturally acceptable approach to primary prevention of components of METS such as dyslipidemia and high waist circumference whereas interventions targeted at prevention of obesity would be important in the prevention of DM2.

Introduction

Metabolic syndrome (METS) is a cluster of risk factors associated with early stages of DM2 [393,394]. Multiple definitions exist for METS with different threshold values for metabolic risks including: hypertension, hyperglycemia, high triglycerides (TG), low high density lipoprotein (HDL), and a high waist circumference (WC) [395,396]. METS, defined using the National Cholesterol Education Program (NCEP) criteria, is prevalent among 44% of US adults ≥ 50 y and according to the harmonized definition of METS was present in 43% of Canadian adults ≥ 30 y [247,397]. The relative risk of developing DM2 in adults with METS as defined by NCEP was estimated to be 2.99 (95% CI: 1.96 to 4.57, *P*= 0.001) [398]. In fact, factors that are components of METS such as dyslipidemia and visceral obesity often are linked to insulin resistance which contributes to the etiopathology of DM2 [399]. Hence prevention of METS or its components represent an early stage of prevention of DM2.

Low vitamin D status has been associated with METS and DM2 in adults [210,400,401], suggesting a role in the pathological evolution of METS and perhaps progression to DM2. Higher serum 25-hydroxyvitamin D (25(OH)D) significantly reduces the risk of METS among Canadian adults > 30 y (OR= 0.76; 95 CI: 0.62 to 0.93) [320]. In addition, serum 25(OH)D was found to be inversely associated with plasma TG, and positively to lipoprotein lipase in a cross sectional study [402], further implying that vitamin D may be protective against chronic disease. In accordance with this, many observational studies indicate that higher concentrations of plasma 25(OH)D associate with lower odds of DM2 [210,403]. However, the relationship between DM2 and serum 25(OH)D is inconsistent in the literature with notable differences observed by ethnic group [210,404]. Recently, a high prevalence of low vitamin D status was reported among Inuit [10].

Vitamin D is obtained from TF which contain other nutrients [118] that help in the prevention of chronic diseases [405].

The Inuit diet was historically deemed to be protective from cardiovascular diseases (CVD) due to its high omega-3 fatty acid content [385,386]. It is also possible that the high vitamin D intake associated with traditional (TF) was protective. This is consistent with the biological effects of vitamin D on pancreatic [406], hepatic [407] and cardiac [408], smooth [409] and skeletal muscle cells [410] that are involved in the etiopathology of METS. The relationship of 25(OH)D and TF to METS, metabolic risk factors and DM2 should be further clarified among Inuit people living in the Arctic. Hence the goals of this study were to assess whether increases in vitamin D status, or intake of TF reduce, the odds of METS and DM2 in Inuit adults while adjusting for obesity as assessed by BMI and fat mass, fatty acid (FA) status (saturated FA [RBC-Sat], omega-3 FA [RBC-Omega-3], trans FA [RBC-Trans] of red blood cells), fish and marine mammals intakes and region of residence in the Arctic.

Methods

Participants and Methods

Study Design

This cross-sectional analysis addressed two outcomes: the first was METS; and the second was DM2 previously diagnosed, or newly diagnosed as a result of the International Polar Year Inuit Health Survey (IPY-IHS) of 2007-2008. The independent variables included 25(OH)D, RBC fatty acids, TF or marine mammals and fish intakes. Covariates included obesity indicators, arctic region, age and sex. The survey included 2595 male and female adults as published in detail elsewhere [353] . In brief, stratified random sampling was used to select households using either a computer generated random numbers or a random digit table. Participant entrance criteria were: ≥ 18 years of age and non-pregnant if female.

To analyze the METS outcome, the inclusion criteria were no prior diagnosis of DM2 and data available for all study variables. The exclusion criteria were limited to pregnancy and lactation for women. Participants with newly diagnosed DM2 as a result of the survey were not excluded [363]. METS was defined according to NCEP (presence of ≥ 3 of the following: abdominal obesity as defined by a WC ≥ 102 cm for men and ≥ 88 cm for women, high TG (≥ 1.7 mmol/L), low HDL (< 1.03 for men and <1.3 mmol/L for women), elevated blood pressure (BP: ≥ 130/85 mm Hg or taking antihypertensive medications), and impaired fasting glucose (≥ 5.6 mmol/L) [209]. High LDL was defined as > 3 mmol/L and elevated total cholesterol > 6.9 mmol/L [411], whereas an elevated LDL/HDL was defined as > 3.3 [412]. Data were available for 1725 adults of which 317 had METS in the analysis for odds of METS. Among the study group of METS 8.1% took anti-hypertensive medications, 1.0% took medications for heart disease, 4.4% took hormones, 3.4% took cholesterol lowering medications and 8.5% took nutrition supplements.

For the DM2 outcome, the inclusion criteria were any participant in the IPY-IHS with data available on all study variables. A prior diagnosis of DM2 if the person identified themselves as having DM2. In addition, new DM2 was identified if fasting plasma glucose (FPG) was ≥ 7 mmol/L, or 2 hour post OGTT plasma glucose ≥ 11.1 mmol/L. The analysis for DM2 involved a total of n=1857 of which 161 had DM2. Among adults with previously diagnosed DM2, 44 out of 117 were taking DM2 medications (n=29 were taking metformin, n=7 were taking insulin, n=6 were taking glyburide, whereas n=4 were taking rosiglitazone, n=7 were taking unknown DM2 medications). In the DM2 analysis group; 10.1% were taking anti-hypertensive medications, 1.3% were taking medications for heart disease, 5.3% took medications for osteoporosis, 4.6% took hormones, and 9.1% took nutrition supplements.

Ethical approval was obtained from the McGill University Faculty of Medicine Institutional Review Board, the Nunavut Research Institute, and the Aurora Research Institute. Signed informed consent was obtained from each participant.

Dietary Assessment

For the analyses in this report, the main dietary variable was frequency of TF intake captured using food frequency questionnaire (FFQ) that reflected one year [352]. Frequency was then expressed per day. Inuit adults were asked about how often in the last year they consumed (in season or off season) TF items from a list of 47 items, or how often they consumed in the last month from a list of 7 store bought items. Total TF, total marine mammals and total fish intakes were included in the present analysis. The 24-h dietary recall was based on the multiple pass technique and food models (Santé Quebec). Methods for dietary assessment were described in detail previously [69]. In brief, the food intake items from the 24-h recall were entered into CANDAT Software (Godin London) and nutrient intake generated using the Canadian Nutrient File (2007b) available at the time of the study [353]. Nutrient content of food items not in the Canadian Nutrient File were analyzed using a School of Dietetics and Human Nutrition in-house food file, food labels, recipes and other resources including the U.S. Department of Agriculture.

Clinical Assessment

Clinical assessment data for the present analyses included standing height (Road Rod 214 Portable stadiometer, Seca, Maryland, USA), weight and body composition (Tanita TBF-300GS), WC (ERP, Laval, Quebec) and BP (Bp TRUTM Vital Signs Monitor MedTech LTD., Coquitlam, BC, Canada) using standard protocols as previously described [353]. Healthy WC for men was defined as < 102 cm and healthy WC for women was defined as < 88 cm according to Health Canada [22]. The definition of elevated (at risk) BP was defined as BP ≥ 130/85 (mm Hg) based on three readings, spaced 2 minutes apart [209].

Biochemical Analysis

Serum 25(OH)D was measured using a Liaison Auto-analyzer chemiluminescent assay (Diasorin Inc, Stillwater, MN, USA) with quality control data meeting the certification criteria of the Vitamin D External Quality Assurance program. Based on the observed standard deviation in the dataset, data were analyzed per 30 nmol/L of 25(OH)D [10]. As an objective measure of TF intakes, RBC membrane FA profiles were explored. The analytical details of the Folch method and the parameters of the gas chromatograph (Varian 3400 GLC Palo Alto, CA) used are already reported [76]. RBC-Sat (14:0 to 24:0) and RBC-Trans were used to reflect intake of market foods [413], whereas RBC-Omega-3 (18:3 n-3 to 22:6 n-3) were used to reflect TF intakes. For the METS components, fasting serum total cholesterol, HDL cholesterol and TG were measured using colorimetric assays [361]; LDL cholesterol was then obtained by calculation (Nutrasource Diagnostics, Guelph, ON) [336]. Plasma glucose was analyzed by the Glucose Hexokinase II method [356].

Statistical Analysis

Data for this study was analyzed for normality using Kolmogorov–Smirnov test, Shapiro-Wilk, and Anderson darling tests. A mixed model ANOVA was used to calculate adjusted least square means for study outcomes in different regions and groups with/without METS and DM2 with Bonferroni post hoc testing used to identify differences among arctic regions. Serum 25(OH)D and frequency of intake of TF were compared among adults grouped according to the number of metabolic risks using mixed model ANOVA and post hoc Bonferroni tests. Moreover, the relationship between serum 25(OH)D (in increments of 30 nmol/L) and cut-point threshold groups of metabolic characteristics with METS, specific metabolic risk factors, total number of metabolic risk factors present, and DM2 was evaluated in multiple logistic regression models, adjusting for covariates. Covariates included: age (y), sex (male/female), arctic region, RBC FA

status (RBC-Sat FA, RBC-Omega-3 and RBC-Trans FA) and total fish and total marine mammals intakes. Similar methods were then used to explore total TF in the models in place of marine mammals and fish specifically. Data was explored separately for prior diagnosis of DM2 and newly diagnosed DM2, but is shown as total DM2 since time since onset was not known and the interpretation was similar. Significance was set at $P< 0.05$.

Results

Metabolic syndrome analysis

The METS analysis involves n=1725 adults (Table 5.1). Median age was 39 y (IQR: 29, 49), BMI 27.1 kg/m^2 (IQR: 23.3, 31.8), 25(OH)D 49.7 nmol/L (IQR: 31.2, 73.6) and FPG 4.9 (4.6, 5.2) mmol/L. METS was present in 317 adults, representing 18.4% (95% CI: 16.6% to 20.2%) of the study group. The percent of adults under 50 y who had METS was 15.0% (95% CI: 13.1% to 16.9%), whereas the percent of adults ≥ 50 y who had METS was 28.8% (121 out of 420; 95% CI: 24.5% to 33.1%). The prevalence of each metabolic characteristic is described in supplemental table 5.1. The leading metabolic abnormalities among adults with METS were: high WC (92.4%), high TG (76.0%), low HDL (68.8%), high BP (61.8%) and high FPG (39.1%). Other metabolic abnormalities included high LDL (54.5%), high LDL/HDL ratio (30.6%) and high cholesterol (6.3%).

The proportion with METS was higher in both Nunatsiavut (29.8%) and Inuvialuit Settlement Region (32.5%) relative to Nunavut (13.9%). Regional differences were also noted in anthropometry, biomarkers of Inuit lifestyle and metabolic health (Table 5.1). With Nunavut as the reference, in Inuvialuit Settlement Region all three measures of obesity (BMI, WC, FM%) were higher and on average participants consumed more total energy. Lower intakes of total TF, including marine mammals, was observed with no difference in fish intake. In addition, higher RBC-Sat and RBC-Trans FA, TG, cholesterol, but lower RBC-Omega-3 and HDL were

observed, with no difference in BP. In Nunatsiavut, all measures of obesity were higher relative to Nunavut, whereas energy from protein was lower. In addition, lower total intakes of TF and marine mammals and higher fish intakes were noticed. With regard to biomarkers, RBC-Omega-3, TG and cholesterol were higher in Nunatsiavut than Nunavut, whereas both RBC-Sat and RBC-Trans FA were lower. In addition, both systolic and diastolic BP were higher in Nunatsiavut than Nunavut. FPG, HDL and LDL were not different between Nunatsiavut and Nunavut. Compared to Nunatsiavut, Inuit in Inuvialuit Settlement Region had higher WC, energy intake, marine mammal intake, RBC-Sat and RBC-Trans and serum TG, but lower RBC-Omega-3, BP and HDL. Regional differences were not observed in 25(OH)D with average values above 50 nmol/L in all three regions.

In view of regional differences in METS and its components, adults with METS were compared to adults without. Relative to adults without METS, adults who had METS were older, more likely to be female, obese and have higher RBC-Sat and they consumed a higher proportion of energy as protein. By design, having METS was associated with deteriorated metabolic profile including higher BP and lower HDL as well as higher serum TG and LDL (Table 5.1). Serum 25(OH)D decreased with increasing number of metabolic risks in a model adjusted for age, sex, BMI, RBC FA, TF and region (Table 5.2). Intake of total TF, marine mammals or fish did not relate to the number of metabolic risk factors.

In a multiple logistic regression model adjusted for age, sex and BMI (model 1), serum 25(OH)D (per 30 nmol/L) reduced the odds of METS, high WC, high TG, low HDL and increased the odds of high LDL (Figure 5.1-b-d-e-f). After further adjustment for region, RBC FA and marine and fish intakes (model 2), serum 25(OH)D lowered the odds of high WC, high TG and low HDL, but not METS (Figure 5.1-a-b-d-e). Hypertension was not affected by serum

25(OH)D (Figure 5.1-c). In contrast, total TF lowered the odds of METS, high TG and low HDL (Figure 2-a-d-e) in models adjusted for age, sex and BMI (model 1). After further adjustment of the model (model 2), TF did not lower the odds of METS overall, or any of its components of METS (Figure 5.2). Supplemental Table 5.2 outlines the details of the fully adjusted model for METS. In a multiple logistic regression model that controlled for age, sex, BMI, serum 25(OH)D, RBC FA and marine mammals and fish intake, factors such as age, being male, FM% and BMI increased odds of METS. In addition, being from Nunatsiavut doubled the odds for having METS. The following did not contribute to explaining the variance in any model: RBC-Sat, RBC-Omega-3, RBC-Trans. In a separate model (supplemental table 5.3) that included age, sex, BMI, RBC FA, serum 25(OH)D and total TF intake, TF lowered the odds for high LDL/HDL ratio, but this did not reach statistical significance (OR: 0.83; 95% CI: 0.69 to 1.004, *P*= 0.055). Repeating the analysis using FM% instead of BMI did not change the interpretation.

The relationship of the components of total TF, total marine mammals and total fish were tested in separate models. Neither marine mammals nor fish related to the components of METS (data not shown). However, in a fully adjusted model (including age, sex, BMI, RBC FA, serum 25(OH)D and marine mammals and fish intake) (Supplemental table 5.3), marine mammal intake increased the odds for high LDL/HDL ratio (OR: 1.71; 95% CI: 1.02 to 2.84, *P*= 0.04), whereas fish lowered the odds for high LDL/HDL ratio (OR: 0.35; 95% CI: 0.17 to 0.69, *P*= 0.003). Repeating the analysis using FM% instead of BMI did not change the interpretation.

Diabetes mellitus analysis

In total n=161 of 1857 had DM2 representing 8.7% (95 % CI: 7.4% to 10.0%). Of these, 117 were previously diagnosed and 44 newly diagnosed. The percent of DM2 for adults <50 y

was 4.1% (95% CI: 3.0% to 5.2%), whereas among adults ≥ 50 y, 20.9% of adults had DM2 (95% CI: 17.4% to 24.4%) and 62.8% of adults with DM2 had METS. In comparison to adults without DM2, adults with DM2 were older, more obese, with higher RBC-Sat, RBC-Omega-3, FPG and serum 25(OH)D. Total TF and energy intake and the proportion from protein and fat were higher with DM2, whereas the proportion of intake as carbohydrate was lower. Having DM2 was associated with higher systolic BP and higher TG (Table 5.3).

For the DM2 subgroup analysis, the proportion having DM2 was not different among the regions: Nunavut (8.5%), Inuvialuit Settlement Region (7.8%) and Nunatsiavut (10.7%). The same pattern of regional differences (Table 5.3) was observed for obesity indicators, dietary patterns and biomarkers associated with metabolic health as was described earlier for METS. Notably, obesity as measured by BMI, WC and FM% and biomarkers including FPG, systolic BP, TG and cholesterol were the lowest in Nunavut relative to both Nunatsiavut and Inuvialuit Settlement Region. On the other hand, intake TF was the highest in Nunavut relative to the other geographical regions. Compared to Nunatsiavut, Inuit in Inuvialuit Settlement Region had higher waist, energy intake, marine mammal intake, RBC-Sat and RBC-Trans and serum TG, but lower RBC-Omega-3, BP and HDL

In a multiple logistic regression model that adjusted for age, sex and BMI serum 25(OH)D was not significantly associated with the odds of having DM2 (OR 1.17; 95% CI 0.99 to 1.38, P= 0.075). Similarly, in model that adjusted for age, sex and BMI, fish and marine mammal were not associated with DM2 (data not shown). In the fully adjusted model (Table 5.4) that controlled for age, sex, BMI, serum 25(OH)D, and marine mammals and fish intakes, RBC-Sat increased the odds for having DM2. The following did not significantly contribute to the model; serum 25(OH)D, RBC-Omega-3, RBC-Trans, fish and marine mammal intakes. Being

from Inuvialuit Settlement Region reduced the odds for having DM2, whereas being from Nunatsiavut increased odds for having DM2 relative to Nunavut. Including either WC or FM% in the model in place of BMI did not change the results. In separate models that were adjusted for similar variables except with total TF in place of marine mammals or fish, TF was still not related to DM2. Lastly, in a model adjusted for age, sex, BMI, serum 25(OH)D (per 30 nmol/L), RBC fatty acids including RBC-Sat, RBC-Omega-3 and RBC-Trans, both fish and marine mammal, having METS was associated with higher odds for having new DM2 (OR 5.9; 95% CI: 2.6 to 13.1, $P< 0.0001$) (Data not shown).

Discussion

The prevalence of DM2 in Canadian Inuit has rapidly increased to 12.2% among adults over 50 y of age [22]. This study addressed a pressing need to identify primary prevention approaches for METS, which is a clustering of metabolic risk factors used to estimate the risk of DM2 [414]. It is notable in the present analysis that in the Inuit there were regional differences in the clustering of metabolic factors and that Nunatsiavut had the highest proportion of METS. Based on these observations regional differences in the Canadian Arctic appear related to METS and DM2. The geographical differences in METS and DM2 could be related to differing degrees of isolation from the general Canadian population and its impact on dietary factors and health services. In testing whether certain components of a traditional lifestyle could be protective against such chronic diseases, it was observed in this analysis that greater intakes of TF and higher vitamin D status were protective from METS, but these associations disappeared in the final models. In addition, higher serum 25(OH)D protects against high WC, TG and low HDL, three commonly observed components of METS in this study population, whereas fish intake was protective from high LDL/HDL ratio. On the other hand age, obesity and RBC-Sat were

among the leading factors that increased the odds of DM2. Taken together, the data of this analysis indicate that higher consumption of TF, especially fish intake, that is rich in vitamin D and other nutrients, continues to protective Inuit from dyslipidemia (higher TG, LDL and lower HDL), whereas nutrients that are usually present in market food such as saturated fatty acids increase DM2.

The risk of METS is reported to progressively decrease with increasing plasma 25(OH)D in general population studies [415]. For example, in the US NHANES, serum 25(OH)D ≥ 96.4 nmol/L versus ≤ 48.4 nmol/L reduced the odds of METS (OR: 0.46; 95% CI: 0.32 to 0.62) [415]. However, in Canada the highest quartile of 25(OH)D was not protective against METS (OR 0.50; 95% CI: 0.24 to 1.06) [248]. Similarly, in the present analysis that included marine mammal and fish intakes as sources of 25(OH)D, the OR for METS did not reach significance (OR 0.86; 95% CI: 0.71 to 1.03). These discrepant results could be due to the observation that METS was predominantly driven by obesity and dyslipidemia in the Inuit population or that by including marine mammal and fish intakes as a covariates, vitamin D status was already captured. Indeed, TF is a rich and natural source of vitamin D for Inuit [10] and marine mammal and fish intakes as estimated for the previous year would have represented a more sustained intake of vitamin D compared to serum 25(OH)D with a short half-life of 14 days [416].

TF is a source of many nutrients that could contribute to reduction of elevated LDL relative to HDL. The diet of Inuit consists of wild animals and plants including various marine mammals, fish and caribou which are eaten fresh, cooked or dried and involve eating skin, blubber, liver and fat in different meals [417]. TF is considered a good source for many nutrients including both vitamin D and highly unsaturated omega-3 FA [418,419]. Diets rich in omega-3 FA inversely relate to markers of chronic diseases such as TG [420,421] and increases in HDL

cholesterol [421]. In the Alaska Native health research study, both LDL and HDL increased while TG decreased with increasing RBC omega-3 FA, but LDL/HDL was not measured [419]. The mechanism of action could include reduction of hepatic production of very low density lipoprotein along with stimulation of plasma lipolytic activity through LPL-mediated clearance. It is possible too, that TF is associated with less consumption of carbohydrates and saturated fat which could otherwise contribute to METS and a higher LDL/HDL ratio [422]. In addition, native food in Alaska contributed 83% of vitamin D intake [418] highlighting the valuable health benefits of a traditional lifestyle.

In this study we noticed a lower odds ratio for having high WC in relation to vitamin D status, but not TF. The relationship of serum 25(OH)D to WC could be protective from future development of DM2 and could have important implications for Inuit health, especially those who do not consume TF as often. Elevated WC has been suggested as a mechanism that most strongly influences the METS [423]. However, elevated WC is not always associated with increases in the risk of METS, for example, in a study among Greenland Inuit, obesity was not associated with the same level of metabolic disturbances as in Europeans [424]. As shown in the present report, METS is common among Inuit in Canada with 92.4% of those with METS having elevated WC. This is important since, having high WC was associated with an almost 4 times increased risk for having DM2 (adjusted OR: 3.7; 95% CI: 0.8 to 16.8), whereas having both high WC-high TG (two of the driving factors in METS) is associated with a 9 times increased risk of DM2 in the same population (adjusted OR: 8.6; 95% CI: 2.1 to 34.6) [22]. The association between serum 25(OH)D and WC in this study could have implications in the prevention of METS and possibly DM2.

Serum 25(OH)D was inversely and significantly associated high TG and low HDL. The relationship between serum 25(OH)D and serum lipids could be mediated by LPL. In a study among Chinese adults, serum 25(OH)D was associated with increases in post-heparin LPL (ß= 0.17, $P< 0.001$) [425] which relates to increases in serum HDL and decreases in serum TG and hyperlipidemia in chronic diseases including DM2 [426]. Other cross sectional studies support that serum 25(OH)D is associated with decreases in serum TG [211,427], whereas similar findings were not evident in a clinical trial of the association of vitamin D and plasma lipids [428]. Similar to our study, in a study by Ford et al., hypertriglyceridemia was significantly reduced by serum 25(OH)D, whereas vitamin D status did not associate with HDL and BP in the US NHANES III data [211]. Based on the present study and others to date, a higher serum 25(OH)D is anticipated to improve serum lipids, both serum TG and HDL.

In contrast to other studies [240,241,429], serum 25(OH)D was not directly associated with DM2. Most of the studies that investigated the relationship between serum 25(OH)D and DM2 had a prospective longitudinal design [240,429,430]. Such designs capture slow changes in serum 25(OH)D over time and seasonal effects, whereas the design of this study is cross sectional and limited to late summer and early fall. Results from clinical trials on the effect of vitamin D on clinical outcomes in adults with DM2 are equivocal, with some studies showing that vitamin D supplementation to DM2 patients lowered HBA1C, HOMA-IR and insulin concentration in Iranians [431,432], but not in Arabs [433]. Further studies from other ethnic groups including other Inuit and Aboriginal groups are lacking. This analysis adjusted for many variables and showed that there is no relationship between serum 25(OH)D and DM2, likely since the disease is already established.

