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Nutritional Intake and Hormone Phenotypes in the Kansas Mennonite

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Nutritional Intake and Hormone Phenotypes in the Kansas Mennonite

By

Christopher E. Barrett

In Partial Completion
of the Requirements for the Degree
Masters of Arts

Kathleen L. Kitto, Dean of the Graduate College

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Master’s Thesis

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Christopher E. Barrett
November 15, 2016
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A Thesis
Presented to
The Faculty of
Western Washington University

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Of the Requirements for the Degree
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By
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November, 2016
Abstract

Nutrition directly shapes phenotype and genetic health, therefore playing a central role in determining health outcomes and disease trajectories. Chronic health problems and nutritional deficits have been rapidly escalating globally as nutritional deficiencies continue to accelerate the onset of illness and suffering. Vitamin D deficiency for example is causative in many pathophysiological disease and metabolic conditions while correction of deficient levels is known to treat cancer and reduce cardio-metabolic risks. Despite the bio-social origins and explicit connections between nutrition and health, explanations remain contentious at best. Enhanced diagnostic and nutritional epidemiological approaches are required in order to delineate any ambiguities. Serving as a follow up study by biological anthropologists into notable longevity among the Kansas Mennonite, The Kansas Nutrition Project (KNP) explores the effects of nutrition and genetics on chronic health. Nutrients and their cellular receptors interact with genes coding for hormones, particularly adiponectin and leptin. Recent studies show vitamin D influences normal levels of adiponectin and leptin both in healthy and unhealthy populations with research benefiting from cross-cultural and population level studies. Mennonite populations of Kansas have experienced several instances of differential bottlenecks and founder effects during their migration histories across Europe and the Americas while maintaining cultural and religious independence. The reliability of working within population isolates in anthropological and epidemiological research is well supported, with linear relationships increasing with increasing population homogeneity. Combining measures of nutritional intake and diagnostic biomarkers, this thesis investigates the relationships between nutritional intake and measures of adiponectin and leptin within a population of semi-
isolate Kansas Mennonite. Using data from the KNP, I construct sex-specific multivariate models of nutritional intake and hormone variation using anthropological and epidemiological rationale. Investigations include the relationships between nutritional intake, adipose traits and micronutrient intake on circulating hormone phenotypes. Operating within a sex-specific and nutrigenetic framework, I predict lipophilic nutrients like vitamins A, D, E and K may play significant roles in the variation of adiponectin and leptin within this population of Mennonite. Results show statistically significant ($p < 0.05$) variation between females and males in almost all categories of variables including anthropometrics, blood lipids, nutrient intake and hormone variation in this population of semi-isolated Kansas Mennonite. Vitamin D intake was significant for adiponectin and leptin levels in men but not women, while folate was significant in leptin level in women but not men.
Acknowledgements

Western Washington University and the University of Kansas are thankful to the Anabaptist Mennonite communities and congregations who consented and participated in the Kansas Nutrition Project. Special thanks to the thesis committee members, Dr. M.J. Mosher, Dr. Joan Stevenson and Dr. Wayne Landis and biological anthropologists Dr. Michael Crawford and Dr. Dario Demarchi.
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Introduction

This thesis attempts to highlight the cultural and biological factors shared between nutrition and chronic disease by examining the associations between nutritional intake and concentrations of adipokine of clinical significance in a sex-stratified semi-isolate population of Kansas Mennonite. It examines the relationships between nutritional intake and hormone levels for possible clinical applications. While both anthropology and epidemiology contribute insight and enhanced understanding of human health and disease, they both have short comings in their structures (Janes, Stall and Gifford, 1987; Trostle, 2005). Anthropology and epidemiology can learn from each other and be combined to enhance outcome efficacy and application (Béhague et al., 2008; Ayeni, 2008). Human nutrition has relied upon epidemiological and interventional studies to understand the mechanism between food and health. A cross-cultural perspective using anthropological techniques within a semi-isolate population will benefit and advance ultimate understandings of diet and disease.