This study has limitations that include its cross sectional design. Furthermore, variables such as smoking, alcohol intake and physical activity were not included due to missing data. Also, aging and other nutrients that exist in TF which associate with metabolic risk among Inuit could have confounded the studied relationships in this paper. Lastly, the FFQ assessment did not fully capture quantity of TF intakes, thus recommendations on quantities of TF associated with health benefits are not possible.

In conclusion, to the authors' knowledge, this is the first study to address the risk of METS and DM2 in relation to serum 25(OH)D and TF among Inuit adults in Canada. According to this study, there was a significant inverse relationship between fish intake and LDL/HDL ratio. Even after adjustment for covariates there was a favorable relationship between serum 25(OH)D and WC and plasma lipid profile. Finally, the data indicate that 25(OH)D could be protective from metabolic risk factors. There were no significant relationships between serum 25(OH)D and TF with DM2. Factors including obesity, RBC-Sat and being from Nunatsiavut were associated with higher odds of DM2. Regions that represent Inuit habitat in the arctic should not be considered homogenous when considering metabolic diseases.

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Conflicts of interest, none.

Table 5.1 Comparison of the study variables according to geographical region and comparison between adults with and without metabolic syndrome.

Variable	Nunavut (n=1276)	Inuvialuit Settlement Region (n=231)	Nunatsiavut (n=218)	Metabolic Syndrome No (n= 1408)	Metabolic Syndrome Yes (n= 317)	*P*-value
Age (y)	39.4 ± 13.9[a]	42.3 ± 14.5[b]	42.9 ± 13.5[b]	39.0 ± 13.7	45.8 ± 13.6	<0.0001
Male %*	509 (39.9%)	71 (30.7%)	83 (38.1%)	560 (39.8%)	103 (32.5%)	0.016
Female %	767 (60.1%)	160 (69.3%)	135 (61.9%)	848(60.2%)	214 (67.5%)	
Obesity						
BMI (kg/m^2)	27.5 ± 6.2[a]	30.4 ± 6.8[b]	29.1 ± 5.7[b]	26.8 ± 5.7	33.8 ± 5.5	<0.0001
Waist Circumference (cm)	90.4 ± 15.3[a]	101.6 ± 16.3[c]	96.0 ± 14.0[b]	89.3 ± 14.3	107.8 ± 13.1	<0.0001
FM %	28.6 ± 11.0[a]	34.7 ± 10.8[b]	32.8 ± 10.1[b]	27.9 ± 10.7	39.3 ± 7.3	<0.0001
24-h Dietary Recall (intake/d)						
Total Energy (kcal)	2132 .0± 1195.9[a]	2436.1 ± 1268.7[b]	2101.6 ± 1071.8[a]	2183.2 ± 1238.5	2107.3 ± 976.3	0.312
% Energy as Protein	20.6 ± 11.3[b]	19.8 ± 9.6[ab]	18.7 ± 9.1[a]	20.0 ± 10.9	21.4 ± 10.8	0.033
% Energy as CHO	47.3 ± 17.5	45.9 ± 15.4	47.8 ± 12.0	47.5 ± 17.1	45.5 ± 14.6	0.054
% Energy as fat	31.0 ± 12.0	33.0 ± 10.7	32.0 ± 9.1	31.4 ± 11.7	31.5 ± 10.5	0.944
FFQ (frequency/d)						
Total TF	1.10 ± 0.92[b]	0.89 ± 0.81[a]	0.92 ± 0.77[a]	1.10 ± 0.90	1.10 ± 0.80	0.430
MM	0.29 ± 0.34[c]	0.19 ± 0.27[b]	0.10 ± 0.22[a]	0.26 ± 0.33	0.23 ± 0.30	0.276
Fish	0.21 ± 0.26[a]	0.25 ± 0.29[ab]	0.28 ± 0.26[b]	0.22 ± 0.27	0.22 ± 0.26	0.833
Biomarkers						
FPG (mmol/L)	4.9 ± 0.7[a]	5.2 ± 0.6[b]	4.9 ± 0.6[a]	4.9 ± 0.5	5.4 ± 0.9	<0.0001
Systolic BP (mm Hg)	113.6 ± 18.4[a]	113.5 ± 18.7[a]	119.7 ± 23.9[b]	112.1 ± 18.3	124.3 ± 20.6	<0.0001
Diastolic BP (mm Hg)	74.1 ± 12.1[a]	74.8 ± 12.3[a]	78.9 ± 15.4[b]	73.3 ± 11.9	81.2 ± 14.0	<0.0001
RBC-Omega-3 (%)	5.8 ± 3.2[b]	2.7 ± 2.6[a]	6.9 ± 2.3[c]	5.5 ± 3.2	5.4 ± 3.5	0.428
RBC-Sat (%)	43.0 ± 4.9[b]	49.6 ± 5.6[c]	40.7 ± 3.4[a]	43.3 ± 5.4	44.9 ± 5.6	<0.0001
RBC-Trans FA (%)	1.37 ± 0.80[b]	1.55 ± 0.52[c]	1.18 ± 0.55[a]	1.38 ± 0.77	1.34 ± 0.64	0.367
25(OH)D (nmol/L)	55.6 ± 33.5	55.1 ± 23.3	58.5 ± 26.4	55.4 ± 32.1	58.2 ± 28.7	0.151
TG (mmol/L)	1.2 ± 0.6[a]	1.6 ± 0.8[c]	1.4 ± 0.7[b]	1.1 ± 0.5	2.2 ± 0.8	<0.0001
HDL (mmol/L)	1.5 ± 0.4[b]	1.4 ± 0.4[a]	1.5 ± 0.5[b]	1.6 ± 0.4	1.2 ± 0.4	<0.0001
LDL (mmol/L)	2.8 ± 1.0	2.9 ± 0.9	3.0 ± 0.9	2.8 ± 1.0	3.2 ± 0.9	<0.0001
Cholesterol (mmol/L)	4.9 ± 1.1[a]	5.0 ± 1.0[ab]	5.1 ± 1.0[b]	4.8 ± 1.1	5.3 ± 1.0	<0.0001

* $P < 0.05$, values with different letters are statistically significant; a<b<c. Data are expressed as means± SD for geographical comparison and for with and without metabolic syndrome. BMI, body mass index; CHO, carbohydrate; FM, fat mass; FPG, fasting plasma glucose; 25(OH)D, 25-hydroxyvitamin D; HDL, high density lipoprotein;; LDL, low density lipoprotein; MM, marine mammal; RBC-Omega-3, red blood cell content of omega-3 fatty acids; RBC-trans FA, Trans fatty acids in red blood cells; RBC-Sat, red blood cell content of saturated fatty acids; TG, triglycerides; WC, waist circumference.

Table 5.2 Adjusted means of serum 25(OH)D and categories of TF intake across metabolic risk groups.

Metabolic risks (n, %)	25(OH)D (nmol/L)	Total TF (frequency/d)	Marine Mammals (frequency/d)	Total Fish (frequency/d)
0 (n=568, 32.9%)	61.0 ± 1.3[b]	1.03 ± 0.03	0.24 ± 0.01	0.26 ± 0.01
1 (n=504, 29.2%)	57.2 ± 1.2[ab]	1.02 ± 0.03	0.24 ± 0.01	0.25 ± 0.01
2 (n=336, 19.5%)	54.1 ± 1.5[a]	0.98 ± 0.03	0.23 ± 0.01	0.27 ± 0.01
≥ 3 (n=317, 18.4%)	53.7 ± 1.5[a]	0.93 ± 0.03	0.27 ± 0.01	0.26 ± 0.01

Data are presented as adjusted means and standard error. Comparisons performed using mixed model ANOVA with random effects of age (y), sex (male/female), BMI, RBC-Omega-3, RBC-Sat, RBC-Trans, TF, marine, fish, serum 25(OH)D and region: Nunavut, Inuvialuit Settlement Region and Nunatsiavut using Bonferroni post hoc test. Within columns, data with different superscript letters are significantly different, $P<0.05$. 25(OH)D: 25-hydroxyvitamin D.

RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, Traditional food.

Table 5.3 Comparison of the study variables according to geographical region and between adults with and without DM2.

Variable	Nunavut (n=1369)	Inuvialuit Settlement Region (n=245)	Nunatsiavut (n=243)	Adults without DM2 n=1696	DM2 n=161	*P*-value
Age (y)	40.5 ± 14.4[a]	43.2 ± 14.8[b]	44.2 ± 14.0[b]	40.0 ± 13.8	55.1 ± 13.6	<0.0001
Male %*	549 (40.1%)	76 (31.0%)	89 (36.6%)	650 (38.3%)	64 (39.8%)	0.722
Female %	820 (59.9%)	169 (69.0%)	154 (63.4%)	1046 (61.7%)	97 (60.2%)	
Obesity						
BMI (kg/m^2)	27.9 ± 6.5[a]	30.5 ± 6.8[b]	29.4 ± 5.8[b]	28.1 ± 6.3	32.0 ± 7.0	<0.0001
Waist Circumference (cm)	91.4 ± 15.7[a]	102.2 ± 16.2[c]	96.9 ± 14.3[b]	92.7 ± 15.8	102.7 ± 16.4	<0.0001
FM %	29.3 ± 11.2[a]	34.9 ± 10.7[b]	33.4 ± 10.1[b]	30.0 ± 11.1	36.7 ± 10.6	<0.0001
24-h Dietary Recall (intake/d)						
Total Energy (kcal)	2120.0 ± 1195.0[a]	2432.1 ± 1275.7[b]	2052.2 ± 1054.9[a]	2181.8 ± 1202.1	1847.9 ± 1048.3	0.0008
% Energy as Protein	20.9 ± 11.4	20.2 ± 9.7	19.1 ± 9.3	20.2 ± 10.8	24.0 ± 12.0	<0.0001
% Energy as CHO	46.7 ± 17.6	45.6 ± 15.3	47.7 ± 12.2	47.2 ± 16.6	41.2 ± 16.4	<0.0001
% Energy as fat	31.3 ± 12.1	33.0 ± 10.5	31.9 ± 9.1	31.3 ± 11.4	34.1 ± 13.1	0.005
FFQ (frequency/d)						
Total TF	1.13 ± 0.92[b]	0.92 ± 0.81[a]	0.93 ± 0.79[a]	1.05 ± 0.94	1.37 ± 0.94	<0.0001
MM	0.30 ± 0.35[c]	0.20 ± 0.27[b]	0.10 ± 0.22[a]	0.25 ± 0.32	0.34 ± 0.38	0.001
Fish	0.22 ± 0.27[a]	0.26 ± 0.29[ab]	0.29 ± 0.27[b]	0.22 ± 0.27	0.29 ± 0.28	0.002
Biomarkers						
FPG (mmol/L)	5.0 ± 0.9[a]	5.2± 0.8[b]	5.2 ± 1.3[ab]	4.9 ± 0.5	6.5 ± 2.2	<0.0001
Systolic BP	114.3 ± 18.7[a]	114.1± 18.9[a]	118.9 ± 23.9[b]	114.1 ± 19.4	122.2 ± 23.7	<0.0001
Diastolic BP	74.2 ± 12.2[a]	75.0 ± 12.2[a]	77.9 ± 16.7[b]	75.0 ± 12.8	75.0 ± 14.2	0.834
RBC-Omega-3 (%)	5.9 ± 3.3[b]	2.7 ± 2.7[a]	6.9 ± 2.3[c]	5.5 ± 3.2	7.0 ± 4.2	<0.0001
RBC-Sat (%)	43.1 ± 5.0[b]	49.6 ± 5.6[c]	40.7 ± 3.4[a]	43.6 ± 5.4	44.7 ± 5.7	0.012
RBC-Trans FA (%)	1.37 ± 0.80[b]	1.60 ± 0.57[c]	1.20 ± 0.65[a]	1.37 ± 0.68	1.40 ± 1.36	0.640
25(OH)D (nmol/L)	57.2 ± 34.3	55.4 ± 23.8	60.2 ± 27.4	55.5 ± 31.0	77.4 ± 38.2	<0.0001
TG (mmol/L)	1.3 ± 0.8[a]	1.8 ± 1.3[c]	1.5 ± 0.9[b]	1.4 ± 0.9	1.7 ± 1.1	<0.0001
HDL (mmol/L)	1.5 ± 0.5[b]	1.4 ± 0.4[a]	1.5 ± 0.5[b]	1.5 ± 0.5	1.4 ± 0.6	0.256
LDL (mmol/L)	2.8 ± 1.0	2.9 ± 0.9	2.9 ± 0.9	2.8 ± 1.0	2.9 ± 1.1	0.582
Cholesterol (mmol/L)	4.9 ± 1.1[a]	5.1 ± 1.0[ab]	5.1 ± 1.0[b]	4.9 ± 1.1	5.1 ± 1.2	0.111

* $P< 0.05$, values with different letters are statistically significant; a<b<c. Data are expressed as means± SD for geographical comparison and for comparison between DM2 (Yes/No). BMI, body mass index; CHO, carbohydrate; FM, fat mass; FPG, fasting plasma glucose; 25(OH)D, 25-hydroxyvitamin D; HDL, high density lipoprotein; LDL, low density lipoprotein; MM, marine mammal. RBC-Omega-3, red blood cell content of omega-3 fatty acids; RBC-trans FA, trans fatty acids in red blood cells; RBC-Sat, red blood cell content of saturated fatty acids; TG, triglycerides; WC, waist circumference.

Table 5.4 Odds Ratios with 95% confidence intervals (95% CI) of DM2 multiple logistic regression association with anthropometric, demographics, serum 25(OH)D, fatty acids, markers of TF variables and DM2.

	DM2					
	BMI Model		FM% Model		WC Model	
Variables	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
BMI (kg/m^2)	**1.09 (1.06, 1.12)**	<0.0001				
FM%			**1.07 (1.05, 1.10)**	<0.0001		
WC (cm)					**1.04 (1.02, 1.05)**	<0.0001
Inuvialuit Settlement Region	**0.51 (0.28, 0.93)**	0.0096	**0.48 (0.26, 0.88)**	0.0099	**0.44 (0.24, 0.81)**	0.004
Nunatsiavut	**1.36 (0.81, 2.27)**	0.026	1.22 (0.73, 2.04)	0.052	**1.24 (0.74, 2.08)**	0.028
Nunavut	ref		ref		ref	
Age (y)	**1.06 (1.05, 1.08)**	<0.0001	**1.06 (1.04, 1.07)**	<0.0001	**1.06 (1.04, 1.08)**	<0.0001
Sex (M vs. F)	1.09 (0.75, 1.58)	0.655	**1.98 (1.27, 3.08)**	0.003	0.92 (0.64, 1.33)	0.658
25(OH)D (per 30 nmol/L)	1.13 (0.93, 1.39)	0.219	1.14 (0.94, 1.40)	0.192	1.14 (0.93, 1.39)	0.205
RBC-Sat (%)	**1.06 (1.01, 1.11)**	0.016	**1.05 (1.004, 1.10)**	0.033	**1.06 (1.01, 1.11)**	0.016
RBC-Omega-3 FA (%)	1.03 (0.95, 1.11)	0.483	1.02 (0.94, 1.10)	0.627	1.03 (0.95, 1.11)	0.443
RBC-Trans FA (%)	1.09 (0.90, 1.33)	0.369	1.11 (0.91, 1.34)	0.302	1.09 (0.90, 1.32)	0.385
FFQ (frequency/day)						
MM	1.42 (0.82, 2.45)	0.209	1.48 (0.86, 2.57)	0.160	1.44 (0.83, 2.47)	0.194
Fish	1.10 (0.58, 2.09)	0.770	1.08 (0.57, 2.05)	0.822	1.09 (0.58, 2.07)	0.784

BMI, body mass index; FFQ, food frequency questionnaire; F, female; FM, fat mass; 25(OH)D, 25-hydroxyvitamin D; M, male; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference.

Supplemental Tables

Supplemental Table 5.1 Distribution of metabolic characteristics between adults with and without METS.

		Metabolic Syndrome		Chi-square *P*-value
	Total (1725)	**Yes (317)**	**No (1408)**	
High WC	809 (46.9%)	293 (92.4%)	516 (36.7%)	<0.0001
High FPG	216 (12.5%)	124 (39.1%)	92 (6.5%)	<0.0001
High TG	379 (22.0%)	241 (76.0%)	138 (9.8%)	<0.0001
Low HDL	424 (24.6%)	218 (68.8%)	206 (14.6%)	<0.0001
High BP	420 (24.4%)	196 (61.8%)	224 (15.9%)	<0.0001
New DM2	42 (2.4%)	25 (7.9%)	17 (1.2%)	<0.0001
High LDL	687 (39.8%)	173 (54.5%)	514 (36.5%)	<0.0001
High cholesterol	65 (3.8%)	20 (6.3%)	45 (3.2%)	0.009
High LDL/HDL	197 (11.4%)	97 (30.6%)	100 (7.1%)	<0.0001

FPG, fasting plasma glucose; TG, triglyceride; HDL, high density lipoprotein; BP, blood pressure; DM, type 2 diabetes mellitus; LDL, low density lipoprotein; High waist is a WC ≥ 102 cm for men and ≥ 88 cm for women, high TG (≥ 1.7 mmol/L), low HDL (< 1.03 for men and < 1.3 mmol/L for women), elevated BP (≥ 130/85 mm Hg or taking antihypertensive medications, and high FPG (≥5.6 mmol/L). High LDL was defined as > 3 mmol/L and elevated total cholesterol > 6.9 mmol/L, whereas an elevated LDL/HDL was defined as > 3.3.

Supplemental Table 5.2 Odds ratios with 95% confidence intervals (95% CI) of multiple logistic regression association of anthropometric, demographics, serum 25(OH)D, fatty acids, markers of TF variables with metabolic syndrome

	Metabolic syndrome			
	Adjusted for BMI		Adjusted for FM%	
Variable	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
BMI (kg/m^2)	**1.20 (1.16, 1.22)**	<0.0001		
FM%			**1.17 (1.14, 1.19)**	<0.0001
Inuvialuit Settlement Region	1.87 (1.21, 2.87)	0.566	1.63 (1.06, 2.51)	0.667
Nunatsiavut	**2.67 (1.79, 3.98)**	0.002	**2.18 (1.46, 3.26)**	0.015
Nunavut	ref		ref	
Age (y)	**1.04 (1.03, 1.06)**	<0.0001	**1.03 (1.02, 1.04)**	<0.0001
Sex (M vs. F)	1.05 (0.77, 1.44)	0.743	**4.67 (3.11, 7.03)**	<0.0001
25(OH)D (per 30 nmol/L)	0.86 (0.71, 1.03)	0.107	0.88 (0.72, 1.06)	0.179
RBC-Sat (%)	1.01 (0.97, 1.05)	0.622	1.0 (0.96, 1.04)	0.919
RBC-Omega-3 FA (%)	0.97 (0.91, 1.04)	0.430	0.96 (0.89, 1.03)	0.221
RBC-Trans FA (%)	0.90 (0.72, 1.13)	0.355	0.91 (0.73, 1.13)	0.380
FFQ (frequency/day)				
MM	1.44 (0.88, 2.35)	0.150	1.56 (0.94, 2.57)	0.083
Fish	0.65 (0.36, 1.15)	0.137	0.67 (0.37, 1.21)	0.184

BMI, body mass index; FFQ, food frequency questionnaire; F, female; FM, fat mass; 25(OH)D, 25-hydroxyvitamin D; M, male; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference.

Supplemental Table 5.3 Odds Ratios with 95% confidence intervals (95% CI) of DM2 multiple logistic regression association with anthropometric, demographics, serum 25(OH)D, fatty acids, markers of TF variables and high LDL/HDL.

		Without TF			**TF**
Variables	OR (95% CI)	*P*-value		OR (95% CI)	*P-value*
BMI (kg/m^2)	**1.10 (1.07, 1.12)**	**<0.0001**		**1.10 (1.07, 1.12)**	**<0.0001**
Inuvialuit Settlement Region	**2.15 (1.33, 3.47)**	**0.022**		**1.87 (1.17, 2.99)**	**0.027**
Nunatsiavut	1.42 (0.88, 2.28)	0.901		1.13 (0.72, 1.79)	0.451
Nunavut	ref			ref	
Age (y)	1.01 (0.998, 1.03)	0.103		1.01 (0.998, 1.03)	0.083
Sex (M vs. F)	**2.73 (1.96, 3.80)**	**<0.0001**		**2.09 (2.01, 4.03)**	**<0.0001**
25(OH)D (per 30 nmol/L)	0.87 (0.71, 1.06)	0.164		0.87 (0.71, 1.06)	0.176
RBC-Sat (%)	1.01 (0.97, 1.05)	0.627		1.01 (0.97, 1.05)	0.607
RBC-Omega-3 FA (%)	**1.08 (1.01, 1.16)**	**0.036**		**1.08 (1.004, 1.16)**	**0.039**
RBC-Trans FA (%)	0.95 (0.75, 1.21)	0.702		0.93 (0.73, 1.18)	0.551
FFQ (frequency/day)					
TF				0.83 (0.69, 1.004)	0.055
MM	**1.71 (1.02, 2.84)**	**0.040**			
Fish	**0.35 (0.17, 0.69)**	**0.003**			

BMI, body mass index; FFQ, food frequency questionnaire; F, female; FM, fat mass; 25(OH)D, 25-hydroxyvitamin D; M, male; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference

FIGURE 5.1 The Association between serum 25(OH)D with (a) metabolic syndrome and risk parameters including (b) high waist WC, (c) hypertension, (d) high TG and (e) low HDL and (f) high LDL. Odds ratios are studied per each 30 nmol/L of 25(OH)D. *** $P< 0.0001$, ** $P< 0.01$, * $P < 0.05$. Model 1: Adjusted for age (y), sex (male, female) and BMI (kg/m^2). Model 2: Adjusted for age (y), sex (male, female) BMI (kg/m^2), total RBC Omega-3 FA, RBC-Sat, RBC-Trans, total marine mammal intake, total fish intake and region (Nunavut, Inuvialuit Settlement Region and Nunatsiavut).

FIGURE 5.2: The Association between total TF intake with (a) metabolic syndrome and risk parameters including (b) high WC, (c) hypertension, (d) high TG and (e) low HDL and (f) high LDL. *** $P< 0.0001$, ** $P< 0.01$, * $P< 0.05$. Model 1: Adjusted for age (y), sex (male, female) and BMI (kg/m^2). Model 2: Adjusted for age (y), sex (male, female) BMI (kg/m^2), RBC-Omega-3 FA, RBC-Sat, RBC-Trans, serum 25(OH)D, and region (Nunavut, Inuvialuit Settlement Region and Nunatsiavut).

Figure 5.1.

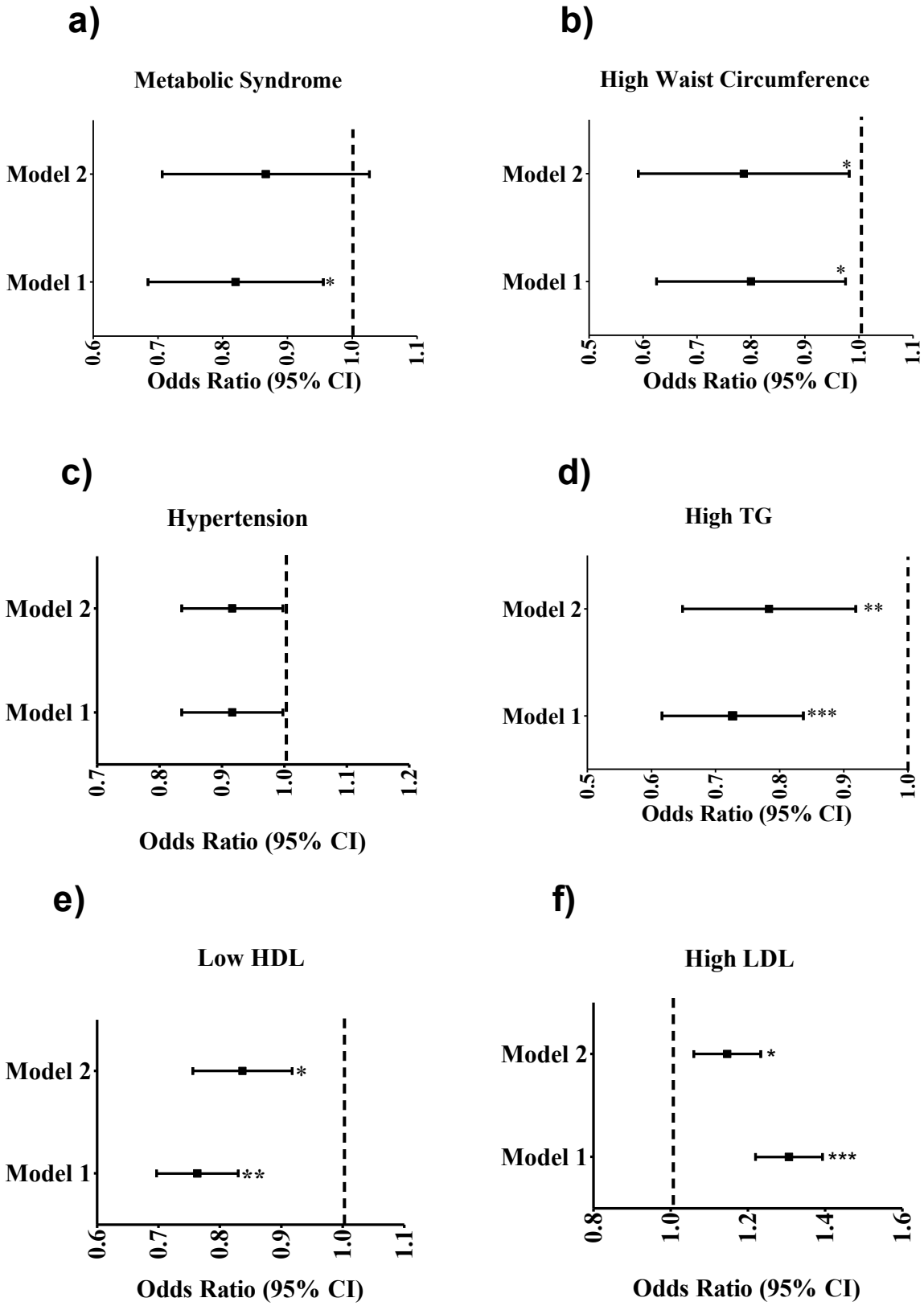
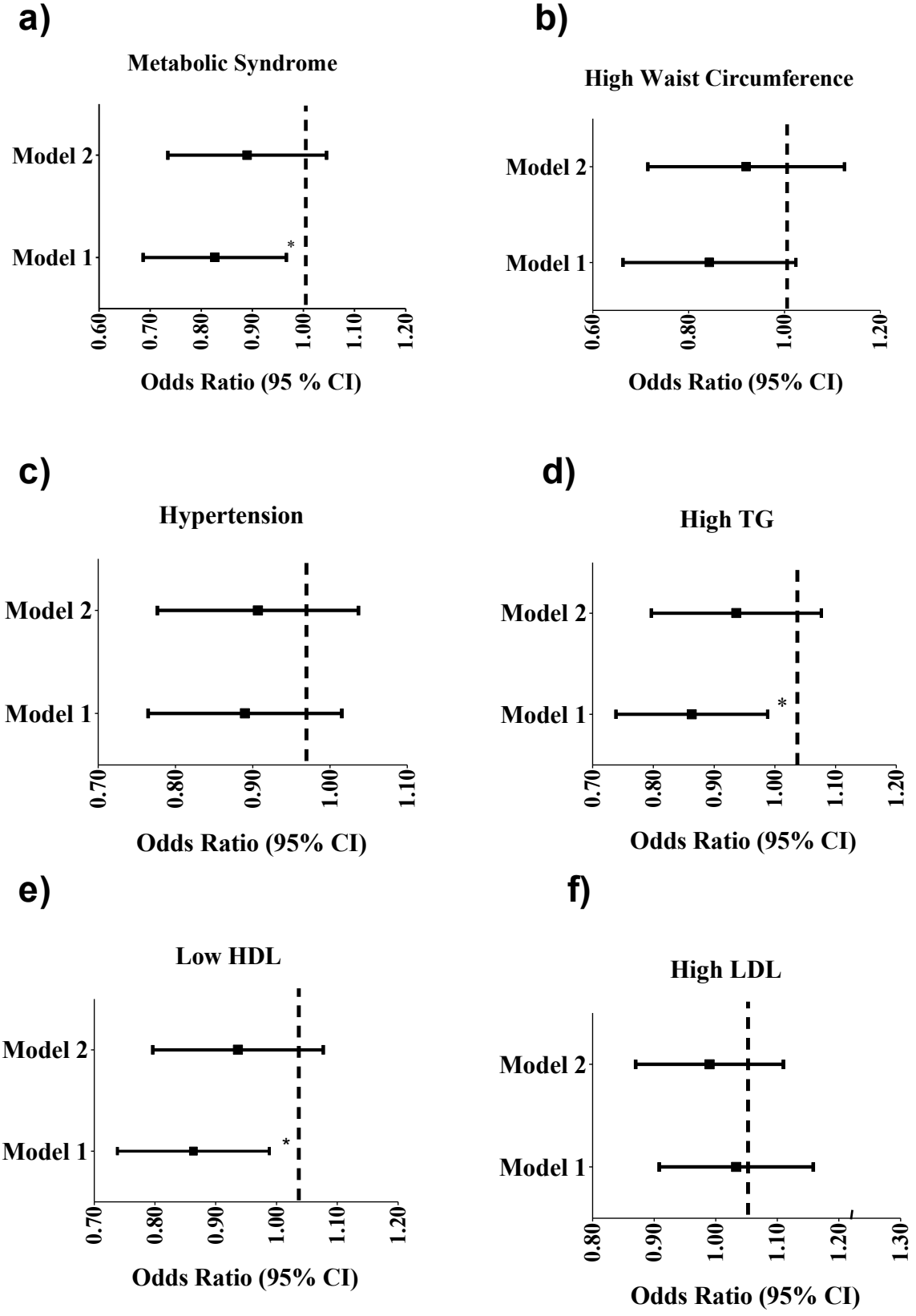

a)
Metabolic Syndrome
Model 2
Model 1
*
0.6
0.7
0.8
0.9
1.0
1.1
Odds Ratio (95% CI)
b)
High Waist Circumference
Model 2
Model 1
*
*
0.5
0.6
0.7
0.8
0.9
1.0
1.1
Odds Ratio (95% CI)
c)
Hypertension
Model 2
Model 1
0.7
0.8
0.9
1.0
1.1
1.2
Odds Ratio (95% CI)
d)
High TG
Model 2
Model 1
**
***
0.5
0.6
0.7
0.8
0.9
1.0
Odds Ratio (95% CI)
e)
Low HDL
Model 2
Model 1
*
**
0.6
0.7
0.8
0.9
1.0
1.1
Odds Ratio (95% CI)
f)
High LDL
Model 2
Model 1
*
***
0.8
1.0
1.2
1.4
1.6
Odds Ratio (95% CI)

Figure 5.2


a)
Metabolic Syndrome
Model 2
Model 1
*
0.60
0.70
0.80
0.90
1.00
1.10
1.20
Odds Ratio (95 % CI)
b)
High Waist Circumference
Model 2
Model 1
0.60
0.80
1.00
1.20
Odds Ratio (95% CI)
c)
Hypertension
Model 2
Model 1
0.70
0.80
0.90
1.00
1.10
Odds Ratio (95% CI)
d)
High TG
Model 2
Model 1
*
0.70
0.80
0.90
1.00
1.10
1.20
Odds Ratio (95% CI)
e)
Low HDL
Model 2
Model 1
*
0.70
0.80
0.90
1.00
1.10
1.20
Odds Ratio (95% CI)
f)
High LDL
Model 2
Model 1
0.80
0.90
1.00
1.10
1.20
1.30
Odds Ratio (95% CI)

Bridge 4

In chapter 5, having a higher vitamin D status slightly lowered the risk of METS and was not related to the risk of DM2 among Inuit. At the same time vitamin D status was associated with increases in HDL, decreases in TG and a lower trend of elevated WC after adjustment for BMI. Although higher vitamin D status was associated with increases in the concentration of LDL, this association did not lead to significant increases in LDL/HDL indicating that higher vitamin D status is in general associated with improved WC and serum lipid profile. Hence, the relationship between insulin resistance and vitamin D status could be mediated by modification of serum lipids. This leads one to expand this theory to include that insulin resistance is related to vitamin D status through modification of adiposity and other body compartments, particularly, the bone which interacts with energy metabolism through various mechanisms such as osteocalcin. Osteocalcin plays a major role in the coordinated metabolism of insulin and glucose [257]. The mechanism by which osteocalcin affects glucose metabolism involves insulin expression, secretion and sensitivity. The relationship between osteocalcin, FPG and HOMA-IR has not been studied among Inuit. The next study will investigate the relationship between osteocalcin and insulin resistance and FPG among Inuit after adjustment for dietary markers of TF including red blood cell content of FA. In addition to fish and marine mammals intakes and markers of obesity, in addition to serum 25(OH)D.

CHAPTER 6: Osteocalcin is not Related to Insulin Resistance among Inuit Women: The 2007-2008 International Polar Year Inuit Health Survey

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Key words: glucose, insulin resistance, adiponectin, osteocalcin, women, Inuit.

ABSTRACT

Background: In human and animal studies, osteocalcin regulates insulin resistance and the osteocalcin gene includes a positive vitamin D response element. Inuit are at risk of low vitamin D status and increasingly insulin resistance and type 2 diabetes mellitus (DM2).

Objective: To test the associations between osteocalcin and insulin resistance, obesity and adipokines.

Methods: The study group consisted of Inuit women (n=275) from Nunavut who participated in the cross-sectional 2007-2008 International Polar Year Inuit Health Survey (IPY-IHS). The dependent variables fasting plasma glucose (FPG) and fasting serum insulin were measured along with the independent variables, adiponectin, leptin, osteocalcin and 25-hydroxyvitamin D (25(OH)D). Anthropometry and fat mass were measured. Fatty acid status was measured using red blood cells (RBC) and dietary data was collected from a food frequency questionnaire. Data were analyzed using multiple regression models for continuous outcomes and adjusted for dietary markers and RBC membrane fatty acids.

Results: Median age was 51 (44, 58) y and BMI 28.8 (23.9, 33.4) kg/m^2. Osteocalcin was not associated with FPG (β= 0.002; 95% CI: -0.104 to 0.108, *P*= 0.964) or homeostatic model of insulin resistance (HOMA-IR) (β= 0.001, 95% CI: -0.023 to 0.025, *P*= 0.913), in a model adjusted for 25(OH)D, intakes of fish and marine mammals and RBC fatty acids including omega-3, saturated and trans fatty acids.

Interpretation: Osteocalcin, which is a bone derived endocrine protein, is not related to insulin resistance and fasting plasma glucose among Inuit women with high prevalence of obesity.

Introduction

Inuit are currently facing a transition that is characterized by increasing rates of obesity [324] and change in dietary habits towards less dependence on traditional food (TF) intake [77,70]. As a consequence, the rate of type 2 diabetes mellitus (DM2: 12.2%) in adults ≥ 50 y from a recent report from International Polar Year Health Survey (IPY-IHS) is now consistent with the general Canadian population [22]. Previously DM2 was rare among this ethnic group [20]. DM2 is characterized by insulin resistance which could be defined as lack of response of peripheral tissues to insulin [434]. One of the methods to assess insulin resistance in the fasting state is homeostatic model of insulin resistance (HOMA-IR) [435]. Identifying measures that lower insulin resistance could help in the prevention and management of DM2.

Recent data suggests the presence of interrelationships between bone metabolism and glucose homeostasis [47]. Osteocalcin which is a bone derived protein is involved in insulin secretion, pancreatic cell proliferation and insulin resistance as shown in knock out mice [48]. The mechanism(s) by which osteocalcin affects glucose metabolism involves insulin expression, secretion and insulin sensitivity. Gene expression analysis of pancreatic cells treated with osteocalcin indicates increases in the expression of insulin genes (Ins1 and Ins2). In addition, osteocalcin enhances the expression of cyclin dependent kinase, cyclin D1 and cyclin D2 which suggests that osteocalcin could regulate β-cell proliferation [275]. The action of osteocalcin on pancreatic cells is mediated by G-protein coupled receptor family C group 6 member (Gprc6a) [13]. In particular, uncarboxylated osteocalcin acts through Gprc6a receptors in epithelial and STC-1 enteroendocrine cells to stimulate the production of glucagon-like peptide-1 which is an insulin secretagogue [276]. However, data from epidemiological research is equivocal as to whether osteocalcin has a role to play in insulin resistance and DM2.

In study among Aboriginal and European adults from Canada, glucose (P= 0.009), HOMA-IR (P= 0.032) and HBA1C (P= 0.008) differed significantly across osteocalcin quartiles with significant impact of ethnicity [281]. In addition, a significant relationship between osteocalcin and HOMA-IR was observed (r= -0.298, P= 0.019) in Korean postmenopausal women [436]. However, osteocalcin was not correlated with HOMA-IR in a study among three generations of non-diabetic females [349].

In Inuit women, osteocalcin values appear to be low [362] in comparison to values reported for both men, women and postmenopausal females from different ethnicities [437,438]. A positive vitamin D response element (VDRE) exists on the promoter region of osteocalcin gene [439]. Whether this is due to low vitamin D status is not clear. Further studies are needed among Inuit to identify levels of osteocalcin in association with glucose metabolism while accounting for vitamin D status. Hence the goals of this study are to test the associations between osteocalcin and insulin resistance, obesity and adipokines among Inuit women in models that adjust for dietary markers such as TF intakes, vitamin D status and red blood cell (RBC) membrane fatty acids.

Methods

Population and Ethics

The study sample consisted of non-pregnant Inuit women from Nunavut who were ≥ 40 years with data available for all study variables during the cross-sectional IPY-IHS of 2007 to 2008. Stratified random sampling was used where communities were strata and homes were randomly selected using a computer random generation of numbers or a random digit table. To be included in this study each woman had to have data available on all study variables.

Detailed methods and population characteristics are described elsewhere [362]. The study protocol was reviewed and approved by the McGill University Faculty of Medicine Research Ethics Board and the Nunavut Research Institutes. Consent forms, questionnaires

and the DVD were translated into different Inuit dialects and all participants signed a written consent form.

The study design involves studying FPG and HOMA-IR as primary outcomes with osteocalcin as the main independent variable. Covariates of the study included age, body composition assessments, RBC fatty acids, intakes of fish and marine mammal, serum 25(OH)D and in subgroup, leptin and adiponectin. To be included in the study, data on osteocalcin and all study variables was required. The studied group involved women with DM2 (n=44 of them 33 had prior DM2) and those without evidence of DM2.

Trained nurses collected data from bilingual questionnaire and performed anthropometric measurements which included; height (Road Rod 214 Portable Stadiometer; Seca, Hanover, MD), weight and fat mass (Tanita TBF-300GS with goal setter; Tanita, Arlington Heights, IL), waist circumference (WC) (ERP, Laval, Canada).

Clinical and Laboratory Measurements

Blood glucose was analyzed using hexokinase II method [22]. The analysis of serum insulin was completed by electrochemiluminescent immuno assay (Roche Modular Analytics E170 platform) at the Institut Universitaire de Cardiologie et de Pneumologie de Québec (Québec City, QC). The interassay CV for the control samples were 2.4% and 2.7% at 219 and 556 pmol/L levels. HOMA-IR was calculated [355]:

HOMA-IR = fasting serum insulin (μIU/mL)*fasting plasma glucose (mmol/L)/22.5.

Adipokines were measured using a Meso Scale Discovery Multi-Array Assay (MSD cat#K151BXC) for adiponectin with a detection limit of 0.005 ng/mL and manual sandwich ELISA assay (Linco cat# EZHL-80SJK, Linco Research, St Charles, Missouri) for leptin with a detection limit of 0.5 ng/mL. For adiponectin, the intra-assay and inter-assay CV were 5% and 15%, respectively, whereas for leptin, the intra-assay and inter-assay CV were 10%. All analyses were performed at Nutrasource Diagnostics, (Guelph, ON) [353]. Serum 25(OH)D and total osteocalcin were measured at McGill University using an autoanalyzer and

chemiluminescent assays (Liaison, Diasorin Inc.). The derived functional sensitivity of osteocalcin is defined as the concentration at which CV% exceeds 20%, the derived functional sensitivity from regression analysis is ≤ 3.0 ng/ml; the inter assay CV was 2.2%. The inter-assay and intra-assay CV were 4.5 % and 11.1 % for the low 25(OH)D control (38.2 nmol/L) and 6.2 % and 5.3 % for the high 25(OH)D control (127.2 nmol/L). The quality control data for serum 25(OH)D are according to Vitamin D External Quality Assessment Scheme program 2009–2010 indicating that 80% or more of the reported results are within 30% of the ALTM [353].

RBC membrane fatty acid (FA) profiles, expressed as percent of total FA, were determined [353] using the methodology of Folch et al. [358] by gas chromatography (Varian 3400, Palo Alto, CA), as described in detail elsewhere [76]. Red blood cell content of saturated FA (RBC-Sat) included 14:0 to 24:0. RBC-Omega-3 included all of the n-3 FA from 18:3 n-3 to 22:6 n-3.

Dietary assessment

To obtain data on nutrition intake, a trained bilingual interviewers collected information using a culturally appropriate food frequency questionnaire (FFQ) [69,352]. The FFQ data reflected past year intake of market or TF items that are available in region of Nunavut. Data for frequency of total TF, total marine mammals and total fish intakes were then expressed per day. Inuit adults were asked about how often in the last year they consumed (in season or off season) TF items from a list of 47 items, or how often they consumed in the last month from a list of 7 store bought items.

Statistical Analyses

Data was analyzed for normality using Kolmogorov–Smirnov, Shapiro Wilk, and Anderson-Darling tests. Medians were used to summarize data not normally distributed and spearman correlations were calculated for the associations between study variables and

osteocalcin. Multiple linear regression associations between osteocalcin and both FPG and HOMA-IR were used after adjustment for obesity as assessed by (BMI, FM% and WC), age, both total fish and total marine mammals intakes, serum 25(OH)D and RBC FA. Total TF was explored, but data not included as the interpretation was the same. Multiple regression models were repeated including serum leptin and adiponectin. The regression models were checked for residual distribution and normality, influential points and co-linearity using tolerance, which is 1/variance inflation factor (VIF). Values were considered significant if $P<0.05$.

Results

The present analysis involves 256 women ≥ 40 y, median age (IQR) was 51 (44, 58) y, HOMA-IR 1.81 (1.20, 2.95) and FPG 5.2 (4.8, 5.7) mmol/L. Osteocalcin was 1.88 (1.47, 2.41) nmol/L and 25(OH)D 72.0 (51.2, 97.2) (nmol/L). DM2 was present in 17% of the study group. Hyperglycemia (FPG≥ 5.6) was present among 17.5% excluding DM2. The subgroup (n=200) had a median leptin concentration of 20.1 (10.6, 34.9) µg/L, adiponectin 11.0 (6.8, 16.0) µg/L. Frequency distributions of leptin, adiponectin and osteocalcin are in Figure 1.

Obesity (BMI ≥ 30 kg/m^2) was prevalent among 44.4% and overweight (30 > BMI ≥ 25 kg/m^2) was prevalent among 24.9%. Having high WC ≥ 88 cm was present in 61.9% of the study group. Median (IQR) values for BMI were 28.8 (23.9, 33.4) kg/m^2, WC 93.4 (81.2, 103.0) cm and FM% 37.7 (30.4, 43.1).

The prevalence of women with serum 25(OH)D ≥ 75 nmol/L was 47.5%, and the groups with serum 25(OH)D between 50 -74.9, 49.9-30 and <30 nmol/L were 28.0%, 16.3% and 8.2% respectively. Frequency of intakes of total TF was a median (IQR) of 0.89 (0.32, 1.64), with marine mammals intake being 0.18 (0.04, 0.56) and fish intake 0.10 (0.01, 0.29).

Other markers of food intake included RBC-Omega-3 7.5 (5.0, 9.6) %, RBC-Sat 42.4 (40.5, 44.7) % and RBC-Trans FA 1.13 (0.68, 1.68) %.

Table 6.1 provides spearman correlation coefficients between osteocalcin and study variables partially adjusted for age. It was observed that osteocalcin was inversely related to both FPG and HOMA-IR in addition to obesity measures (BMI, FM% and WC). Osteocalcin was not correlated with any markers of TF intake or adipokines. In terms of FA, osteocalcin only positively correlated with RBC-Trans FA. Serum 25(OH)D was not significantly correlated with osteocalcin.

In the basic multiple regression model adjusted for age and BMI, osteocalcin was not significantly associated with FPG (β= -0.008; 95% CI -0.110 to 0.094, *P*= 0.877) or HOMA-IR (β= 0. 005; 95% CI -0.007 to 0.017, *P*= 0.997). Osteocalcin remained unrelated to FPG (Table 6.2) or to HOMA-IR after further adjusting for obesity including WC and FM%, marine mammals and fish intakes, in addition to RBC-Sat, RBC-Omega-3 and RBC-Trans FA and serum 25(OH)D (Table 6.3). Upon including leptin and adiponectin in the models (Table 6.4-6.5), osteocalcin remained unrelated to FPG and HOMA-IR. The only factors that were significantly related to FPG were anthropometry including BMI, and WC, whereas factors that were significantly related to HOMA-IR included anthropometry and leptin. Variables such as age, serum 25(OH)D, fatty acids including RBC-Omega-3, RBC-Sat and RBC-Trans FA, adiponectin and both marine and fish intakes did not enter as significant variables in models of both FPG and HOMA-IR. For all models, repeating the analysis without including participants with DM2 did not change the results.

Discussion and Conclusion

The potential for osteocalcin to modulate glucose metabolism was explored in this homogenous group of Inuit women ≥ 40 y. In the present analysis, all of the women were from Nunavut with a high prevalence of obesity yet relatively low values for both osteocalcin

and HOMA-IR. This could be ascribed in part to the very good vitamin D status observed in this segment of the IPY-IHS as both serum 25(OH)D and osteocalcin [50] are typically inversely associated HOMA-IR. Accordingly, osteocalcin was not among factors that were associated with insulin resistance. Rather obesity as measured by BMI, WC and FM% in addition to leptin were the driving factors for those with evidence of insulin resistance among the Inuit women studied. Measures of obesity were inversely related to osteocalcin probably due to suppression of bone turnover [440]. In addition, dietary factors both as assessed by FFQ and RBC FA were not related to osteocalcin. Furthermore adipokines, including both leptin and adiponectin, were not related to osteocalcin.