Conceptual Model: Nutritional Phenotype and Epidemiology

Interactions between nutrition and genetics may be epidemiologically studied with the collection of biological samples (Bingham, 2002). The mechanisms underlying individual health status can be complex when asking about nutritional effects. Voruganti et al. (2013) find that diet shapes chronic disease and mitigates the effects of genetics on disease traits in a population of baboon (Papio) (Higgins et al., 2010; McCurdy et al., 2009). These diet-gene interactions are paralleled in humans as diet actively shapes biological traits and genetic expression (Johnson and Belshaw, 2014; Mead, 2007). The nutritional phenotype is a varying function of diet, integrating the effects of diet on disease
or wellness and is the quantitative indication of the paths by which genes and environment exert health effects (Mosher, 2012) (see figure 1). These processes are individual and collectively spatio-temporally relative, predicated by social and biological factors as sex, age, developmental state, stress, health status and timing of environmental exposure (Mosher, 2012). More focus and work is needed on larger populations and different ethnicities to resolve these controversies. Conceptualizing a nutritional phenotype gives form and substance to the recognized future of nutritional science in confronting disease and health and for this there is a need for valid, reliable measures of the nutritional environment (Glanz, 2009; Green and Glanz, 2015; Zeisel et al., 2005).

![Figure 1. Theoretical illustration of the nutritional phenotype.](image)

Genetic epidemiology is similar to traditional epidemiology in its efforts to understand the spread, containment and potential removal of a disease within a given community also using principles and methods from population genetics (Khoury et al.,
1993; Morton et al., 1967). While diseases and disease traits are of interest they are not of
exclusive focus in genetic epidemiology which also examines anthropometric and
behavioral factors in disease etiology (DeWan, 2010; Rao, 1983). Where human genetics
focuses on classic concepts of Mendelian diseases, diseases with a single gene culprit,
genetic epidemiology examines complex diseases involving many genes such as chronic
disease. These complex diseases manifest in many different constellations of symptoms
and pathologies. Understanding the genetic determinants of chronic disease requires
working within populations of similar genetics in order to make population level claims
using any genetic results. For this reason, working within geographic, cultural and
linguistic isolates is far more ideal (Hatzikotoulas et al., 2014; Kristiansson et al., 2008;
Peltonen et al., 2000). The reliability of working within population isolates in
epidemiological research is well supported, with linear relationships increasing with
genetic homogeneity (Biino et al., 2013; Bulayeva, 2006; Crawford, 2000; Kristiansson et
al., 2008). Testing nutritional environmental hypotheses that underlie chronic disease risk
within these Mennonite populations provides a unique opportunity to study these complex
relationships.

**Nutrition and Disease**

Nutrition and diet are frontline in the prevention, intervention, and treatment of
chronic disease and conditions (Jones et al., 2013; Konstantinidou et al., 2014; Ness,
2004). Some of the earliest recorded work of nutrition curing disease was in 1753 by
Scottish physician, James Lind (Baron, 2009; Lind, 1772; Willett, 2014). While traditional
roles of nutrition in disease may bring forth canonical examples of rickets, scurvy or a
form of anemia the contemporary focus in nutritional epidemiology is on chronic diseases
and diseases of Western civilization (Carrera-Batos et al., 2011; Melnik et al., 2011; Weisberg et al., 2004; Willett, 2014; Yasmin et al., 2016). Early epidemiological work demonstrates the direct implication of a nutritional component in disease etiology and individual genetic health (Alam et al., 2016; Mead, 2007; Fenech et al., 2011; Lind, 1772; Bartholomew, 2002). Nutrients and their receptors interact with genes coding for hormones, particularly adipokines, which are central to chronic disease and aging biology (Krawczynska et al., 2014; Nimitphong et al., 2009; Xiong et al., 2014). Vitamin D may influence normal levels of adipokines adiponectin and leptin (Bidulescu et al., 2014; Ghavamzadeh et al., 2014). Cheng et al. (2010) noted that variation in the amounts of adipose tissue reflected also the actions of vitamin D after controlling for physical activity, smoking, drinking, sex and age. This nutrition-genetic or nutrigenetic approach provides exciting potentials in finally placating some long persisting global health problems (Barish, 2006; Gillies, 2003; Kaput, 2004).

In the U.S., nutritional or dietary factors are explicitly modifiable especially within the context of nutritional deficiencies. Determining individual nutrition health status however is challenging due the complex and diversity of diagnostic methods as well as poor standardization of measures (Alberti et al., 2006; Subar et al., 2003; Willett, 2014). Chronic and metabolic diseases are often comorbid health outcomes resulting from nutritional deficiencies, nutritional access and bodily absorption (Via, 2012). While synthetic and fortified foods have been instrumental in the global management of nutrient deficiencies and disease they are not sufficient substitutes for quality dietary sources that supply adequate energy, protein, essential fats and other food constituents required for optimal health (Darnton-Hill et al., 2002; WHO, 2009). Sufficient nutritional intake means
satisfying three major areas of nutrient responsibility: 1) consumption of enough energy to satisfy the amount of energy spent, 2) maintaining physiological processes during basal and resting metabolic states processing, 3) replenishing stores within high energy demanding tissues and cells such as adipose, neurological and musculoskeletal (Hall et al., 2012). These three major areas of nutritional function to ensure a robust life and health span.

Individual dietary intake and food preferences are however results of social processes and not explicitly biological (Lind et al., 2016; Mintz and Du Bois, 2002; Robinson et al., 2014). Authorities on nutritional knowledge can be found in many fields of study from the nutritional or biomedical science as well as social disciplines including psychology and anthropology (Ferraro et al., 2016; Melnik et al., 2011). Navigation of nutritional knowledge can be challenging considering the variation in nutritional information and cohesion of nutritional information. Some insufficiencies of knowledge about nutritional strategies in treating chronic disease are attributed to poor characterization of subpopulations, lack of comparisons between obese and healthy individuals, small samples sizes, as well as social and genetic heterogeneity (Bidulescu et al., 2014; Edita et al., 2014; Kim et al., 2013; Kou, 2014). What may be defined as a healthy or unhealthy diet is multifactorial, determined by location of food sources, lifestyles and beliefs, socioeconomics and biology (Fildes et al., 206; Larson et al., 2009; Lind et al., 2016).

Some nutrients, like vitamin D, have a direct effect on many systems but importantly adipose tissue and adipose hormones suggesting a possible nutritional approach to disease and MetS (Belenchia et al., 2013; Cai et al., 2015; Cheng et al., 2015;
Maggi et al., 2013; Wood, 2008). These associations between vitamin D, adipose and adipose hormones remain poorly understood however, largely due to biological and environmental confounders (Bidulescu et al., 2014; Edita et al., 2014; Jamal-Allial et al., 2014; Kou, 2014).

**Risk Factors of Metabolic Syndrome**

Chronic disease management of dysfunctional adipose tissue is estimated to cost the U.S. upwards of 140 billion dollars annually (Zamosky, 2013). Obesity is the single greatest risk factor for MetS, unfortunately however, obesity is diagnosed using the flawed body mass index (BMI) method resulting in likely misleading reports (Bennasar-Veny et al., 2013; Burkhauser and Cawley, 2008; Pories et al., 2010; Rothman, 2008). Obesity also increases risk of other chronic disease including cardiovascular disease and cancer (Belardi et al., 2013; Roger et al., 2003). What is certain however is that adipose tissue clearly plays a significant role in the mechanisms behind chronic and metabolic disease (Tchkonia et al., 2010). Epidemiological data suggest that concentrations of vitamin D are inversely correlated with obesity parameters including BMI, waist circumference, fat mass or percent body fat while have a positive association with increase insulin sensitivity (Belenchia et al., 2013; Koszowska et al., 2014).