Osteocalcin is an osteoblast derived hormone hypothesized to improve insulin expression and secretion from β-cells and improves insulin sensitivity in target tissues [48,257]. Osteocalcin was inversely correlated with HOMA-IR after age adjustment, but this relationship did not remain after multiple adjustment. In a study by Rui et al., osteocalcin was significantly inversely correlated with HOMA-IR, yet it was not among the final correlates in a multivariate model [441]. Similarly, in a study among Chinese postmenopausal women, osteocalcin was not significantly related to either insulin or HOMA-IR [442]. In contrast to these studies, the relationship between osteocalcin and insulin resistance was observed to be significant even after adjustment for BMI in Chinese men not taking DM2 medications [443]. In a multiethnic study by Weiler et al. both serum 25(OH)D and osteocalcin were related to measures of insulin resistance and secretion, in both white and First Nations women [444]. Compared to the First Nations study, only people of Inuit ethnicity were included precluding a comparison group and the value for HOMA-IR was lower in the present study indicating that this was a relatively healthy group of women. For these reasons, it is thus reasonable that no association between osteocalcin and HOMA-IR was observed.

In this study osteocalcin was inversely correlated with FPG, but this relationship did not last in multiple regression models. Similarly, various forms of serum osteocalcin did not correlate with glucose among three generations of women in a family study [445]. However other studies showed that serum osteocalcin is inversely related to fasting plasma glucose, for example osteocalcin was an independent potential predictor of FPG (β= -0.210; 95% CI -0.129 to -0.018) among postmenopausal Chinese women [446]. Relative to our study, both BMI and FPG were lower, whereas age was higher among the postmenopausal Chinese women. In another study, among 160 middle-aged Tibetan men and upon adjustment for age and BMI, serum total osteocalcin was inversely correlated with both FPG and post challenge glucose. Whether sex hormones play any role in the association of FPG and osteocalcin needs to be clarified [447].

Osteocalcin protein undergoes posttranslational gamma carboxylation at glutamic acid residues [445]. Almost 40% or more of circulating osteocalcin in humans is uncarboxylated osteocalcin [445]. Total osteocalcin represents both uncarboxylated and carboxylated osteocalcin. In a meta-analysis of epidemiological studies, both total osteocalcin and uncarboylated osteocalcin correlated inversely with FPG [448]. However, data from experimental animal models show that it is the uncarboxylated part of osteocalcin that has effects on insulin secretion and sensitivity [449]. In contrast to animal studies, a study in middle aged men found an inverse association between both carboxylated and uncarboxylated osteocalcin with plasma glucose and HOMA-IR [450]. Further studies are needed to clarify the potential differences in different forms of osteocalcin in Inuit adults.

The present analysis group of women had high rate of obesity (44.4%) and central obesity (61.9%). It is well known that leptin resistance and possible concomitant increase in leptin concentration accompany conditions of obesity [451]. The regulation of glucose metabolism is reciprocal between osteocalcin and leptin; leptin seems to suppress insulin

secretion [452], whereas osteocalcin favors it [453]. Under the control of leptin, the sympathetic nervous system may regulate osteoblast metabolism in a way that suppresses the synthesis and release of osteocalcin [454]. At the same time, obesity is associated with lower secretion of adiponectin [455]. Adiponectin is associated with osteoblast proliferation and associated with dose dependent increase in osteocalcin secretion [456]. Hence the lower adiponectin and increased leptin associated with obesity could have precluded the associations between osteocalcin and FPG and insulin resistance in this study.

Osteocalcin correlated inversely significantly with BMI, WC and FM%. Conversely, in a study among postmenopausal Chinese women, a low osteocalcin value was an independent risk factor for abdominal obesity (OR: 0.97; 95% CI 0.953 to 0.991, *P*= 0.004). Genetically modified mice that lack the osteocalcin gene demonstrate elevated fat pad mass [48]. Infusion of osteocalcin in wild type mice reduces obesity induced by a high fat diet [457]. However, a causal relationship cannot be inferred from this study due to its cross sectional design.

This study is limited by its cross sectional design, and by the lack of measurement of other uncarboxylated and carboxylated osteocalcin. The sample for osteocalcin was small and included only women ≥ 40 y from one arctic region. Furthermore, the measures of HOMA-IR are considered proxy measures of insulin resistance and secretion. Moreover, the information regarding menopausal status was not available. Further studies using more robust methods are needed to elaborate upon the relationships between osteocalcin and insulin resistance among Aboriginal men and women.

In summary among a relatively homogenous group of Inuit women, osteocalcin is inversely related to obesity as assessed by BMI and FM%, but not to FPG and insulin resistance after adjustment for covariates. Finally, obesity including the adipose derived adipokines leptin continue to be the driving factors of insulin resistance among Inuit women.

Table 6.1 Spearman correlation coefficients between study variables and osteocalcin adjusted for age (n=256)

Variable	Spearman correlation coefficient (*P*-value)
Age (y)	0.40 (<0.001)
Anthropometry	-0.20 (0.002)
BMI (kg/m^2)	
WC (cm)	-0.15 (0.015)
FM (%)	-0.19 (0.003)
FFQ (frequency/d)	
Total TF	0.02 (0.722)
MM	-0.04 (0.509)
Total Fish	-0.04 (0.494)
Biomarkers	
FPG (mmol/L)	-0.14 (0.023)
HOMA-IR	-0.17 (0.005)
RBC-Sat (%)	-0.01 (0.935)
RBC-Omega-3 (%)	-0.11 (0.068)
RBC-Trans (%)	0.15 (0.016)
25(OH)D (nmol/L)	-0.08 (0.189)
Adiponectin (µg/L) (n=200)	0.10 (0.168)
Leptin (µg/L) (n=200)	-0.13 (0.075)

BMI, body mass index; FFQ, food frequency questionnaire; FM, fat mass; FPG, fasting plasma glucose HOMA-IR, homeostatic model of insulin resistance; 25(OH)D, 25-hydroxyvitamin D; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference.

Table 6.2. Beta coefficient with 95 % confidence intervals of multiple regression models between FPG and osteocalcin, obesity and dietary variables.

FPG (mmol/L)

Variables (n=256)	BMI Model β-coefficient (95% CI)	P-value	FM% Model β-coefficient (95% CI)	P-value	WC Model β-coefficient (95% CI)	P-value
BMI (kg/m^2)	0.043(0.025, 0.061)	<0.0001				
FM%			0.029 (0.014, 0.044)	0.0002		
WC (cm)					0.018 (0.010, 0.026)	<0.0001
Age (y)	0.017 (0.003, 0.031)	0.014	0.017 (0.003, 0.031)	0.014	0.015 (0.001, 0.029)	0.033
Osteocalcin (nmol/L)	0.002 (-0.104, 0.108)	0.964	0.002 (-0.104, 0.108)	0.964	-0.004 (-0.11, 0.102)	0.935
25(OH)D per 30 nmol/L	0.008 (-0.146, 0.162)	0.917	0.008 (-0.147, 0.163)	0.917	0.025 (-0.132, 0.182)	0.753
RBC-Sat (%)	-0.009 (-0.040, 0.022)	0.550	-0.009 (-0.040, 0.022)	0.550	-0.008 (-0.037, 0.021)	0.753
RBC-Omega-3 FA (%)	-0.010 (-0.061, 0.041)	0.688	-0.010 (-0.061, 0.041)	0.688	-0.012 (-0.063, 0.039)	0.635
RBC-Trans FA (%)	-0.100 (-0.261, 0.061)	0.209	-0.103 (-0.264, 0.058)	0.209	-0.090 (-0.470, 0.286)	0.260
FFQ (frequency/day)						
MM	-0.060 (-0.440, 0.316)	0.755	-0.060 (-0.436, 0.316)	0.755	-0.061 (-0.440, 0.320)	0.752
Fish	0.082 (-0.477, 0.641)	0.773	0.082 (-0.477, 0.641)	0.773	0.070 (-0.490, 0.630)	0.805
R-square	0.134		0.135		0.127	

Data are expressed as β-values (95% CI) and *P* values. BMI, body mass index; FFQ, food frequency questionnaire; FM, fat mass; FPG, fasting plasma glucose HOMA-IR, homeostatic model of insulin resistance; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference.

Table 6.3. Beta coefficient with 95 % confidence intervals of multiple regression models between HOMA-IR and osteocalcin, obesity and dietary variables.

HOMA-IR						
	BMI Model		**FM% Model**		**WC Model**	
Variables (n=256)	**β-coefficient (95% CI)**	**P-value**	**β-coefficient (95% CI)**	**P-value**	**β-coefficient (95% CI)**	**P-value**
BMI (kg/m^2)	0.026 (0.022, 0.030)	<0.0001				
FM%			0.020 (0.016, 0.024)	<0.0001		
WC (cm)					0.012 (0.010, 0.014)	<0.0001
Age (y)	0.001 (-0.001, 0.003)	0.473	-0.0004 (-0.003, 0.003)	0.813	-0.0002 (-0.003, 0.003)	0.906
Osteocalcin (nmol/L)	0.001 (-0.023, 0.025)	0.913	0.011 (-0.015, 0.036)	0.385	-0.002 (-0.025, 0.021)	0.855
25(OH)D per 30 nmol/L	-0.002 (-0.037, 0.033)	0.909	0.0003 (-0.036, 0.036)	0.987	0.009 (-0.026, 0.044)	0.632
RBC-Sat (%)	0.003 (-0.004, 0.010)	0.339	0.003 (-0.068, 0.074)	0.987	0.004 (-0.003, 0.011)	0.258
RBC-Omega-3 FA (%)	0.003 (-0.008, 0.014)	0.661	-0.0004 (-0.012, 0.012)	0.950	0.001 (-0.011, 0.013)	0.859
RBC-Trans FA (%)	-0.024 (-0.059, 0.011)	0.197	-0.019 (-0.056, 0.018)	0.312	-0.017 (-0.054, 0.020)	0.357
FFQ (frequency/day)						
MM	0.036 (-0.046, 0.118)	0.398	0.057 (-0.031, 0.145)	0.207	0.037 (-0.049, 0.123)	0.405
Fish	-0.029 (-0.152, 0.094)	0.653	-0.042 (-0.170, 0.088)	0.527	-0.035 (-0.160, 0.092)	0.596
R-square	0.428		0.384		0.404	

Data are expressed in form of β (95% CI) and *P* values. BMI, body mass index; FFQ, food frequency questionnaire; FM, fat mass; FPG, fasting plasma glucose HOMA-IR, homeostatic model of insulin resistance; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference. HOMA-IR was log transformed prior to analysis.

Table 6.4. Beta coefficient with 95 % confidence intervals of multiple regression models between FPG and osteocalcin, obesity, adipokines, and dietary variables including serum 25(OH)D.

FPG (mmol/L)						
	BMI Model		**FM% Model**		**WC Model**	
Variables (n=200)	**β-coefficient (95% CI)**	**P-value**	**β-coefficient (95% CI)**	**P-value**	**β-coefficient (95% CI)**	**P-value**
BMI (kg/m^2)	0.045 (0.011, 0.078)	0.008				
FM%			0.023 (-0.002, 0.048)	0.078		
WC (cm)					0.019 (0.005, 0.033)	0.013
Age (y)	0.016 (-0.001, 0.033)	0.061	0.015 (-0.003, 0.033)	0.105	0.014 (-0.003, 0.031)	0.121
Osteocalcin (nmol/L)	0.002 (-0.120, 0.123)	0.971	0.007 (-0.12, 0.134)	0.915	-0.003 (-0.124, 0.118)	0.959
Leptin (µg/L)	0.001 (-0.010, 0.012)	0.847	0.005 (-0.006, 0.016)	0.407	0.002 (-0.008, 0.012)	0.719
Adiponectin (µg/L)	0.001 (-0.017, 0.019)	0.901	0.002 (-0.017, 0.021)	0.872	0.002 (-0.017, 0.188)	0.874
25(OH)D in 30 nmol/L	0.036 (-0.158, 0.230)	0.716	0.045 (-0.150, 0.24)	0.652	0.054 (-0.140, 0.248)	0.585
RBC-Sat (%)	-0.006 (-0.043, 0.031)	0.749	-0.006 (-0.043, 0.031)	0.753	-0.004 (-0.041, 0.033)	0.816
RBC-Omega-3 FA (%)	-0.007 (-0.070, 0.056)	0.821	-0.010 (-0.076, 0.056)	0.777	-0.008 (-0.072, 0.057)	0.813
RBC-Trans FA (%)	-0.078 (-0.273, 0.117)	0.432	-0.070 (-0.268, 0.128)	0.489	-0.070 (-0.261, 0.126)	0.515
FFQ (frequency/day)						
MM	-0.024 (-0.489, 0.441)	0.920	-0.001 (-0.475, 0.473)	0.997	-0.023 (-0.489, 0.443)	0.924
Fish	-0.210 (-0.950, 0.530)	0.586	-0.250 (-1.000, 0.501)	0.512	-0.221 (-0.775, 0.524)	0.561
R-square	0.128		0.110		0.125	

Data are expressed in form of β (95% CI) and *P* values. BMI, body mass index; FFQ, food frequency questionnaire;. FM, fat mass; FPG, fasting plasma glucose HOMA-IR, homeostatic model of insulin resistance; 25(OH)D, 25-hydroxyvitamin D; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference.

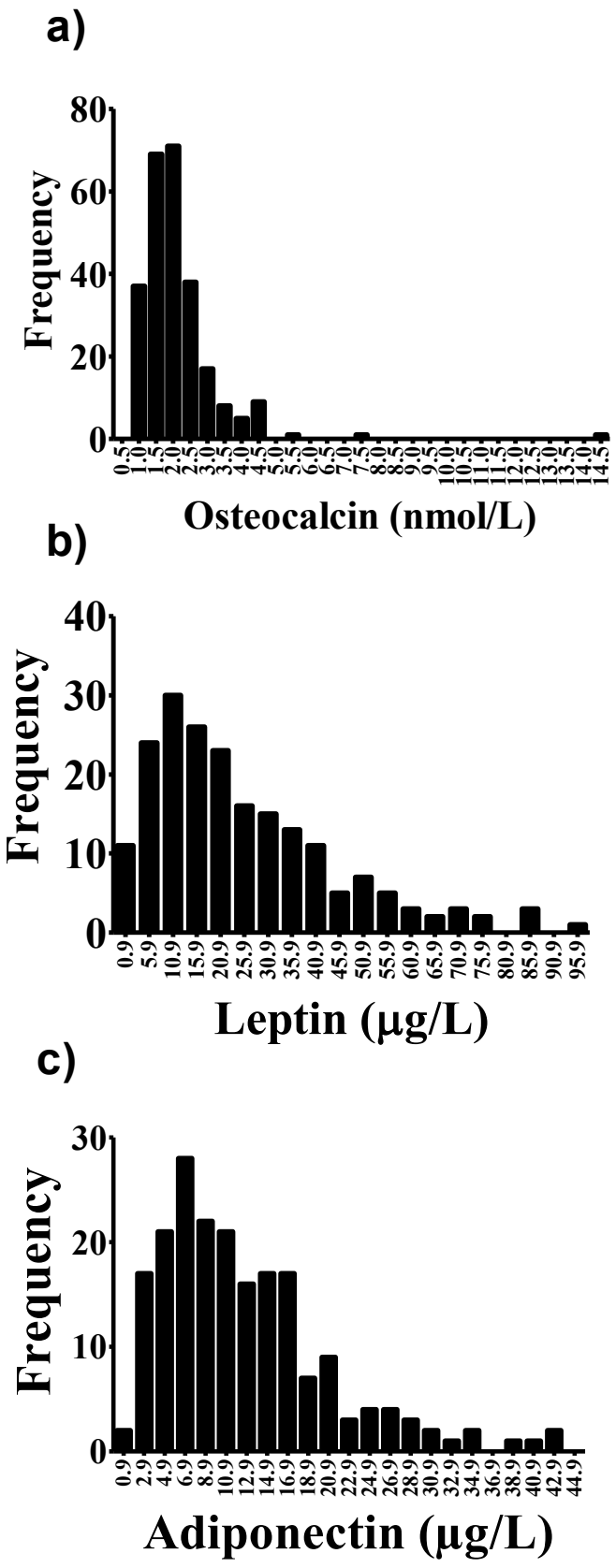
Table 6.5. Beta coefficient with 95 % confidence intervals of multiple regression models between HOMA-IR and osteocalcin, obesity, adipokines, and dietary variables including serum 25(OH)D.

HOMA-IR						
	BMI Model		**FM% Model**		**WC Model**	
Variables (n=200)	β-coefficient (95% CI)	P-value	β-coefficient (95% CI)	P-value	β-coefficient (95% CI)	P-value
BMI (kg/m^2)	0.017(0.010, 0.024)	<0.0001				
FM%			0.011(0.005, 0.017)	0.0001		
WC (cm)					0.007(0.004, 0.010)	<0.0001
Age (y)	0.004(0.0005, 0.008)	0.052	0.003(-0.001, 0.007)	0.174	0.003(-0.001, 0.007)	0.153
Osteocalcin (nmol/L)	0.0006(-0.024, 0.026)	0.964	0.005(-0.022, 0.032)	0.715	-0.002(-0.027, 0.023)	0.855
Leptin (µg/L)	0.004(0.002, 0.006)	0.0001	0.005(0.003, 0.007)	<0.0001	0.005(0.003, 0.007)	<0.0001
Adiponectin (µg/L)	-0.004(-0.008, -0.0002)	0.073	-0.003(-0.007, 0.001)	0.110	-0.003(-0.007, 0.001)	0.084
25(OH) in 30 nmol/L	0.0001(-0.039, 0.039)	0.995	0.004(-0.037, 0.045)	0.847	0.007(-0.034, 0.048)	0.746
RBC-Sat (%)	0.001(-0.007, 0.009)	0.806	0.001(-0.007, 0.009)	0.891	0.002(-0.006, 0.010)	0.662
RBC-Omega-3 FA (%)	0.0007(-0.012, 0.014)	0.912	-0.001(-0.015, 0.013)	0.864	0.001(-0.013, 0.015)	0.875
RBC-Trans FA (%)	-0.019(-0.060, 0.022)	0.365	-0.014(-0.055, 0.027)	0.483	-0.014(-0.055, 0.027)	0.507
FFQ (frequency/day)						
MM	0.024(-0.072, 0.12)	0.632	0.037(-0.063, 0.137)	0.461	0.022(-0.076, 0.12)	0.654
Fish	-0.022(-0.175, 0.131)	0.836	-0.040(-0.197, 0.117)	0.614	-0.028(-0.18, 0.13)	0.723
R-square	0.481		0.456		0.461	

Data are expressed in form of β (95% CI) and *P* values. BMI, body mass index; FFQ, food frequency questionnaire; FM, fat mass; FPG, fasting plasma glucose HOMA-IR, homeostatic model of insulin resistance; 25(OH)D, 25-hydroxyvitamin D; MM, marine mammal; RBC-Omega-3 FA, red blood cell content of omega-3 fatty acids; RBC-Sat, red blood cell content of saturated fatty acids; RBC-Trans FA, red blood cell content of trans-fatty acids; TF, traditional food; WC, waist circumference. HOMA-IR was log transformed prior to analysis.

FIGURE 6.1 Distribution of osteocalcin (n=256), leptin (n=200), adiponectin (n=200) among Inuit women ≥ 40 y.

Figure 6.1.


a)
Frequency
80
60
40
20
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
11.0
11.5
12.0
12.5
13.0
13.5
14.0
14.5
Osteocalcin (nmol/L)
b)
Frequency
40
30
20
10
0
0.9
5.9
10.9
15.9
20.9
25.9
30.9
35.9
40.9
45.9
50.9
55.9
60.9
65.9
70.9
75.9
80.9
85.9
90.9
95.9
Leptin (µg/L)
c)
Frequency
30
20
10
0
0.9
2.9
4.9
6.9
8.9
10.9
12.9
14.9
16.9
18.9
20.9
22.9
24.9
26.9
28.9
30.9
32.9
34.9
36.9
38.9
40.9
42.9
44.9
Adiponectin (µg/L)

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Conflicts of interest: None.

Bridge 5

In the previous paper, osteocalcin correlated inversely with insulin resistance and fasting plasma glucose, but was also inversely related to obesity markers, indicating a possible link of bone to energy metabolism. However, the association between glucose metabolism and bone in Inuit has not been reported. Various previous studies in other ethnicities point to a positive association between DM2 and bone mineral density probably due to higher BMI and insulin concentration.

The relationship between both prediabetes and DM2 to bone health could be through various physiological pathways or through lifestyle factors that marks the life of Inuit. For example the intake of traditional food or market food which can be assessed by fatty acid content in the red blood cells and food frequency questionnaire and marks particular pattern of distribution of both DM2 and prediabetes. Hence, it was important to try explore the association of the lifestyle markers that accompany prediabetes and DM2 with FaBMD and fracture among Inuit. The next chapter addresses the relationship of FaBMD, T-score of FaBMD, osteoporosis and fracture to DM2, prediabetes and dietary factors that accompany impaired glucose conditions among Inuit women.

CHAPTER 7: The Association of DM2 and Impaired Fasting Glucose with Bone Mineral Density and Fracture among Inuit Women: The 2007-2008 International Polar Year Inuit Health Survey

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Key words, Type 2 diabetes mellitus, impaired fasting glucose, bone mineral density, traditional food, marine mammals, fish.

ABSTRACT

Background: Bone mineral density (BMD) and fracture rates are elevated in adults with impaired fasting glucose (IFG) or type 2 diabetes mellitus (DM2).

Objective: To examine markers of bone health in Inuit women with IFG or DM2.

Methods: This is a nested case control study of women ≥ 40 y of age with IFG (n=41) or DM2 (n=72) and data available for BMD from the 2007-2008 International Polar Year Inuit Health Survey (IPY-IHS). Cases of IFG and DM2 were matched with controls (1:2) on the basis of body mass index (BMI) and age (n= 226). Bone mineral density of the distal non-dominant forearm (FaBMD) and T-scores (FaBMDT) were measured using a peripheral instantaneous x-ray imager (PIXI, GE/Lunar, Fort Myers, FL). Anthropometric measurements were collected along with fasting plasma glucose, adiponectin, leptin, osteocalcin and 25-hydroxyvitamin D (25(OH)D). A food frequency questionnaire was used to capture traditional food (TF) intake patterns. Data was analyzed using Wilcoxon two sample test comparison test and multiple regression models for continuous outcomes.

Results: The median age for the IFG group was 52 (48, 57) y and for the respective control 52 (46, 56) y, whereas the median age for DM2 56 (48.5, 62.5) y and for the control 54 (47, 62) y. Neither FaBMD nor FaBMDT were different between cases of IFG and DM2 and their respective controls, even with adjustment for arctic region and medication use. In contrast, being free of osteoporosis (T-score less than -2.5) was more common in DM2 cases versus controls (OR: 12.8; 95% CI: 1.5 to 109.9, *P*= 0.016). There were no differences in fracture prevalence between groups. The frequency of consuming marine mammals over the past year was positively related to FaBMD (ß= 0.048; 95% CI: 0.015 to 0.081, *P*= 0.005) in the control groups combined whereas fish intake was positively related to FaBMD in women with DM2 (ß= 0.057; 95% CI: 0.002 to 0.112, *P*= 0.043).

Interpretation: The presence of DM2 and greater fish intake were found to be protective against low bone mass in Inuit women. TF intake, particularly fish, should be encouraged as it may lower the risk of IFG and DM2 and possibly fracture.

Introduction

Ethnic variation in bone mineral density (BMD) is commonly ascribed to many factors including genetic, anthropometric and lifestyle factors [458]. In the International Polar Year Inuit Health Survey (IPY-IHS), a study among pre and postmenopausal Inuit females indicated that fat mass and age were the most important predictors of BMD [362]. Similarly, in Greenland, age was the most important predictor of fragility fracture independent of family history, smoking, alcohol intake, steroid intake, low sun exposure and dairy intake along with age at menopause [459]. No previous studies have examined the association between BMD and impaired fasting glucose (IFG) and type 2 diabetes mellitus (DM2) among Inuit, both of which increase with adiposity and age.

The prevalence of DM2 used to be rare among Inuit, for example no cases were reported in a population assessment in early sixties [20], but currently it occurs at a rate of at least 12.2% (95% CI: 8.7% to 15.7%) among adults ≥ 50 y [22]. In a meta-analysis and review by Vestergaard, DM2 was associated with higher BMD [57]. Among 30 East Indian postmenopausal women with DM2, the BMD T-score was higher relative to 30 age matched postmenopausal women without DM2 (-2.84 ± 0.42 vs. -3.22 ± 0.74, $P < 0.05$) [460]. The positive association between BMD and DM2 could be related to elevated adiposity as assessed by BMI [305], or hyperinsulinemia which leads to increases in BMD [283, 284, 285].

Despite elevated BMD in DM2, studies report increases in fracture risk at the hip [301,302], proximal humerus [302, 303], foot, and all non-vertebral fractures [302,303,304]. Having First Nations ethnicity was among important predictors of fracture ($P < 0.0001$) in an age, sex and region matched case control study in Manitoba [461]. In that study, having DM2 increased risk of any fracture (rate ratio: 1.26; 95% CI: 1.19 to 1.34), but not at all anatomical sites (hip fracture and spine fracture). Similarly, the incidence of fracture was higher for Inuit women

over 64 y in Alaska relative to white US women and the rate of fracture increased from 1979-89 to 1996-99 [462].

Other factors that accompany IFG and DM2 among Inuit such as nutrition transition, variable traditional food (TF) consumption [463] and associated exposure to contaminants [33], disturbances in adipokines [464] and fatty acids [465] levels have not been studied in relation to BMD among Inuit. These factors associate with DM2 [463, 33,464] and could at the same time be associated with bone health as TF represent a source for polyunsaturated fatty acids [466], vitamin D [362] and contaminants [467] which could influence BMD. In addition higher leptin associates with increased BMD and lower fracture risk, whereas higher adiponectin associates with decreased BMD and increased fracture risk [468]. Data on fracture, BMD and the association with IFG and DM2 in Inuit women is scarce. Hence the goals of this study are: 1) to compare forearm BMD (FaBMD) and FaBMD T-score (FaBMDT) between Inuit women with IFG to age and BMI matched control or with DM2 to age and BMI matched control women; and 2) to assess whether other metabolic factors that accompany IFG and DM2 relate to BMD.

Methods

Participants and Data Collection:

Data was obtained from a cross-sectional survey of Inuit residing in 36 Arctic communities in the late summer and early fall of 2007 (August-September) and 2008 (August-October). Details of the IPY-IHS survey are published in full elsewhere [22]. The survey included all communities in three jurisdictions: Inuvialuit Settlement Region (ISR), Nunavut, and Nunatsiavut. The communities recruited for the study are located between 54° 10' and 74° 43' north. Stratified random sampling was used to select households where communities were strata and where homes were randomly selected using either a computer random generation of numbers or a random digit table [10]. The household participation rate was 68% and 2595 male and non-pregnant female adults were included in the main survey.

Assessment of FaBMD was performed on n=570 women (22.0% of the survey participants) ≥ 40 y of age. Supplemental table 7.1 includes a comparison between women ≥ 40 y of age who had data on BMD relative to women who did not. In comparison with women who did not have FaBMD measurements, women who had this test done were less obese, with higher markers of TF intake as both biological markers such as serum 25 hydroxyvitamin D (25(OH)D) and omega-3 fatty acid content of red blood cells (RBC-Omega-3). Also, red blood cell content of saturated acid (RBC-Sat), red blood cell content of trans-fatty acid (RBC-Tran FA), leptin, adiponectin, medication use, and region were significantly different between women with and without measurements of FaBMD (Supplemental table 1).

From the total survey, women with IFG or DM2 (defined as cases) with data available for FaBMD were matched to control women (absence of IFG or DM2) on the basis of 2:1 control:case and, but with similar age (within 5 years) and body mass index (BMI; within 3 units). This selection criterium yielded 133 cases and 266 controls. Impaired fasting glucose (IFG, n= 41) was defined by values between ≥ 5.6 to 6.9 mmol/L [198], whereas DM2 (n= 72) was ascertained if a participant had a prior diagnosis of DM2; or if the survey results for FPG exceeded 7 mmol/L a new diagnosis was made as was the case for a sub-group of the population undergoing OGTT if 2-h plasma glucose exceeded 11.1 mmol/L [214]. Among adults with a prior diagnosis of DM2: n=21 of 48 were using medications (n=17 were using metformin, n=2 were using glyburide and n=1 was using insulin, in addition to 4 with non-specified type of medications) as some were taking more than one medication at the same time. Ethics for the study was approved from McGill University, Faculty of Medicine Research Ethics Board, the Nunavut Research Institute, and the Aurora Research Institute. Signed informed consent was obtained from each participant [10].

Dietary Assessment

Data on dietary habits was collected using a food frequency questionnaire (FFQ) and 24-h dietary recall administered by trained bilingual (English and Inuit dialects) interviewers. Portion sizes were estimated by food model kits (Santé Quebec). The dietary information was entered into CANDAT Software (Godin London), and nutrient intake generated according to the 2007b Canadian Nutrient File. Nutrient content of food items that were not located in the Canadian Nutrient File, were imported from an additional School of Dietetics and Human Nutrition in-house food file. Food labels, recipes, and other resources such as nutrient values from the U.S. Department of Agriculture were used to estimate nutrient composition that was not otherwise available. The FFQ was customized to capture information within the past year about Inuit TF items that are abundant in regions of ISR, Nunavut and Nunatsiavut. Participants were given pictures to quantify the usual size of their servings. Data for frequency of TF intakes was then expressed per day [352]. Inuit adults were asked about how often in the last year they consumed (in season or off season) TF items from a list of 47 items, or how often they consumed in the last month from a list of 7 store bought items. Data was combined into total TF, total marine mammals and total fish per day.

Clinical Assessment

A portable stadiometer (Road Rod 214 Portable stadiometer, Seca, Maryland, USA) was used to measure height to the nearest 0.1 cm. Body weight was recorded to the nearest 0.1 kg and 0.4 kg was subtracted to account for clothes; body fat % was measured using the same scale (Tanita TBF-300GS with goal setter, Tanita Corporation of America Inc. Arlington Height, Illinois). Participants who had pacemakers had their weight measured by a Seca Scale (Medical Scale Model 214, Seca Corp., Toronto, Ontario), BMI was then calculated**.**

Areal bone mineral density of the distal one third forearm (FaBMD) was evaluated using a peripheral instantaneous x-ray imager (PIXI; GE/Lunar, Fort Myers, FL). Before performance of any assessment, the equipment underwent quality control using the forearm phantom every day; long-term precision error was 0.5 %. In vivo variability was calculated as percent coefficient of variation (CV) by doing three repeated measures on randomly selected patients per day. One of the patients was measured in the beginning of the day and the other at the end of the day; the average CV was 1.7 %. All reference data were collected in the United States using PIXI systems. T-scores were calculated using reference data of ambulatory white premenopausal women who were 20–45 years of age with no history of chronic disease or medications affecting bone and no history of symptomatic, atraumatic fractures. The WHO definition for osteoporosis and osteopenia was used [469]: a FaBMD T score of less than -2.5 was considered as osteoporosis, whereas a FaBMD T score of -1 to -2.5 was considered to be osteopenia and values above -1 considered normal [469]. Fractures and medication use were ascertained from medical records and patients answers. Fractures were classified into low or high trauma by the research team.

Laboratory Analysis

Serum 25(OH)D reflects both endogenous and exogenous vitamin D sources. Serum total 25(OH)D and intact parathyroid hormone (PTH) 1-84 were measured using LIAISON total 25(OH)D and PTH chemiluminescent assays (Diasorin Inc, Stillwater, MN, USA) at McGill University [10]. The inter-assay and intra-assay coefficient of variation (CV) % were 4.5 and 11.1% for the low 25(OH)D control (38.2 nmol/L) and 6.2 and 5.3% for high control (127.2 nmol/L), respectively. The inter-assay CV% for PTH was 19.1 (5.2 pmol/L) for the low control and 8.7 (52.1 pmol/L) for the high PTH control. The accuracy using the mid-range of the manufacturer specifications was 95% for 25(OH)D and 86.7% for PTH. The laboratory that performed the measurement for 25(OH)D was certified by the Vitamin D External Quality Assessment Scheme for the year 2009-2010 which reflects that at least 80%

of the results in this report are within 30% of all laboratory trimmed mean. Osteocalcin was also measured at McGill University using an autoanalyzer (Liaison, Diasorin Inc.). The derived functional sensitivity of osteocalcin is defined as the concentration at which CV% exceeds 20%, the derived functional sensitivity from regression analysis is ≤ 3.0 ng/mL; the inter assay CV was 2.2% [353].

Adiponectin was measured using an Ultra-Sensitive Human Cytokine Assay kit using a Meso Scale Discovery Multi-Array Assay (MSD cat#K151BXC) System and a detection limit of 0.005 ng/mL. Leptin was measured in the serum using a manual sandwich ELISA assay (Linco cat# EZHL-80SJK, Linco Research, St Charles, Missouri) with a detection limit of 0.5 ng/mL. For adiponectin, the intra-assay and inter-assay CV were 5% and 15%, respectively, whereas for leptin, the intra-assay and inter-assay CV were 10%. All of these analyses were conducted by Nutrasource Diagnostics, Guelph, ON [353].

Red blood cell (RBC) membrane fatty acid (FA) profiles, expressed as percent of total FA, were determined [353] using the methodology of Folch et al. [358] and fatty acid methyl esters were prepared by using standard techniques [359] with boron trichloride-methanol to reduce artifact formation [360] and separated on a Varian 3400 GLC with a 60-m DB-23 capillary column (0.32 mm diameter). Methods are described in detail elsewhere [76]. Red blood cell content of saturated fatty acid (RBC-Sat) included FA from 14:0 to 24:0. RBC-Omega-3 included all of the n-3 FA from 18:3 n-3 to 22:6 n-3.

Samples for plasma FPG and serum insulin were kept at 4ºC, until plasma was separated and frozen at -80ºC, then assayed for glucose by Glucose Hexokinase II (GLUH) method [354]. These analyses were conducted by Nutrasource Diagnostics, Guelph, ON.

Statistical Analysis

Continuous variables were checked for normality using Kolmogorov–Smirnov, Shapiro-Wilk, and Anderson Darling tests and descriptive statistics were presented as

proportions for the data, or medians (inter-quartile range; IQR) if not normally distributed. Medians were compared between case and control groups using nonparmetric Wilcoxon two sample test. In addition, adjusted least square means were obtained by mixed multiple regression models and were compared between case and control groups using Bonferroni post hoc testing. The association between study variables and FaBMD and T-scores were checked using spearman correlation adjusted for age. Then the same associations were studied separately for controls, IFG and DM2 groups using multiple linear regression models adjusted for age and BMI, Arctic region and medication use. Final regression models were checked for residual distribution and normality, influential points, and co-linearity using tolerance which is 1/variance inflation factor (VIF). Logistic regression models were used to assess the association between fracture and study variables, or the association between classifications of T-score of FaBMD in relation to case/control group. The fit of logistic regression models were checked using the Hosmer and Lemeshow test. Relationships were considered significant if *P*-value < 0.05, after adjustments for multiple comparisons where appropriate.

Results

This report includes n=72 cases of DM2, n= 41 cases of IFG and n=226 control women. Age, adiposity and anthropometric measures were similar between the IFG and DM2 cases and their respective control groups (Table 7.1). Only serum 25(OH)D and RBC-Omega-3 were slightly lower in controls versus IFG cases whereas intakes of fish, TF and dietary fat were significantly higher in control versus IFG case groups. In DM2 cases versus the respective control group, intake of energy from fat was lower and energy from protein was higher in control versus cases. Usage of hormones, cholesterol medications and supplements were slightly higher in the control group and the distribution of arctic region was significantly different between IFG cases and controls. Thus a model was generated to adjust

for arctic region and medications use (Table 7.2) resulting in no differences between IFG cases and the control group except for TF, fish and RBC-Trans FA which were higher in controls. Fracture was not reported in the control group for IFG, whereas 5 cases of fracture were reported in IFG group. No differences were observed in FaBMD or FaBMD T-scores among cases of DM2 and IFG with their respective controls (Figure 7.1).

With regard to DM2, both RBC-Trans FA and osteocalcin were slightly higher in the control versus DM2 case group. Taking antihypertensive, heart and anticholesterol medications were more common in the DM2 cases versus the respective control group. No differences were observed in FaBMD or FaBMD T-scores among DM2 cases and controls (Figure 7.1). In multiple logistic regression analyses that accounted for arctic region and medications use, not having osteoporosis (being normal relative to osteoporosis) was more common in cases of DM2 relative to their controls (OR: 12.8; 95% CI: 1.5 to 109.9, P= 0.016). Fracture was not different between groups, likewise, the odds ratio for fracture was not significant (OR: 2.79; 95% CI 0.93 to 8.34, P= 0.066) in models adjusted for region and medication use.

The various regressions models explaining FaBMD are summarized in Table 7.3. Each variable was tested in a separate model while accounting for age, BMI, region of the arctic and medication use. This analysis shows that only height and intake of marine mammals were significantly and positively related to FaBMD in the combined control group while adiponectin and fish were significantly positively related FaBMD in DM2. No other study variables were related to FaBMD after adjustment for geographical region and medication use. Repeating this analysis for FaBMDT (Supplemental Table 7.2) similarly showed that BMD is positively associated with marine mammals and height for the controls; and fish and adiponectin for those with DM2. The prevalence of fracture was not significantly associated with any of the studied variables, but was reduced by fish intake

(Supplemental Table 7.3). A description of the anatomical sites of fractures are summarized in (Supplemental Table 4). The most common site of fracture was the wrist.

Discussion

This study is novel in being the first study to address the relationship between IFG, DM2 and their risk factors with bone health measures among Inuit in the Canadian Arctic. For the first time we show in Inuit women ≥ 40 y, that eating TF such as fish and marine mammals could be associated with increased BMD. In fact the intake of fish and TF were higher for Inuit women in this study versus Inuit in the total survey, for fish (women of this study had fish intake (median (IQR) of 0.24 (0.07, 0.47) versus the whole population 0.11 (0.02, 0.30, $P< 0.0001$) which could help explain better FaBMD in this group. Interestingly, FaBMD was not significantly elevated in women with IFG or DM2, this contrasts what others observe in terms of a positive association between DM2 and BMD [57,460]. Nonetheless, we were able to show that it is 12 times more likely for a woman to be free of osteoporosis if she has DM2.

As our design carefully matched for age and BMI, the difference in osteoporosis between cases and controls is not likely related to higher mechanical loading due to higher body weight. It is well known that insulin secretion may increase to compensate for insulin resistance causing hyperinsulinemia in conditions of glucose intolerance [470]. Insulin is known to promote an increase in BMD [471]. It is important to mention that BMD is not an indicator of bone quality, as DM2 is associated with accumulation of end glycated products [306] and increased porosity [307] in bone which may increase the risk of fracture. However, fracture was not related to DM2 and IFG in this study, but further studies are needed to assess the longitudinal impact of impaired glucose metabolism on fracture risk in this group, or to assess the association between BMD and fracture at other skeletal sites. In a study in First Nations adults, the association between DM2 and fracture was most common in hip and

spine, but not wrist and craniofacial sites [461]. Thus future studies should incorporate assessments of the axial skeleton and femoral neck regions.

This study is among very few studies that characterizes BMD among Inuit. In study by Andersen et al. BMD was assessed in the distal forearm and calcaneus and was found to be higher than the BMD measured in forearm in this study. However, the study by Andersen did not address the glucose metabolism aspect and its impact on BMD [55]. Similarly, some of the data in the present analysis stems from previously published work [362], and uniquely addresses glucose metabolism and TF intakes on BMD. Up to the authors' knowledge, there is no other study on the association between IFG or DM2 and BMD among Aboriginal adults in the USA or Canada. A meta-analysis that pooled many studies, regardless of ethnicity which was mainly Caucasian, found no association between FaBMD and DM2, but a 0.04 g/cm^2 increase in BMD at femoral neck and a 0.06 g/cm^2 increase in BMD at the total hip and lumbar spine have been observed in other studies [472]. Similar to our study, a study among Chinese women 40 y or older showed that the rate of BMD-based assessments of osteoporosis is lower in women with DM2 relative to age-matched control [473]. In another study among African, Mexican and Caucasian American men and women, being white was linked to having a positive association between DM2 and BMD, whereas in non-white men there was no significant difference in BMD in DM2 cases versus controls [474]. Hence more studies are needed to clarify the ethnic differences in the association between DM2 and BMD.

Among the variables that were different between case and control groups, only frequency of intake of marine mammals and fish were positively related to BMD, whereas fish was related to lower fracture. Similar to our findings, in postmenopausal Chinese women BMD was 3.2-6.8% higher in top quintile to lowest quintile of sea fish intake [475]. It is well known that marine mammals and fish are rich in omega-3 polyunsaturated FA [476] and

vitamin D [118] that are known to be beneficial to bone health [466,477]. At the same time, marine mammals being higher in the food chain could be more prone to contaminants [478] which negatively impact the bone strength although this was not confirmed in another study of Inuit women [479]. Both serum 25(OH)D and RBC-Omega-3 were not related positively to FaBMD among our Inuit women. It is, however, important to highlight the biological marker such as RBC-Omega-3 and 25(OH)D reflects a shorter exposure relative to the one year assessment of TF intake.

The observation that RBC-Trans FA, TF and fish intakes were significantly higher in controls versus the case group of IFG, whereas both RBC-Omega-3, marine mammal intake and serum 25(OH)D were not different was unexpected. We cannot preclude the hypothesis that specific parts of animals that contain higher amounts of omega-3 fatty acids and vitamin D such as the blubber [480] and liver [481] of marine mammals and caribou could be also rich in saturated fat at the same time. In a study of TF food samples caribou and seal meat had similar content of fat to beef [482]. In fact, both TF intake and fat intake were higher in control versus cases of IFG. We did not examine which type of mammal and which specific mammals were eaten between cases and controls. The type of food eaten could be affected by arctic region too as we showed that Inuit intake of TF differed by region [483]. Furthermore, this study indicates that fish intake was higher in the control group relative to the adult and was associated with lower fracture, which emphasizes the fact that the benefits of TF, especially fish which is lower in food chain should be highlighted as source for omega-3 and vitamin D.

This study is not without limitations including the small sample size and inclusion of women 40 y of age and older. In addition, the only assessment for bone that was available was FaBMD, the study of other sites is definitely needed to build upon the results of this study. In addition, the FFQ assessed the frequency of TF over a year based on recall and may

thus not have accurately captured the variability of food intake. The assessment of other biomarkers of bone metabolism would have improved our understanding of the relationship between bone health, IFG and DM2. Finally, data on the duration of DM2 was not available.

In summary, in this nested case control study we found that FaBMD and FaBMD T-scores were not different between cases of DM2 and IFG compared to matched control groups. However osteoporosisas defined by a T-score of -2.5 or less, was less common in women with DM2 than those who did not have DM2. Only frequency of fish and marine mammal intakes were associated positively with FaBMD, whereas a higher fish intake was associated with less prevalent fractures. It seems that TF and especially fish is protective from IFG and DM2 [484], and may confer protection to bone as well. Therefore, the intake of TF, particularly fish should be encouraged among Inuit women.

Table 7.1 Comparison between case and control groups.