Metabolic syndrome (MetS) is a constellation of chronic conditions such as hypertension, high levels of cholesterol, cardiovascular disease, and obesity. Obesity replaced undernutrition and infectious disease as leading contributor to ill health at the turn of the last century (Kopelman, 2000). Flegal et al. (2016) found obesity, the single greatest risk factor for MetS. Obesity is clinically defined as the pathological accumulation of fat (adipose) tissue. Obesity levels correlate with cancer rates. The prevalence for obesity is
35 to 40% in the USA (Flegal et al., 2016). Rates of obesity have doubled between 1980 and 2014 (Calle et al., 2003; Belardi et al., 2013; WHO, 2014; WHO, 2015). Chronic disease management of the health impacts of obesity is estimated to cost the U.S. upwards of 140 billion dollars annually (Zamosky, 2013). Surgery is how some individuals adjust to obesity but it is costly and not without risk. Thus, in 2016 there are no successful long-term treatment options for obesity (Tam et al., 2011). MetS is alarmingly prevalent in the general population carrying with it significant morbidity, representing an orchestration of advanced aging which facilitates multi-organ disease and all-cause mortality (Fadini et al., 2011). A considerable degree of ambiguity surrounding MetS lies in the various sex-specific ways it may present itself (see table 1).

<table>
<thead>
<tr>
<th>Feature</th>
<th>Diagnostic Clinical Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Lipids</td>
<td>HDL Cholesterol (&lt;40 mg/dL males; &lt;50 mg/dL females)</td>
</tr>
<tr>
<td></td>
<td>Triglycerides ≥150 mg/dL</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>≥102 cm males; ≥88 cm females</td>
</tr>
<tr>
<td>Fasting Glucose</td>
<td>≥100 mg/dL</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>≥85 mmHg diastolic; ≥130 mmHg systolic</td>
</tr>
</tbody>
</table>

*Table 1. The constellation of sex-specific phenotypes indicative of MetS. Greater physician consideration of sex-specific differences in diagnostic cutoffs is needed (Jin and Benyshek, 2013 pg.97).*

MetS proves to be an increasing problem of global proportions (Grundy, 2008). MetS and its associated diseases or conditions result from interactions between genetics and environment (Corella and Ordovas, 2004; Go et al., 2005; Ordovas, 2008; Xia et al., 2016). Prior to data collection there was an estimated 20% prevalence of MetS among the greater U.S. populace with variations in results depending on nationality, age, socioeconomic status, and other predictor variables (Park et al., 2003). Comparative
follow-ups find an unyielding increase of MetS within U.S. citizens and stress its seriousness as the leading global public health concern (Mozumdar and Liguori, 2011). Beltrán-Sánchez and colleagues emphasize the increasing prevalence of MetS and the greatest risk factors; highlight the urgency in addressing MetS as a healthcare priority (2013). The World Health Organization (WHO) asserts that research in MetS should focus on developing and evaluating population-based strategies (Simmons et al., 2009). In order to do so, epidemiological studies of nutrition and chronic disease must take note of the complex gene-environment interactions experienced in the biocultural environment of humans.

**Human Variation in Metabolic Disease Risk**

In a large genome-wide analysis, Randall and colleagues (2013) demonstrate the genomic determinants of anthropometric traits are likely sex-specific. While adipose genes are clearly implicated in many metabolic pathways, the ultimate expression of the adipose phenotype varies dramatically by age, sex, nationality, social status, environmental endocrine blockers and decreased nutrient content of foods (Grün and Blumberg, 2009; Karastergiou et al., 2012; Lee et al., 2007; Schug et al., 2011; Wells, 2007; Wells, 2012).

Individual differences and inter-personal variation in leptin and adiponectin secreted by adipose is determined by biological sex and age (Guerra et al., 2008; Kristensen et al., 2000; Lönnqvist et al., 1997; Peake et al., 2005; Wildman et al., 2013). Consuming alcohol also impacts circulating adiponectin and leptin concentrations (Makita et al., 2013; Otaka et al., 2007). Dimorphic and ethnic differences exist in vitamin D levels with women being more likely to be deficient or in states of hypovitaminosis (Bidulescu et al., 2014; Smith, 2010; Zadshir et al., 2005). There are sex-specific variations in vitamin D
specific to age and developmental period (Dam et al., 2009; Jungert and Neuhäuser-Berthold, 2015). In general, young people have higher levels of vitamin D than older people and males have higher levels than females. The strong sex and age specific factors must be considered when designing interventional or therapy responses (Nesby-O’Dell et al., 2002). While alcohol consumption and smoking tobacco also effect vitamin D levels the strength of these effects are also sex-specific (Brot et al., 1999; Lee, 2012). Yannakoulia et al. (2003) colleagues report sex-specific results in relation to body mass, adipokine levels and nutrient intake.