Variable	n	Case IFG	n	Control	n	Case DM2	n	Control
Age (y)	41	52 (48, 57)	82	52 (46, 56)	72	56 (48.5, 62.5)	144	54 (47, 62)
Height (m)	41	1.53 (1.50, 1.55)	82	1.52 (1.49, 1.58)	72	1.53 (1.49, 1.56)	144	1.52 (1.49, 1.57)
Adiposity Indicators								
BMI (kg/m^2)	41	29.3 (23.9, 33.4)	82	28.4 (24.2, 33.8)	72	30.7 (27.0, 36.3)	144	31.7 (26.9, 35.6)
Waist circumference (cm)	39	92.0 (81.0, 106.2)	81	94.0 (82.1, 104.0)	72	100.0 (91.5, 110.0)	141	98.0 (89.0, 108.0)
FM (%)	41	38.4 (28.7, 43.2)	82	37.5 (29.4, 42.4)	72	40.2 (36.7, 45.2)	144	40.8 (34.4, 44.9)
Biochemistry								
Fasting plasma glucose (mmol/L)	41	5.8 (5.7, 5.9)	76	4.8 (4.6, 5.1)***	69	6.0 (5.3, 6.9)	139	5.0 (4.7, 5.3)***
Fasting serum insulin (μIU/mL)	40	12.0 (6.9, 16.8)	74	7.4 (4.6, 15.3)*	69	13.1 (8.2, 18.7)	132	8.6 (5.5, 13.5)***
RBC-Trans FA (%)	41	0.95 (0.60, 1.18)	78	1.40 (1.15, 1.88)**	70	1.02 (0.70, 1.50)	140	1.20 (0.85, 1.60)*
RBC-Omega-3 (%)	41	8.1 (6.5, 10.5)	78	7.0 (5.3, 9.7)*	70	7.5 (5.8, 10.4)	140	7.7 (5.6, 9.5)
RBCSAT (%)	41	41.7 (40.0, 42.7)	78	41.6 (38.2, 45.3)	70	42.4 (39.8,4 5.2)	140	42.1 (40.2, 44.0)
Serum 25(OH)D (nmol/L)	41	77.6 (59.4, 92.9)	78	65.3 (52.9, 88.9)*	69	79.1 (59.2, 114.3)	142	75.8 (55.7, 95.8)
PTH (pmol/L)	40	4.3 (3.2, 5.3)	78	4.5 (3.7, 5.7)	68	4.3 (3.6, 5.2)	139	4.2 (3.1, 5.7)
Adiponectin **(**μg/L)	31	11.4 (6.7, 13.5)	78	11.8 (8.1, 18.8)	59	9.5 (6.1, 14.0)	125	11.0 (7.5, 17.6)
Leptin **(**μg/L)	31	18.8 (7.3, 33.9)	78	18.3 (9.4, 33.9)	59	27.6 (14.4, 42.7)	125	26.6 (13.7, 39.1)
24-h Dietary Recall								
Vitamin D (μg)	36	3.4 (1.7, 6.1)	80	4.2 (1.5, 9.0)	64	3.9 (1.9, 6.6)	128	3.7 (1.5, 8.1)
Calcium (mg)	36	329.0 (212.8, 476.9)	80	399.7 (249.5, 598.3)	64	413.4 (276.8, 680.4)	128	360.0 (206.4, 512.0)
Total Energy (kcal)	36	1457.5 (1053.9, 2482.4)	80	1723.3 (1251.8, 2624.6)	64	1685.4 (1297.8, 2220.2)	128	1719.0 (1109.4, 2252.6)
% Energy from protein	36	19.8 (15.1, 26.2)	80	19.1 (12.2, 28.1)	64	18.8 (15.4, 22.1)	128	21.9 (15.3, 29.8)*
%Energy from CHO	36	46.6 (31.3, 57.1)	80	42.8 (28.9, 57.2)	64	42.5 (33.1, 52.2)	128	44.5 (31.4, 55.7)
% Energy from fat	36	29.5 (22.8, 34.2)	80	32.7 (27.7, 41.1)*	64	35.9 (26.7, 43.7)	128	30.8 (22.3, 38.7)*
FFQ (per day)								
Total TF	35	0.76 (0.38, 1.21)	80	1.31 (0.67, 2.17)**	66	1.16 (0.60, 1.89)	125	0.97 (0.55, 1.94)
MM	35	0.14 (0.04, 0.34)	80	0.06 (0.02, 0.30)	66	0.14 (0.02, 0.61)	125	0.11 (0.02, 0.35)
Fish	35	0.10 (0.03, 0.24)	80	0.31 (0.10, 0.86)***	66	0.10 (0.03, 0.24)	125	0.31 (0.10, 0.86)

Medications								
HTN	9/41	22%	12/82	14.6%	35/72	48.6%	29/144	20.1%***
Heart	0/41	0	3/82	3.7%	6/72	8.3%	1/144	0.7%*
Hormones	1/41	2.4%	6/82	7.3%*	10/72	13.9%	10/144	6.9%
Cholesterol	6/41	14.6%	3/82	3.7%***	24/72	33.3%	16/144	11.1%***
Steroids	1/41	2.4%	3/82	3.7%	4/72	5.6%	2/144	1.4%
Nutrition Supplements	1/41	2.4%	12/82	14.6%*	12/72	16.7%	23/144	16%
Region								
Nunavut	36/41	87.8%	64/82	78.1%*	49/72	68.1%	110/144	76.4%
Inuvialuit Settlement Region	0/41	0	12/82	14.6%	3/72	4.2%	12/144	8.3%
Nunatsiavut	5/41	12.2%	6/82	7.3%	20/72	27.8%	22/144	15.3%

Data are median (IQR) for continuous variables and as proportions for categorical variables.
Comparisons were performed by non-parametric measurements for continuous variables and by
Chi-square for categorical variables. *P< 0.05, **P< 0.001, ***P< 0.0001.
Antihypertensive and heart agents included medications such as diuretics, calcium channel blockers, beta blockers and angiotensin converting enzymes (e.g. hydrochlorothiazide, nifedipine, atenolol, and enalapril); cholesterol lowering agents mainly HMG-CoA inhibitors (e.g Atorvastatin); hormones included thyroxin, estrogens and progesterone as birth control (e.g., Eltroxin, estradiol, and provera); steroids were mainly prednisolone and topical corticosteroids; nutrition supplements included, calcium and vitamins. BMI, body mass index; DM2, type 2 diabetes mellitus; FM, fat mass; FFQ, food frequency questionnaire, HTN, hypertension; 25(OH)D, 25-hydroxyvitamin D; IFG, impaired fasting glucose; MM, marine mammals; PTH, parathyroid hormone; RBC-Omega-3,Omega-3 fatty acid content of red blood cells; RBC-SAT, saturated fatty acid content of red blood cells; RBC-Trans, trans fatty acids content of red blood cells; TF: traditional food.

Table 7.2 Adjusted comparison of study variables between women in case group versus control group.

Variable	n	IFG Cases	n	Controls	n	DM2 Cases	n	Controls
Biochemistry								
Serum 25(OH)D (nmol/L)	41	80.8 ± 9.0	78	72.1 ± 8.3	69	78.8 ± 7.0	142	75.0 ± 7.0
PTH (pmol/L)	40	4.1 ± 0.7	78	4.5 ± 0.7	68	4.9 ± 0.5	139	5.0 ± 0.5
Osteocalcin (nmol/L)	33	2.1 ± 0.2	6	2.4 ± 0.4	42	1.9 ± 0.5	84	1.9 ± 0.4
Bone Mineral Density								
FaBMD (g/cm^2)	41	0.430 ± 0.030	82	0.420 ± 0.03	72	0.440 ± 0.020	144	0.420 ± 0.020
FaBMD-T Score	41	-0.920 ± 0.490	82	-1.18 ± 0.46	72	-0.070 ± 0.260	144	-0.130 ± 0.27
WHO Classification of T-Score[#]								
Normal	30/41	73.3%	51/82	62.26%	52/72	72.2%	91/144	63.2%*
Osteopenia	7/41	17.2%	19/82	23.2%	19/72	26.4%	38/144	26.4%
Osteoporosis	4/41	9.8%	12/82	14.6%	1/72	1.4%	15/144	10.4%
Overall Fracture[#]								
Total	5/41	12.2%	0/82	0*	7/72	9.7%	28/144	9.7%
Unspecified	1/41	2.4%	0/82	0*	1/72	1.4%	8/144	5.6%
Low Trauma	2/41	4.9%	0/82	0	3/72	4.2%	12/144	8.3%
High Trauma	2/41	4.9%	0/82	0	3/72	4.2%	8/144	5.6%

Data are mean ± SE. Means are adjusted for region (Nunavut, Inuvialuit Settlement Region, Nunatsiavut), hypertensive medications, heart and cholesterol medications. Means were compared using post-hoc Bonferroni testing. [#] Data are presented as unadjusted proportions which means that data is the prevalence rate without adjustment for any variable including region and medications use. FaBMD,forearm bone mineral density; FaBMD-T, T-score of forearm bone mineral density; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone. *P< 0.05,**P< 0.001,***P< 0.0001.

Table 7.3 Beta coefficient with 95 % confidence intervals of multiple regression associations between FaBMD with dietary markers and adipokines.

Variable[1]	n	Control β-coefficient (95% CI), *P*-value	n	IFG β-coefficient (95% CI), *P*-value	n	DM2 β-coefficient (95% CI), *P*-value
Height	226	0.233 (0.066, 0.400), *P*=0.007	41	-0.21 (-0.60, 0.18), *P* =0.296	72	0.031 (-0.230, 0.294), *P* =0.819
RBC-Trans FA (%)	218	-0.008 (-0.022, -0.006), *P* =0.199	41	0.025 (-0.004, 0.054), *P* =0.101	70	-0.007 (-0.021, 0.007), *P* =0.320
RBC-Omega-3 (%)	218	-0.0007 (-0.004, 0.002), *P* =0.680	41	0.0001 (-0.006, 0.006), *P* =0.972	70	0.003 (-0.001, 0.007), *P* =0.279
RBC-SAT (%)	218	0.002 (0.0001, 0.004), *P* =0.217	41	0.003 (-0.003, 0.009), *P* =0.248	70	0.0003 (-0.003, 0.003), *P* =881
Adiponectin (μg/L)	203	0.0004 (-0.0006, 0.002), *P* =0.551	31	-0.002 (-0.007, 0.003), *P* =0.240	59	-0.002 (-0.004, -0.0001), *P* =0.006
Leptin (μg/L)	203	0.001 (-0.0006, 0.003), *P* =0.135	31	-0.002 (-0.004, -0.0001), *P* =0.116	59	-0.0005(-0.001, 0.0003), *P* =0.272
Serum 25(OH) per 30 nmol/L	220	0.001 (-0.011, 0.013), *P* =0.877	41	0.005 (-0.018, 0.028), *P* =0.692	69	0.008 (-0.004, 0.020), *P* =0.199
FFQ (per day)						
Total TF	205	0. 008 (-0.004, 0.020), *P* =0.134	35	-0. 003 (-0.030, 0.024), *P* =0.847	66	0. 010 (-0.006, 0.026), *P* =0.233
MM	205	0.048 (0.015, 0.081), *P* =0.005	35	0.022 (-0.045, 0.089), *P* =0.528	66	0.036 (-0.005, 0.077), *P* =0.126
Fish intake	205	-0.010 (-0.041, 0.021), *P* =0.535	35	0.024 (-0.135, 0.183), *P* =0.769	66	0.057 (0.002,0.112), *P* =0.043

1 Each variable in the following table was included separately in a model adjusted for age, BMI, region and medication use

Multiple regression models are adjusted for age (y), BMI (kg/m^2), region (Nunavut, Inuvialuit Settlement Region, Nunatsiavut), hypertensive medications, heart and cholesterol medications.

25(OH)D, 25-hydroxvitamin D; MM, marine mammals; RBC-Omega-3, Omega-3 fatty acid content of red blood cells; RBC-SAT, saturated fatty acid content of red blood cells; RBC-Trans FA: trans fatty acid content of red blood cells; TF, traditional food.

Supplemental Tables:

Supplemental Table 7.1 Comparison of study variables between women ≥40 y who have or do not have data on BMD.

Variable	n	BMD Group	n	no BMD Group	*P* -value
Age (y)	570	51 (45, 60)	252	50 (44, 62)	<0.315
Adiposity Indicators					
BMI (Kg/m^2)	564	28.9 (23.9, 34.0)	125	30.2 (26.4, 35.5)	0.009
Waist circumference (cm)	557	94.0 (81.0, 105.6)	121	100.0 (91.0, 109.0)	<0.0001
FM (%)	566	38.0 (29.2, 43.2)	125	40.1 (34.5, 45.2)	0.0005
Biochemistry					
RBC-Trans FA (%)	556	1.15 (0.80, 1.55)	157	1.54 (1.18, 2.03)	<0.0001
RBC-Omega-3 (%)	556	7.6 (5.5, 9.6)	157	2.13 (0.87, 5.77)	<0.0001
RBCSAT (%)	556	41.6 (39.6, 43.9)	157	51.0 (44.1, 54.7)	<0.0001
Serum 25(OH)D (nmol/L)	557	73.6 (54.4, 95.1)	157	60.7 (41.9, 80.6)	<0.0001
PTH (pmol/L)	547	4.2 (3.3, 5.5)	157	4.3 (3.5, 5.7)	0.206
Adiponectin **(**µg/L)	491	11.1 (7.3, 17.7)	150	9.4 (6.0, 15.9)	0.005
Leptin **(**µg/L)	488	19.1 (9.2, 34.1)	150	26.9 (14.4, 41.2)	0.0001
Osteocalcin **(**nmol/L)	308	1.8 (1.5, 2.4)	72	2.0 (1.5, 2.5)	0.186
DM2	565	85/565 (15%)	799	42/799 (5.3%)	p<0.0001
FFQ (per day)					

Total TF		512	1.0 (0.46, 1.80)	134	0.71 (0.20, 1.66)	0.0005
MM		512	0.10 (0.02, 0.35)	134	0.07 (0.01, 0.29)	0.010
Fish		512	0.18 (0.07, 0.43)	763	0.05 (0.005, 0.29)	<0.0001
Medications						
HTN		570	126/570 (22.1%)	252	41/252 (16.3%)	0.055
Heart		570	14/570 (2.5%)	252	3/252 (1.2%)	0.240
	Hormones	570	39/570 (6.8%)	252	16/252 (6.4%)	0.925
	Cholesterol	570	67/570 (11.8%)	252	12/252 (4.7%)	0.002
Steroids		570	14/570 (2.5%)	252	2/252 (0.8%)	0.111
Nutrition Supplements		570	76/570 (13.3%)	252	41/252 (8.4%)	0.042
Region		570				
Nunavut			429/570 (75.3%)	252	129/252 (51.2%)	<0.0001
Inuvialuit Settlement Region			37/570 (6.5%)	252	102/252 (40.5%)	
Nunatsiavut			104/570 (18.3%)	252	21/252 (8.3%)	

Data are median (IQR) for continuous variables and as proportions for categorical variable.
Comparison is performed by non-parametric measurement for continuous variables and by Chi-square for categorical variables.
Antihypertensive and heart agents included medications such as diuretics, calcium channel blockers, beta blockers and angiotensin converting enzymes (e.g. hydrochlorothiazide, nifedipine, atenolol, and enalapril); cholesterol lowering agents mainly HMG-CoA inhibitors (e.g Atorvastatin); hormones included thyroxin, estrogens and progesterone as birth control (e.g Eltroxin, estradiol, and provera); steroids were mainly prednisolone and topical corticosteroids; nutrition supplements included iron, calcium and vitamins. BMI, body mass index; DM2, type 2 diabetes mellitus; FM, fat mass; FFQ, food frequency questionnaire, HTN, hypertension; 25(OH)D, 25-hydroxyvitamin D; MM, marine mammals; PTH, parathyroid hormone; RBC-Omega-3,Omega-3 fatty acid content of red blood cells; RBC-SAT, saturated fatty acid content of red blood cells; RBC-Trans, trans fatty acids content of red blood cells; TF: traditional food.

Supplemental Table 7.2 Beta coefficient with 95 % confidence intervals of multiple regression associations between FaBMD-T score with dietary markers and adipokines.

Variable[1]	**n**	**Total Control**	**n**	**IFG**	**n**	**DM2**
		β-coefficient (95% CI), *P*-value		**β-coefficient (95% CI), *P*-value**		**β-coefficient (95% CI), *P*-value**
Height	226	3.97 (1.21, 6.73), *P*= 0.005	41	-3.52 (-10.09, 3.05), *P*= 0.303	72	0.49 (-3.90, 4.88), *P*= 0.827
RBC-Trans FA (%)	218	-0.15 (-0.36, -0.06), *P*= 0.176	41	0.42 (-0.07, 0.91), *P*= 0.101	70	-0.11 (-0.33, 0.11), *P*= 0.328
RBC-Omega-3 (%)	218	-0.010 (-0.069 ,0.049), *P*= 0.737	41	0.001 (-0.093, 0.095), *P*= 0.978	70	0.043 (-0.033, 0.119), *P*= 0.283
RBCSAT (%)	218	0.025 (-0.016, 0.066), *P*= 0.231	41	0.052 (-0.032, 0.136), *P*= 0.244	70	0.005 (-0.056, 0.066), *P*= 0.875
Adiponectin (μg/L)	203	0.007 (-0.015, 0.029), *P*= 0.522	31	-0.034 (-0.095, 0.027), *P*= 0.269	59	-0.038 (-0.063, -0.013), *P*= 0.007
Leptin (μg/L)	203	0.011 (-0.003, 0.025), *P*= 0.131	31	-0.028 (-0.061, 0.005), *P*= 0.116	59	-0.008 (-0.023, 0.007), *P*= 0.294
Serum 25(OH)D per 30 nmol/L	220	0.016 (-0.182, 0.214), *P*= 0.877	41	0.093 (-0.32, 0.506), *P*= 0.664	69	0.139 (-0.069, 0.347), *P*= 0.197
FFQ (per day)						
Total TF (per d)	205	0.15 (-0.03, 0.33), *P*= 0.117	35	-0.06 (-0.52, 0.41), *P*= 0.815	66	0.17 (-0.10, 0.44), *P* = 0.224
MM (per d)	205	0.80 (0.25, 1.35), *P*= 0.005	35	0.57 (-0.12, 1.26), *P*= 0.552	66	0.60 (-0.15, 1.35), *P*= 0.120
Fish intake (per d)	205	-0.15 (-0.70, 0.40), *P*= 0.581	35	0.37 (-2.31, 3.05), *P*= 0.789	66	0.97 (0.07, 1.87), *P* = 0.040

[1]Each variable in the following table was included separately in a model adjusted for age, BMI, region and medication use.
Multiple regression includes BMI (kg/m^2) and age (y), region (Nunavut, Inuvialuit Settlement Region, Nunatsiavut), hypertensive medication, heart medications and cholesterol medications.
25(OH)D, 25-hydroxvitamin D; MM, marine mammals; RBC-Omega-3, Omega-3 fatty acid content of red blood cells; RBC-SAT, saturated fatty acid content of red blood cells; RBC-Trans FA: trans fatty acid content of red blood cells; TF, traditional food.

Supplemental Table 7.3 Odds ratio and 95 % confidence intervals of multiple logistic regression associations between **no fracture** with dietary markers and adipokines.

		Control		DM2
Fracture [1]	**n**	**OR (95% CI), *P-value***	**n**	**OR (95% CI), *P-value***
RBC-Trans FA (%)	218	1.21 (0.65, 2.25), *P*= 0.543	70	3.56 (0.40, 31.6), *P*= 0.254
RBC-Omega-3 (%)	218	1.02 (0.89, 1.18), *P*= 0.748	70	1.43 (0.95, 2.14), *P*= 0.086
RBCSAT (%)	218	0.98 (0.88, 1.10), *P*= 0.762	70	1.03 (0.77, 1.37), *P*= 0.855
Adiponectin (μg/L)	203	1.02 (0.97, 1.08), *P*= 0.364	59	0.86 (0.72, 1.03), *P*= 0.102
Leptin (μg/L)	203	0.99 (0.95, 1.03), *P*= 0.504	59	1.00 (0.89, 1.12), *P*= 0.979
Serum 25(OH)D per 30 nmol/L	220	0.85 (0.52, 1.38), *P*= 0.500	69	1.64 (0.63, 4.31), *P*= 0.310
FFQ (per day)				
Total TF	205	1.27 (0.76, 2.11), *P*= 0.356	66	1.25 (0.36, 4.29), *P*= 0.722
MM	205	0.97 (0.24, 3.93), *P*= 0.970	66	5.89 (0.38, 92.36), *P*= 0.206
Fish intake	205	21.28 (2.39, 189.63), *P*=0.006	66	0.18 (0.006, 5.44), *P*= 0.328

[1]Each variable in the following table was included separately in model adjusted for age, BMI, region and medication use. Multiple regression includes BMI (kg/m^2) and age (y), region (Nunavut, Inuvialuit Settlement Region, Nunatsiavut), hypertensive medication, heart medications and cholesterol medications. 25(OH)D, 25-hydroxvitamin D; MM, marine mammals; RBC-Omega-3, Omega-3 fatty acid content of red blood cells; RBC-SAT, saturated fatty acid content of red blood cells; RBC-Trans FA: trans fatty acid content of red blood cells; TF, traditional food.

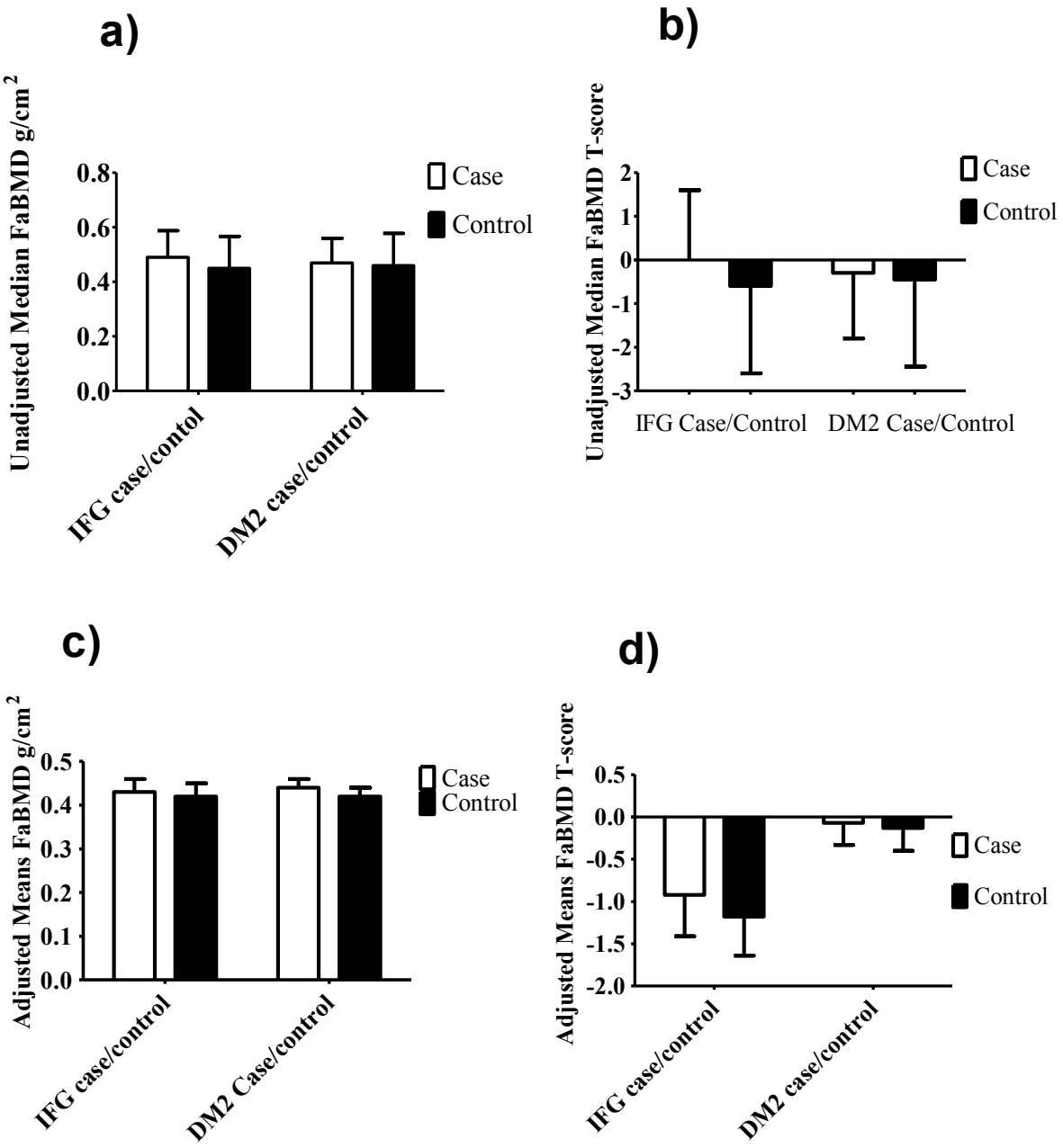
Supplemental Table 7.4 Anatomincal sites of fracture in cases (DM2, IFG) versus controls

Type of fracture	Total Control	DM2	IFG
Ankle	4	2	0
Arm	1	1	2
Clavicle	3	0	0
Fibula	1	0	0
Foot	2	1	0
Forearm	1	0	0
Jaw	1	0	0
Hip	1	0	0
Knee	0	0	1
Leg	0	1	0
Multiple	1	0	0
Shoulder	1	0	0
Spine	1	0	0
Tibia	0	0	1
Wrist	10	1	0
Unknown	1	1	1

DM2, type 2 diabetes mellitus; IFG, impaired fasting plasma glucose.

Figure 7.1. Comparison of FaBMD and FaBMD T-scores between cases (of DM2 relative to controls and IFG relative to controls). Adjusted multivariate mixed regression models accounted for region (Nunavut, Inuvialuit Settlement Region, Nunatsiavut), hypertensive medications, heart medications and cholesterol medications.The variables were compared using Bonferroni post hoc test for least mean squares. The comparison group consisted of IFG cases (n=41), control for IFG (n=82), DM2 (n=72), control for DM2 (n=144).

Figure 7.1.


a)
Unadjusted Median FaBMD g/cm 2
0.8
0.6
0.4
0.2
0.0
Case
Control
IFG case/contol
DM2 case/control
b)
Unadjusted Median FaBMD T-score
2
1
0
-1
-2
-3
Case
Control
IFG Case/Control
DM2 Case/Control
c)
Adjusted Means FaBMD g/cm 2
0.5
0.4
0.3
0.2
0.1
0.0
Case
Control
IFG case/control
DM2 Case/control
d)
Adjusted Means FaBMD T-score
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Case
Control
IFG case/control
DM2 case/control

Bridge 6

Two of the studies in this thesis collectively indicate an inverse association between vitamin D status and insulin resistance and deteriorated metabolic profile. In fact, there is a consistent inverse association between vitamin D status and obesity in the literature that could be related to many plausible physiological mechanisms that are discussed in this thesis. However, vitamin D status could be a marker of TF which although still protective from adverse lipid profile as it was seen in terms of the relationship between TF and LDL/HDL and METS. In addition, the relationship between osteocalcin, bone health and DM2 and insulin resistance was elaborated in this thesis in Inuit women. The studied group of Inuit women seemed to have healthy bone that is not affected by the complication of DM2 probably due to higher intake of TF in general, particularly fish which was positively associated with BMD.

The association between insulin resistance and vitamin D status should be viewed in the context of racial differences among various ethnic groups. Race is related to physiological differences in various aspects of vitamin D, obesity, bone metabolism and insulin resistance. However, various factors could interfere such as cultural and sociodemographic factors, geographical location that could affect dietary sources, physical activity and even sun exposure.

Thus in total, the analysis in this work highlighted the importance of both vitamin D status and TF, particularly fish in the metabolic and skeletal health of Inuit residing Canadian arctic region, yet it has to be emphasized that Inuit in arctic regions were not similar in terms of metabolic and dietary markers. The analysis performed was rigorous in terms of accounting for various markers of obesity and diet. It opens the door for studies, particularly clinical to try to understand the importance of vitamin D in both metabolic and skeletal health using different methods.

CHAPTER 8: Discussion

8.1 Significance and Hypothesis Tested.

It is projected that 3.7 million Canadians will have type 2 diabetes mellitus (DM2) by 2018 which is almost 10% of Canadians in contrast to 2008/2009, when only 6.8 % of Canadians were diagnosed with DM2 [485]. In particular, the prevalence of DM2 among Aboriginal communities undergoing continuous transition toward a greater burden of chronic disease is of concern. Despite the fact that Inuit were found to have similar trends of DM2 to the general population (incidence of 10%) [485], this should be viewed in the context of previous rarity of the disease among this population in the 1960(s) with incidence of almost zero [20]. Thus efforts should be made to identify the possible root causes towards such shifting trends in the DM2 burden among the Inuit population. This is especially important as DM2 is associated with premature death especially due to cardiovascular disease (CVD) and renal failure, in addition to increased cost to quality of life and the health care system [486]. Although not focused on Inuit populations per se, a study by Naqshbandi et al. showed that complications of DM2 are especially higher in the Indigenous population in general worldwide [24].

DM2 is a disease that consists of multiple disturbances including mainly insulin resistance in multiple tissues and progressive decline of β-cell function [487]. In this thesis, a major aim was to study the association between vitamin D status and traditional food (TF) with markers of insulin resistance. First, it was hypothesized that insulin resistance markers including fasting plasma glucose (FPG) and homeostatic model of insulin resistance (HOMA-IR) would be inversely associated with both vitamin D status among Inuit who participated in the International Polar Year Inuit Health Survey (IPY-IHS). In addition, it was hypothesized that adiponectin would relate inversely to insulin resistance, while leptin would relate positively to insulin resistance. Second, it was hypothesized that both vitamin D

status and TF would be inversely related to metabolic syndrome (METS) and its individual components. METS confers a 5 fold increase in the risk of DM2 and 2 fold increase in the risk for CVD over 5 to 10 y [383,384]. This is consistent to the finding in this thesis that METS is associated with 5 times higher odds for having new DM2. Insulin resistance in addition to central obesity are thought to be the underlying causes behind the clustering of METS risk factors [383,384]. It was also hypothesized that osteocalcin will be related inversely to FPG and HOMA-IR. Finally it was hypothesized that DM2, impaired fasting glucose and risk factors associated with DM2 would be associated with increases in forearm bone mineral density (FaBMD).

8.2 Major Findings

In the first study in adults without diagnosed DM2, vitamin D status was inversely associated with HOMA-IR, but not FPG. The negative association between insulin resistance and vitamin D status was significant even upon adjustment with covariates including BMI, waist circumference (WC) and fat mass percent (FM%), fatty acid content of red blood cell membranes (RBC), marine mammal and fish intakes and land claim region of the Arctic. Thus, the first hypothesis is partially accepted, whereas the second hypothesis with regard to the relationship between insulin resistance and TF is rejected as greater frequency of marine mammal intakes were associated with deterioration in insulin resistance as assessed by FPG and HOMA-IR. In contrast, fish consumption was not associated with impaired insulin resistance. It was interesting that trans fatty acids (RBC-Trans) were inversely associated with insulin resistance; an observation that requires further study to explain.

In the second study, serum 25-hydroxyvitamin D (25(OH)D) was not associated with METS and DM2, but lowered high waist circumference (WC), triglyceride (TG), and increased high low density lipoprotein (LDL) and low high density lipoprotein (HDL) odds, whereas TF was not related to METS and high low density lipoprotein to HDL ratio

(LDL/HDL) ratio. Thus the hypothesis is rejected. Again fish intake was neutral and was not associated with METS or serum lipids and lowered the odds of having a high LDL/HDL ratio and thus represented a healthy source for vitamin D. In both the first and second study, arctic region was found to be significantly related to insulin resistance and metabolic outcomes with healthiest communities located in Nunavut.

Aside from the association of vitamin D with FPG and insulin resistance, the part of the hypothesis regarding the relationship between insulin resistance and leptin and adiponectin could be accepted. The role of adipokines in insulin resistance could indicate the central significance obesity has in the transition towards chronic diseases among Inuit adults living in the Arctic. This could be useful in selecting pharmacological agents that could help to modify adipokine release to treat DM2 among Inuit. It is important to emphasize that the analysis for chapter 4 was performed for adults without known or newly diagnosed DM2. In chapter 6, excluding adults with DM2 did not show any difference (data not shown) in the association between insulin resistance and adipokines.

In the third study osteocalcin was not related to insulin resistance as assessed by HOMA-IR and FPG in all adjusted models, thus the hypothesis was rejected. Both HOMA-IR and osteocalcin were low indicating probably low bone turnover that is associated with healthy insulin resistance. Including leptin and adiponectin in the studied relationship did not change the significance of the results. Obesity as assessed by BMI, WC and FM% were the only driving factors for insulin resistance. Similarly, FaBMD was not different between cases of DM2 and IFG and controls and thus hypothesis is rejected. However, having DM2 was associated with lower odds for having osteoporosis. In addition, fish and marine mammal were associated positively with BMD and fish was related to lower fracture.

A common theme across all of the studies in this thesis was to test for benefits of TF overall as well as according to sources of both vitamin D and omega-3 fatty acids, marine

mammals or fish. It was observed that marine mammal intakes were positively associated with FPG, HOMA-IR and LDL/HDL ratio whereas fish intake was not associated with insulin resistance, FPG, METS and serum lipids but associated with a lower LDL/HDL ratio. However both marine and fish intakes had a beneficial relationship with BMD among women. In fact, older women tend to consume more TF and DM2 increase with age, this could explain the observation that DM2 was associated with lower odds for osteoporosis. Our findings highlight the importance of fish as neutral source for both vitamin D and omega-3 fatty acids.

8.3 The Association between Vitamin D, TF and Insulin Resistance

Consistent with this study, other studies in Caucasians and multiethnic groups showed inverse associations between vitamin D status and insulin resistance markers [11,50,178,217,228,232]. However, in other ethnic groups the association between insulin resistance and vitamin D status was not observed in some groups including African Americans [40], and Cree Canadians [17], but was present in two other studies among Arab Americans [231] and Korean adults [233]. The variations in the observations do not seem to be related to divergent vitamin D status among populations as vitamin D status in this thesis research and in the study among a Cree population [17] were comparable to many studies that found significant relationships [229,232], but lower than values reported for 25(OH)D) in the Canadian Health Measures Survey 2007 to 2009 (67.7; 95% CI 65.3 to 70.1 nmol/L) [488]. It is noteworthy that in this thesis, vitamin D source in Inuit could have modified the results, especially with regard to FPG. Vitamin D status in this group is controlled by intake of TF such as marine mammals which was positively linked to FPG.

Most of the studies done on nutrition transition in the Arctic have addressed the fact that nutrition transition affects nutrient intakes including many micronutrients such as vitamin D, iron, zinc, copper, magnesium, manganese, phosphorus, potassium and diet

quality such as higher intake of added sugar with a modern diet, in addition to increased rates of obesity associated with poor quality market food [31]. However, to the best of the author's knowledge very few studies addressed the association between insulin resistance and nutrition transition among Inuit and this is important as it affects the wellbeing of young and future generations of Inuit. The intake of TF as assessed by marine and fish intakes among Inuit seemed to play multiple contradictory roles in this thesis:

- First, less TF intake could be an indicator of nutrition transition in the younger generation and more TF intake among older age group. Chapter 4 showed that vitamin D status is inversely associated with HOMA-IR. This association indirectly reflects the possibility that higher vitamin D status could be a response to higher consumption of TF which is indicative of less nutrition transition and also less insulin resistance. Hence consumption of TF such as fatty fish even among overweight and obese adults should be emphasized.
- Second, higher TF and hence higher vitamin D status was more prevalent in the older group. This may indicate that age overrides the inverse association between vitamin D status and FPG.
- Third, TF is rich in omega-3 fatty acids and many other nutrients that are linked to insulin resistance [489,490]. Thus the model was adjusted for RBC-Omega-3 as both an indicator of TF intake and nutrition transition; as such some of the interpretations were slightly modified by this adjustment especially FPG which was inversely related to RBC-Omega-3.
- Finally, it is hard to understand why marine mammal intake was related to increases in FPG, but this finding seems to be consistent with the literature. In a study by Jeppesen et al. Inuit in Greenland who consumed TF had higher

FPG (mean 5.73 (95% CI: 5.68 to 5.78 mmol/L, $P< 0.0001$) and higher odds for IFG and DM2 [491]. Also, in a study among 835 First Nations adults from the James Bay region of northern Québec, serum insulin increased with increases of both TF and snack foods of high energy and low nutrient density [492]. This could be related to contamination of TF as among Inuit in Greenland, persistent organic pollutants were associated with a decrease in stimulated insulin concentration and homeostatic model of beta cell function [493]. Indeed, our results indicated TF and marine intake were associated with an increase in FPG, whereas fish intake was not associated with deteriorated FPG.

The finding with regard to a positive association between FPG and HOMA-IR with marine mammal intakes raises the concern of possible contamination of natural resources in the Arctic. Methyl mercury, arsenic, persistent organic pollutant could be considered as endocrine disruptor that contributes to obesity and DM2 in the Canadian Arctic [33]. Consequently, not only the intake of TF is important, but possibly the way it is eaten. Further research regarding consumption of muktuk with whale blubber and how they affect nutrients and contaminants intake is needed. Skin of animals such as mattak of white whale has high content of selenium [494]. Selenium and particularly naturally occurring compounds in the skin of whales was shown to protect from contaminants such as methyl mercury [495]. In the present thesis, marine mammals intake did not distinguish among the various edible components: skin, blubber, or meat. The intake of marine mammals and the way it is consumed in association with glucose metabolism needs further research. The same is true for other sources of TF. For example, land animals such as caribou could be very different than marine animals such as whale and seal in terms of nutrient content and contaminants nature, yet the studies in this regard are lacking.

One important covariate in the association between insulin resistance and vitamin D is obesity. BMI in the studied group was within the overweight range with a median of 27.8 kg/m^2 (IQR: 23.9, 32.3). Most studies adjust for obesity by using BMI [31,50,178,217,228,232]. In only one study that adjusted for intra-abdominal fat, there was no significant association between HOMA-IR and vitamin D status among 25 African and 25 Caucasian American females, probably due to the small sample size [40]. Inuit were reported to have shorter leg lengths and high trunk-to-stature proportions [327], but a recent work from IPY-IHS showed that sitting height does not affect the estimation of obesity [327]. Therefore, use of BMI was appropriate in this thesis. In addition, in analyses where WC and FM (%) were included as possible covariates, the same pattern was observed between vitamin D and HOMA-IR, which is a clear strength of this thesis. Fat mass was the most common predictor of insulin resistance among Inuit men and women in other studies. In women, % body fat, BMI and WC predicted HOMA-IR and 2 h insulin sensitivity index, whereas in men, % body fat predicted HOMA-IR and WC and BMI predicted 2 h insulin sensitivity index in other Inuit groups [496].

Another important covariate in the models was Arctic region as markers of insulin resistance were significantly different across regions, with values of insulin resistance highest for Inuvialuit Settlement Region. Although vitamin D status was not significantly different among regions, vitamin D sources could differ among regions as RBC-Omega-3 which is a marker of nutrition transition and TF intake varied significantly, with Inuvialuit Settlement Region having the lowest value. Similarly, food insecurity varied significantly among regions in IPY-IHS report by Egeland et al. which could indicate that access for food and nutrients is different among regions [329].

With regard to interrelationships among vitamin D and adipokines, vitamin D remained associated with insulin resistance even after adjustment of the model for both leptin

and adiponectin, indicating that vitamin D status may act independent of adipokines. However, in a separate analysis serum 25(OH)D was inversely related to leptin (β=-1.35 (95% CI -1.38 to -0.85, *P*= 0.007). Vitamin D receptors are expressed in preadipocytes [497,498] and it is possible that the active form of the vitamin D is involved in regulating the expression of adiponectin genes [178]. In addition, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) was found to regulate the expression of leptin gene in osteoblast and adipocyte cells [499]. Vitamin D could thus act on insulin resistance partially through reducing leptin and enhancing adiponectin.

8.4 The Association between Vitamin D, TF and Metabolic Abnormalities

Besides the various associations of insulin resistance to adiponectin, leptin and serum 25(OH)D, insulin resistance is strongly associated with elevated cardiometabolic risk factors such as serum TG, fasting glucose, fasting insulin, HDL and other CVD risks (*P*< 0.0001) among non-diabetic American Indians [500]. In the same study, risk of METS increased across tertiles of HOMA-IR (13.4% in the first tertile, 32.6% in the second tertile, and 58.9% in the third tertile) [500]. In 1988, Reaven suggested that hypertension, dyslipidaemia and glucose intolerance were the main components of the METS [383]. Insulin resistance and central obesity are the most commonly suggested mechanisms that could provide an explanation for the pathophysiology of the METS [501]. In fact, both HOMA-IR and METS can independently predict DM2 and CVD [502,503]. As was shown in this thesis METS was associated with 5 times increase odds for having DM2. A meta-analysis of 28 studies of various epidemiological designs, but mainly cross sectional, indicated that higher vitamin D status is associated with 51% reduced risk of METS and 55% reduction in the risk of DM2 [504]. Unlike other studies, DM2 and METS were not related to serum 25(OH)D among Inuit.

According to Yhun et al., METS is related to plasma 25(OH)D in cross-sectional studies, not in longitudinal studies. Moreover, the association was stronger in the elderly in populations with METS using the National Cholesterol Education Program Adult Treatment

Panel III (NCEP-ATP III) definition in Western populations and in locations north of latitude 38°N. Further studies, particularly longitudinal studies, are needed to allow for more deeper analyses, more accurate estimates of associations and a better understanding of the potential role of 25(OH)D levels in the risk of METS [505].

To understand the weak association between METS and vitamin D status in comparison to other studies, the associations between vitamin D status and the individual components of METS were explored. In addition to the nonsignificant association between vitamin D status and FPG, the association between vitamin D status and hypertension was not significant in the fully adjusted model. Those results could be related to the fact that both vitamin D and marine mammal and fish were included in the same model, possibly capturing the same variable. In comparison to vitamin D status which reflect no more than two to four weeks intake of vitamin D, FFQ-derived marine and fish intakes reflect vitamin D intake over prolonged durations of time [416].

The relationship between vitamin D status and blood pressure is mediated by its receptors on the endothelial cells, smooth muscle cells [506]. Increases in vitamin D status positively improve endothelial function [506,507], and decrease the activity of renin -angiotensin-aldosterone system [506]. In a recent meta-analysis of clinical trials of vitamin D supplementation, vitamin D did not change systolic blood pressure (BP) (effect size, 0.0 [95% CI: -0.8 to 0.8] mm Hg; *P*= 0.97) or diastolic BP (effect size, -0.1 [95% CI: -0.6 to 0.5] mm Hg; *P*= 0.84) [508]. However, vitamin D status is associated with reduction in BP in observational studies, but those results could be driven by confounding variables of the epidemiological designs [232,509]. In the METS component analysis, there was no association between vitamin D and BP.

In addition, vitamin D status was associated with lower WC in Inuit adults herein, this trend was significant in the final adjusted model. This is important because abdominal

obesity, visceral as opposed to subcutaneous fat, appears to be important in the development of insulin resistance [510]. In a study conducted in 1999-2000, a larger proportion of Inuit women were centrally obese relative to Danish women (58.1% vs.17.8%, $P< 0.001$) as were significantly more Inuit men than Danish men (15.9% vs. 8.3%, $P< 0.001$). Although central obesity was not significantly associated with deteriorated metabolic profile in Inuit compared to Danish populations [511], in this thesis central obesity seemed to drive METS with 92.4% of adults with METS having high WC. This is despite the fact that those with METS had a modestly high BMI of 27.1 (IQR 23.3, 31.8 kg/m^2). Furthermore, in a study that compared Inuit to First Nations, it was found that central obesity had significant impact on BP parameters ($P< 0.0001$), HDL ($P< 0.0001$), and fasting insulin ($P< 0.0001$), but not TG in Inuit [512]. Thus the finding that serum 25(OH)D lowers WC could have implication for protection from METS and DM2.

Vitamin D status was associated with improved TG and HDL profile, but with significant increases in LDL without altering LDL/HDL ratio. The findings in regard to TG and HDL could be related to plausible physiological mechanisms including that plasma 25(OH)D is positively associated with post-heparin lipoprotein lipase (LPL) (B= 0.17, $P< 0.001$) [425]. Post-heparin serum LPL is related to increases in serum HDL and decreases in serum TG and hyperlipidemia in diseases such as uremia, DM2 and coronary artery disease [426]. The results regarding LDL are consistent with results from other cross sectional and clinical trial studies [427,428]. In total, serum 25(OH)D protects from dyslipidemia which has implications for protection from DM2 and CVD. It is also plausible that the inverse association between vitamin D status and TG, WC and the positive association with HDL in the present analyses are related to the fact that vitamin D status could be linked to healthier lifestyle in terms of food intake and physical activity.

The intake of long chain polyunsaturated fatty acids of the omega-3 series, which is usually present in marine-based TF, has been linked to high LDL concentrations [513,514]. On the other hand, another study in Alaskan Yupik showed no association between LDL and TF, although the particular type of TF was not specified and may not have been of marine origin [515]. In a group of 133 adult Greenland Inuit from Nanortalik, the concentrations of plasma cholesterol and LDL (6.39 and 4.39 mmol/L, respectively) were slightly higher than "normal" values found in western societies, whereas the HDL cholesterol level was markedly higher (1.64 mmol/L) [516]. Despite the general increase of LDL with TF intake, as was shown in literature, fish intake in our study protected from high LDL/HDL highlighting its important functional role in prevention of dyslipidemia.

Besides lowering LDL/HDL, TF was not associated with METS [516]. In another study, Greenland Inuit women who consumed a traditional diet versus western diet had lower TG (0.32 ± 0.24 to 0.61 mmol/L versus 0.88 ± 0.65 to 3.4, $P< 0.05$), lower LDL (15.0 ± 4.2 versus 37.0 ± 4.2, $P< 0.05$), whereas HDL was higher in men who consumed TF compared to men who consumed a western diet (32.5 ± 8.0 versus 25.3 ± 9.3, $P< 0.05$). The fact that both serum 25(OH)D and fish and marine intake were included in the same model could be the reason why we were unable to identify associations between METS and serum lipids with TF.

Intake of vitamin D and fish seemed to play a protective role from METS, LDL/HDL ratio, high TG and low HDL. Both TF and vitamin D were not protective from DM2 in total regardless of whether DM2 was previously or newly diagnosed. Obesity as assessed by BMI, FM% and WC continued to be the driving force for both METS and DM2. Being from Nunatsiavut relative to Nunavut was associated with increase in odds for both METS and DM2. Indicating that geographical regions in the Arctic confers protection or deterioration of metabolic profile. RBC-Omega-3 fatty acids were protective from high TG but was not

related to other outcomes, whereas RBC-Sat was associated with increase in the odds for DM2. In sum this indicates that a healthy lifestyle is more related to TF intake relative to market food intake. Table 8.1 and 8.2 give a comparison between participants who were included or excluded from both analysis for DM2 and METS. In general the study groups were representative of the survey, except that vitamin D status and TF intakes were higher in excluded groups.

8.5 Insulin Resistance and Osteocalcin

Building on the metabolic associations with serum 25(OH)D, there is a link between metabolic regulation of insulin resistance and bone that could be mediated by osteocalcin. In the present work regarding osteocalcin and adipokines, age, BMI and serum 25(OH)D, marine and fish intakes and RBC fatty acids were used as covariates and the analysis represented a homogenous population in terms of ethnic, arctic environment and lifestyle habits. It is therefore not plausible that osteocalcin acts independent of obesity on insulin resistance among Inuit women. Osteocalcin, as derived mainly from bone, was inversely related to insulin resistance, but this relationship also became insignificant. Again, these results should be interpreted with caution as the sample included only females ≥ 40 y. However, the results could indicate that the putative relationship between insulin resistance and osteocalcin is mediated by obesity. Similar to the present thesis, total osteocalcin was not associated with insulin sensitivity in obese adults [517]. In another study, carboxylated osteocalcin was associated with lower insulin and HOMA-IR, but total and undercarboxylated osteocalcin were not associated with changes in HOMA-IR [518]. Moreover, in 129 patients with DM2, insulin resistance as assessed by hyperinsulinemic clamp technique was not correlated with osteocalcin [519]. In addition, various forms of osteocalcin were not associated with development of DM2 in community based adult population [520]. It has to be emphasized that most of the studies that addressed the

associations between osteocalcin and insulin resistance are post-hoc analysis of data that was intended to assess the role of osteocalcin.

8.6 Impaired Glucose Metabolism and Bone Health

The relationship between vitamin D status and glycemia and metabolic profile could impact bone [521]. In a meta-analysis, DM2 was associated with increases in bone mass [57]. The possible reasons for increases in bone mass were summarized in the introductory sections of this thesis, and they include having higher BMI and hyperinsulinemia in adults who are obese and with DM2. However, other studies suggest that there is an increase in fracture rates with DM2 despite the increased bone mass [522]. The increase in the rate of fracture could be related to accumulation of advanced glycation end products [523], in addition there is an increase in the risk of falls associated with DM2 as a result of polyneuritis and hypoglycemic episodes [297]. In fact in chapter 7, the odds for osteoporosis decreased significantly in adults with DM2 relative to normal controls. However, there was no relationship to fracture risk in this case control study among Inuit. This is in contrast to other studies where there was an increase in fracture with DM2 after a 7 year of follow up in postmenopausal women (relative risk: 1.20; 95% CI 1.11 to 1.30) [524]. In conclusion, DM2 could be associated with increase in BMD, but with associated decrease quality in other populations, however this is not the pattern observed in Inuit women.

One strength of the study is that it is very unlikely that fat layering affected the precision of measurement of BMD as forearm was assessed where adiposity is less extensive as compared to abdominal adiposity and the impact upon lumbar spine assessments [313]. Furthermore, the site most commonly observed to have fractured was the wrist, thus the

Table.8.1: A comparison between adults included and excluded from the metabolic syndrome analysis.

Variable	Study group		Excluded Group		*P*-value
		n		n	
Male n (%)	38.4%	663	38.7%	281	
Female n (%)	61.6%	1062	61.3%	445	0.900
Age (y)	39 (29, 49)	1725	41(30, 52)	726	0.001
BMI (kg/m^2)	27.1 (23.3, 31.8)	1725	27.1 (22.8, 32.8)	320	0.988
FM%	29.7 (21.1, 39.0)	1725	28.9 (20.0, 39.4)	324	0.370
Waist Circumference (cm)	91.0 (80.0, 104.0)	1725	91.4 (78.3, 104.3)	304	0.650
Systolic BP (mm Hg)	113.3 (104.3, 124.7)	1725	112.2 (102.3, 125.3)	346	0.121
Diastolic BP (mm Hg)	75.0 (68.0, 82.3)	1725	74.3 (65.3, 81.7)	347	0.020
Biochemistry					
TG (mmol/L)	1.14 (0.83, 1.60)	1725	1.22 (0.89, 1.76)	336	0.019
HDL (mmol/L)	1.42 (1.16, 1.74)	1725	1.45 (1.20, 1.81)	334	0.559
LDL (mmol/L)	2.74 (2.15, 3.44)	1725	2.83 (2.12, 3.48)	315	0.835
Cholesterol (mmol/L)	4.87 (4.19, 5.60)	1725	5.09 (4.30, 5.81)	336	0.023
Serum 25(OH)D (nmol/L)	49.7 (31.2, 73.6)	1725	60.0 (38.2, 87.0)	320	<.0001
Fasting Plasma Glucose (mmol/L)	4.9 (4.6, 5.2)	1725	5.0 (4.6, 5.4)	334	0.010
TF Intake (Frequency/d)					
Total	0.81 (0.32, 1.56)	1725	1.10 (0.44, 1.93)	220	0.0004
Marine Mammals	0.10 (0.02, 0.35)	1725	0.11 (0.02, 0.48)	220	0.101
Total Fish	0.12 (0.02, 0.30)	1725	0.14 (0.03, 0.43)	220	0.027
Medications					
HTN	8.1%	139	8.9%	65	0.469
Heart	1.0%	17	1.65%	12	0.164
Hormones	4.4%	76	4.1%	30	0.756
Cholesterol	3.4%	59	2.9%	21	0.499
Nutrition Supplements	8.5%	135	7.3%	47	0.014

Data is expressed as median (IQR) for continuous variables and as proportions for categorical variable. Comparison is performed by non-parametric measurement. For continuous variables and by chi-square for categorical variable. Antihypertensive and heart agents included medications such as diuretics, calcium channel blockers, beta blockers and angiotensin converting enzymes (e.g. Hydrochlorothiazide, nifedipine, atenolol, and enalapril); cholesterol lowering agents mainly HMG-CoA inhibitors (e.g Atorvastatin); Hormones included thyroxin forms, estrogens and progesterone as birth control (e.g Eltroxin, estradiol, and provera); Steroids mainly prednisolone and topical corticosteroids; Nutrition supplements included iron, calcium and vitamins. BMI, body mass index; BP, blood pressure; FM, fat mass; HDL, high density lipoprotein; HTN, hypertension; 25(OH)D, 25 hydroxyvitamin D; LDL, low density lipoprotein. TG: triglyceride. TF: traditional food

Table 8.2: A comparison between adults included and excluded from the DM2 analysis.

Variable	Study group	n	Excluded Group	n	*P*-value
Male	38.5%	714	38.6%	285	0.937
Female	61.6%	1143	61.4%	453	
Age (y)	40 (30, 51)	1857	41 (30, 54)	738	0.013
BMI (kg/m^2)	27.5 (23.4, 32.3)	1857	26.7 (22.7, 32.3)	321	0.093
FM%	30.5 (21.9, 39.7)	1857	28.4 (19.4, 38.5)	326	0.011
Waist Circumference (cm)	92.0 (80.5, 105.0)	1857	90.0 (78.0, 103.0)	307	0.036
Systolic BP (mm Hg)	114.0 (104.3, 125.3)	1857	112.7 (102.7, 124.7)	351	0.051
Diastolic BP (mm Hg)	75.0 (68.0, 82.3)	1857	73.5 (65.0, 80.7)	352	0.004
Biochemistry					
TG (mmol/L)	1.16 (0.83, 1.65)	1857	1.18 (0.87, 1.68)	345	0.568
HDL (mmol/L)	1.41 (1.15, 1.73)	1854	1.47 (1.23, 1.84)	346	0.033
LDL (mmol/L)	2.75 (2.15, 3.44)	1835	2.78 (2.07, 3.46)	341	0.725
Cholesterol (mmol/L)	4.88 (4.19, 5.62)	1857	5.03 (4.19, 5.72)	345	0.140
Fasting Plasma Glucose (mmol/L)	4.9 (4.6, 5.3)	1857	5.0 (4.6, 5.4)	341	0.111
25(OH)D (nmol/L)	51.4 (32.2, 76.4)	1857	62.6 (40.7, 89.9)	329	<0.0001
TF (frequency/d)					
Total	0.84 (0.33, 1.63)	1857	1.08 (0.46, 1.97)	224	0.0007
Marine Mammals	0.10 (0.02, 0.37)	1857	0.12 (0.03, 0.56)	224	0.070
Total Fish	0.13 (0.03, 0.32)	1857	0.14 (0.03, 0.43)	224	0.100
Medication Usage					
HTN	10.1%	196	10.0%	74	0.684
Heart	1.3%	24	1.8%	13	0.365
Cholesterol	5.3%	98	4.2%	31	0.252
Osteoporosis	0.3%	5	0.8%	6	0.055
Hormones	4.6%	86	4.6%	34	0.974
Nutrition Supplements	9.1%	155	7.5%	49	0.036

Data is expressed as median (IQR) for continuous variables and as proportions for categorical variable. Comparison is performed by non-parametric measurement for continuous variables and by chi-square for categorical variable. Antihypertensive and heart agents included medications such as diuretics, calcium channel blockers, beta blockers and angiotensin converting enzymes (e.g. Hydrochlorothiazide, nifedipine, atenolol, and enalapril); cholesterol lowering agents mainly HMG-CoA inhibitors (e.g Atorvastatin); Hormones included thyroxin forms, estrogens and progesterone as birth control (e.g Eltroxin, estradiol, and provera); Steroids mainly prednisolone and topical corticosteroids; Nutrition supplements included iron, calcium and vitamins. BMI, body mass index; BP, blood pressure; FM, fat mass; HDL, high density lipoprotein; . HTN; hypertension; 25(OH)D; 25-hydroxyvitamin D; LDL; low density lipoprotein; TG, triglyceride; TF, traditional food

forearm measure was most appropriate to test whether BMD and fracture risk were altered by IFG or DM2.

There are very few studies that characterize BMD among Inuit. It seems that age (inverse correlate) and adiposity (positive correlate) indicators are the only factors that were found to be associated with BMD as measured at the forearm among Inuit women 40 y of age and over [362] . In addition, both insulin resistance and DM2 seem to be linked positively to BMD in unadjusted models. As was shown in chapter 7, further adjustment of the models to factors such as arctic region and medication use made the association between DM2, prediabetes and BMD insignificant. However, the odds of lack of osteoporosis were higher in DM2 cases relative to controls. Overall these results are in contrast of the general body of literature in terms of the positive association between DM2 and BMD [57]. Enhancement of BMD is one of the main functions of serum 25(OH)D, but in the case-control design in Inuit women there was no association between serum 25(OH)D and FaBMD [362].

In support of this finding, in chapter 7 there were no associations between other markers of food intake such as various fatty acid composition of red blood cells and FaBMD, indicating that those biological biomarkers could have reflected short term cross-sectional exposure or that limited intake of dietary calcium among Inuit limit the beneficial effects of healthy nutrients. However, long term dietary exposure as assessed by FFQ for both fish and marine mammal intakes indicated that those types of food, which are rich sources of nutrients such as omega-3 fatty acid and vitamin D [118], were in fact linked positively to FaBMD regardless of the contamination in the Arctic. Long chain polyunsaturated fatty acids (PUFA) such as omega-3 fatty acids increase bone formation and decrease bone loss as was shown using bone mineral densitometry [525]. Studies performed on animals support a beneficial role

of omega-3 fatty acids on bone health [526]. Self-Reported omega-3 intake among older men and women was associated significantly with 6% of variance in BMD of femoral neck [527].

It was observed that greater frequency of marine mammal intakes was associated with increase in both FPG and HOMA-IR. In an analysis of thesis data, it was clear that the highest quintile of marine mammal intake was associated with increases in total dietary fat intake in grams and monounsaturated fatty acid (MUFA) intake in grams and in % of energy intake, but saturated fat (SFA) and PUFA were not different across quintiles of marine mammal intake (Table 8.3). However, data from the United States Department of Agriculture indicate some marine mammal parts such as muktuk, meat dried in oil and blubber of whale are exceptionally high in fat and relatively high in SFA (Table 8.4). In a study among 544 healthy women, an increase in total fat intake by 20 grams was associated with 9% increase in insulin concentration ($P< 0.001$) prior to adjustment for obesity and 6% increase in insulin concentration ($P< 0.01$) after adjustment to obesity [528]. Although substituting SFA with MUFA was associated with better insulin sensitivity, this relationship was not seen when fat intake was high [529]. Saturated fat was positively associated with BMI and waist to hip ratio in a study among 878 males [530]. In fact, abdominal obesity was the main contributor to METS in this study. Thus, it could be that higher intakes of marine mammal observed in the present research that were associated with higher fat intake had consequent detrimental effects on FPG and METS. In a national survey of adult Canadian undertaken in 1997 to 1998 [531], the total intake of fats in comparison to Inuit in the current survey appears to be similar (Table 8.5).

In summary, the research presented in this thesis indicates that insulin resistance is inversely associated with serum 25(OH)D in Inuit adult, whereas consumption of marine mammals was associated with increases in FPG and HOMA-IR. The relationship between insulin resistance and vitamin D status seems to be present despite adjustment for obesity.

Table 8.3 Comparison between different types of intakes across quintiles of marine mammal intake.

	Quintiles of Marine intake (frequency per day)				
Grams of	**Q1 (0.004 ± 0.004)**	**Q2 (0.029 ± 0.012)**	**Q3 (0.102 ± 0.03)**	**Q4 (0.299 ± 0.094)**	**Q5 (0.869 ± 0.179)**
Fat	70.2 ± 50.2[a]	78.4 ± 53.2[ab]	79.2 ±57.6[ab]	80 ± 60.1[ab]	88.9 ±75.8[b]
SFA	23.2 ± 16.2	26.1 ± 18.0	25.3 ± 18.1	25 ± 18.7	26.2 ± 21.2
PUFA	13.6 ± 14.0	14.3 ± 12.4	13.9 ± 11.4	12.8 ± 10.2	13.8 ± 12.0
MUFA	26.9 ±18.6[a]	30.3 ± 21.6[a]	31.4 ± 23.0[ab]	31.4 ± 24.8 [ab]	35.6 ± 30.2[b]
% Energy:					
Fat	31.2 ± 10.8	31.6 ± 11.0	31.0 ± 11.2	31.5 ± 11.8	32.9 ± 13.0
SFA	10.6 ± 4.3	10.7 ± 4.2	10.1 ± 3.9	10.1 ± 4.3	10.0 ± 4.5
PUFA	5.8 ± 3.5	5.7 ± 3.2	5.5 ± 2.8	5.2 ± 2.7	5.3 ± 2.9
MUFA	12.1 ± 4.9[a]	12.2 ± 4.9[ab]	12.3 ± 5.4[ab]	12.4 ± 5.7[ab]	13.3 ± 6.1[b]

Letters that carry different letters are statistically significant $P<0.05$ within rows. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fat.

Table 8.4 Fat composition of selected Alaskan native TF[1]

		g/100 g		
Marine mammals food items	**Total fat**	**Total SFA**	**PUFA**	**MUFA**
Whale Bowhead				
skin and subcutaneous fat (muktuk)	46.1	6.6	8	28.1
subcutaneous fat (blubber)	96.5	NA	NA	NA
Whale Beluga				
meat, dried	5.34	1.2	0.3	3.3
meat, raw	0.5	0.1	0.02	0.3
liver	3.9	NA	NA	NA
Seal				
bearded, meat dried	2.3	0.6	0.4	1.3
bearded, meat dried in oil	25.1	3.9	1.2	11.2
ringed, meat	3.2	0.8	0.06	1.7
ringed,liver	3.3	1.2	1	1.1
Walrus				
meat, dried	2.6	NA	NA	NA
meat,raw	13.6	2.6	2.6	8.4
meat and subcutaneous fat	24.1	NA	NA	NA
liver, raw	3	NA	NA	NA

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fat.
[1]Source [532].

Table 8.5 A Comparison of fat intakes between Inuit and Canadian

	Canadian*					
Type of Fat	18-34 y		35-49 y		50-65 y	
	M (n= 125)	F (n= 207)	M (n= 266)	F (n= 459)	M (n= 181)	F (n= 306)
Total Fat (% Energy)	29.1	28.8	30.2	29.7	30.6	28.9
SFA (% Energy)	10.2	9.5	9.6	9.7	9.8	9.5
MUFA (% Energy)	11.1	10.6	11.6	11.5	11.7	10.7
PUFA (% Energy)	4.5	5	5.1	5.2	5.3	5
	Inuit data from this thesis**					
Type of Fat	18-34 y		35-49 y		50≥ y	
	M (n= 225)	F (n= 423)	M (n= 276)	F (n= 411)	M (n= 197)	F (n= 288)
Total Fat (% Energy)	31.8 ± 11.0	29.7 ± 11.0	31.4 ± 12.2	32.3 ± 11.3	33.1 ± 12.1	32.5 ± 12.0
SFA (% Energy)	10.3 ± 4.3	9.8 ± 4.1	10.5 ± 4.4	10.4 ± 4.1	10.9 ± 4.7	10.1 ± 4.2
MUFA (% Energy)	12.6 ± 5.6	11.6 ± 5.1	12.4 ± 5.7	12.7 ± 5.4	13.4 ± 5.6	12.7 ± 5.4
PUFA (% Energy)	5.7 ± 3.4	5.4 ± 3.3	5.1 ± 2.5	5.8 ± 3.1	5.5 ± 3.0	5.5 ± 2.7

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fat.
* data is expressed as weighted means, source [531].
** data is expressed as mean ± SD, source IPY-IHS.

Both leptin and adiponectin were significantly related to insulin resistance in final models. Chapter 5 showed that vitamin D was associated with lower TG and increased HDL. In addition, TF was not associated with a reduction in METS and improved plasma lipid profile, whereas serum 25(OH)D was not associated with METS. Both osteocalcin and BMD were related to insulin resistance before adjustment with obesity (data not shown), but upon adjustment for obesity the relationship became insignificant.

In chapter 7, BMD was not different between cases of impaired fasting glucose and DM2 relative to control, whereas FaBMD was positively linked to marine mammal and fish intake. Vitamin D status is a marker of a traditional way of living which is associated with lower adiposity and hence better insulin sensitivity. A novel finding of this thesis was the clustering of deteriorated metabolic profile in both Inuvialuit Settlement Region and Nunatsiavut relative to Nunavut. Previous work done in IPY-IHS indicated the presence of regional differences in terms of food security [329] and consumption of RBC-Omega-3 fatty acids were higher in Nunatsiavut and part of Nunavut in comparison to Inuvialuit Settlement Region [76].

Vitamin D itself is linked to lower adiposity through various molecular mechanisms. As it was found that $1,25(OH)_2D$ could modulate adipogenesis by regulating peroxisome proliferation [533] and the fact that reduced vitamin D can cause excessive differentiation of preadipocytes into adipocytes. In addition, vitamin D receptor null mice models suggest a role of VDR in energy regulation [534]. Moreover, long-term suppression of parathyroid hormone may lead to obesity prevention through sympathetic nervous system [535]. However, data from a meta-analysis on the effect of vitamin D supplementation on obesity is not conclusive [536]. This association with lower adipogenesis and obesity could be associated with improved metabolic profile and insulin resistance as was shown in this thesis.

8.7 Limitations of the Thesis Research

The studies presented in this thesis are not without limitations. One major drawback is the use of fasting proxy measures of insulin resistance, mainly HOMA-IR. Insulin resistance is a very important component of the etiology of DM2. Reciprocally, researchers have used the concept 'insulin sensitivity' which could be understood as 'a quantitative measure of the biological effect of insulin' [537]. Homeostatic assessment model is a mathematical model to measure insulin resistance and β-cell function from fasting concentrations of insulin and glucose [345], because insulin secretion is pulsatile, the optimal sample should be a mean of three results at 5 minutes intervals (0, 5, and 10 minutes intervals) [345], but most epidemiological studies as in this thesis used only one sample for simplicity of sampling. HOMA-IR measures basal insulin resistance and not stimulated insulin resistance.

Other measurements of insulin resistance and sensitivity include methods such as minimal model frequently sampled intravenous glucose tolerance test, euglycaemic clamp and hyperglycaemic clamp. It is well known that the euglycaemic clamp is the gold standard to assess insulin resistance, but it is labor intensive and is likely not feasible in remote Arctic regions, thus methods such as HOMA-IR are the most suitable for large epidemiological studies [538]. Measurement of glucose after the oral glucose tolerance test (OGTT) was not available for most of the study participants and represented only one time-point (2 h). Also, post OGTT insulin was not assessed which limits the utilization of post OGTT measurements in this thesis which represented the only means to assess stimulated insulin sensitivity.

Another limitation is that to assess vitamin D status, the study used a Liaison auto-analyzer assay which is a chemiluminescent immunoassay co-specific for both $25(OH)D_2$ and $25(OH)D_3$ [539]. Other direct methods to assess vitamin D status include HPLC and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [540,541]. HPLC follows a UV

detection and is highly repeatable and is considered the gold standard [542]. However, participation in the Vitamin D External Quality Assessment Scheme (DEQAS) helps bring strength to the serum 25(OH)D analyses. Also, the same method we used in assessing serum 25(OH)D was used in CHMS [140] and thus the results of the present research were comparable and practical in an epidemiological settings.

In addition, total osteocalcin was used to evaluate the relationship between osteocalcin and insulin resistance, but osteocalcin exists in many forms that may behave differently clinically. Studies showed that uncarboxylated osteocalcin, but not carboxylated osteocalcin is capable of inducing adiponectin and insulin secretion [457], whereas total osteocalcin was associated with metabolic traits in adult populations [517,543,544]. However, more studies are needed to determine the association between osteocalcin carboxylation and its role in insulin resistance.

Furthermore, both 24-dietary recall and FFQ were used to capture TF intake. This was performed a single time which indicates that the data regarding the dietary intake is not necessarily reflective of the whole year; especially in the Arctic where time of the year could change the type of TF intake gathered. Also, the daily variation of food consumption through the year was not clear. To adjust for longer-term dietary intake, RBC fatty acids were examined as a physiological marker of dietary TF intake, and to assess the physiological impact of omega-3 fatty acids and nutrition transition and lastly factors such as calcium intake were not included. The reason for exclusion is that data on calcium intake was collected from single 24 hour dietary recall and could include sporadically high values for calcium intake that could limit the analyses. However, calcium intake was on average low among the study participants (less than 600 mg). Moreover, sociodemographic information was explored and found not to change the interpretation of the results. Also, data on alcohol intake and smoking were excluded for reliability and sample size considerations. Males were

under-represented in most of the analyses and they were not part of analysis on bone and osteocalcin. PTH data existed for only limited number of IPY-IHS and although was explored was not presented in this thesis.

One weakness of the study is that data for the study of DM2 and bone included only females, hence one cannot make a conclusion about the relationship between insulin resistance and BMD in the context of the male sex hormones. Male sex hormones help protect bone, in comparison to females who have estradiol [545] of which the decline at menopause is associated with more impact on bone loss. We explored the relationships with adjustment for walking as a measure of physical activity and did not find differences in the data interpretation, but this analysis was not shown due to the drastic decrease in sample size (almost 20% of loss in sample).

8.8 Concluding Remarks and Future Directions

Based on the IPY-IHS there appears to be divergent relationships where the emphasis on insulin resistance was most obvious, as vitamin D status was not related to FPG and inversely related to HOMA-IR. Also, vitamin D status was associated with improved serum TG, HDL, but not with other metabolic outcomes. Both adiponectin (inversely) and leptin (positively) were significantly associated with insulin resistance, with leptin inversely related to serum 25(OH)D. However, both BMD and osteocalcin were not related to insulin resistance. Although osteoporosis was less common in Inuit women with DM2, there was no significant difference in FaBMD between DM2 or IFG cases relative to controls. The fact that vitamin D in the Arctic is mainly derived from TF could indicate that some of the relationships that were observed are related to vitamin D status as a marker of traditional way of living; for example marine mammal intake were associated positively to FPG, increased HOMA-IR and FaBMD. With regard to METS studies, more data are needed such as kidney function and serum calcium to expand the analysis. Finally, the analysis for BMD and

osteocalcin should be performed using data on both males and females, adjusting for sex hormones, using data on BMD at other anatomical sites.

Studies on the associations between various genetic polymorphisms and insulin resistance are needed among Inuit living in Canadian Arctic. One polymorphism in the TBC1D4 gene was associated with DM2 traits in Inuit from Greenland, particularly that post OGTT plasma glucose (β= 3.8 mmol/L, *P*= 2.53 $X10^{-35}$), serum insulin (β= 165 pmol/L, *P*= 1.5 $X\ 10^{-20}$) were higher, whereas FPG (β=-0.18, *P*= $1.1X10^{-6}$), and fasting serum insulin (β=-8.3 pmol/L, *P*=0.0014) were lower. The risk of adults for having DM2 in association with the polymorphism in TBC1D4 was highly increased (OR= 10.3, *P*= $1.6\ X\ 10^{-24}$) [546]. In this thesis, data for post OGTT plasma glucose was available for 6 Inuit participants with DM2; the median was 12.1 (range 11.1, 13.2) mmol/L. Future studies are needed to understand whether this high level of post OGTT plasma glucose is related to genetic polymorphisms.

In conclusion, through this work, it is clear that it is imperative that better knowledge is required to differentiate the effect of vitamin D status from TF intake in future studies, for example to design clinical trials that will provide vitamin D in a form of supplementation and assess its impact on insulin resistance in the Inuit population. Such clinical trial should particularly target the younger population which is more prone to nutrition transition and lower consumption of TF. In addition, insulin resistance in Inuit is poorly characterized in the scientific literature and performance of clinical studies using more sophisticated methods to assess insulin sensitivity in this population are needed; these however may not be feasible in all communities or well accepted. Furthermore, to address the impact of TF and nutrition transition on markers of insulin resistance, studies should be designed to capture data at multiple times of the year.

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