Provided the extensive sex differences in chronic health outcomes, there is a clear need for integrated research that focuses on distinguishing the underlying biological and social determinants of chronic disease (Goldman et al., 2004). Reliable analyses therefore require strict research protocol, multi-method sampling, and ideal populations with social and genetic homogeneity (Bansal et al., 2007; Palmer et al., 2011; Polasek, 2009). These caveats make studying the multifactorial etiologies of MetS in population isolates ideal (Bellia et al., 2009; Benton et al., 2015; Biino et al., 2015; Kolčić et al., 2006; Missoni, 2009). This thesis examines data collected from a semi-isolate population in the form of anthropometric body measures, blood lipid levels, adipokines and nutritional intake values.

**Biological Anthropology and Biological Aging**

Few anthropologists, and specifically biological anthropologists, have focused on the socio-demographic and developmental biology associated with human aging (Ice, 2005). Biological anthropology investigations of aging, reproductive ecology and chronic disease have been conducted on several Anabaptist Mennonite samples from the Midwest U.S. (Crawford, 2000; Devor et al., 1985; Duggirala et al., 2002; Everson et al., 1995;
Stevenon et al., 1990; Stevenson et al., 1994; Stevenson et al., 2004). Within one Mennonite community, the three leading causes of death were found to be analogous to the greater U.S. population; cardiovascular disease, cancer and stroke (Melton, 2006). Biological anthropological responses to aging and the associated developmental and physiological changes focus on how these processes occur within the context of individual variation and various social and biological environments (Crews, 2005). Efforts are spent to understand the multiple coordinated systems permitting individual maintenance of homeostasis across many environments and stressors during critical developmental and reproductive periods. Within context of adipose tissue, central in chronic and metabolic disease, its function changes dramatically throughout life (Tchkonia et al., 2010). The observed increase in the expression of adipose with age is a product of extensive increases or decreases in select hormonal function and production (Michalakis et al., 2013). The endocrinology of aging seeks to understand how these hormonal changes accelerate or slow the aging process in response to environment (Michael, 2010). A better understanding of the dynamics between adipose tissue and the nature of the nutritional environment may enhance studies of human variation in chronic disease.

Chapter 2 covers vitamin D and its synthesis, variation, clinical levels and role in the production of adipose. Chapter 3 discusses adipose tissue physiology, its functions as a hormone organ and how the body makes new adipose. Chapter 4 talks about adipokines adiponectin and leptin, their principle functions as environmental signals and how they can be used to address MetS and chronic disease. Chapter 5 focuses on the methods, research design of the study as well as a brief ethnohistorical survey of the Anabaptist Mennonite. Chapters 6, 7 and 8 consist of results, discussion and conclusion, respectively.
Vitamin D

Highlighted in this chapter and several others are the interactions between vitamin D and key biomarkers of chronic disease and individual health. Vitamin D is two things as once; a nutrient that has been essential to life for hundreds of millions of years as well as a steroid-hormone (Holick, 2003b). It is unique in being the only nutrient that is independently made by the body using light as well as obtained from animal and plant food sources. A majority of vitamin D’s potential in being used as a biomedical target lies in the combined efforts of vitamin D and its nuclear receptor (DeLuca, 2004). Once vitamin D is consumed and bound to its receptor, this vitamin D-receptor complex is able to directly modify genetic activity. Either through binding to additional transcription factors, coactivators, or corepressors, vitamin D regulates the expression of over 1,000 genes (Carlberg, 2014). Established properties of vitamin D now include roles in the immune function, regulation of cellular division and modifying neurohormones (DeLuca, 2004; Bikle, 2010).

Evolution of Vitamin D

Vitamin D is one of the oldest hormones that have been made in the earliest life forms for over 750 million years (Holick, 2003b). Vitamin D has been central in the evolution of many animals long before humans, primates or mammals (Holick, 2003a). The human evolutionary diet, or publicly perceived natural diet, in most human populations contains little vitamin D (Naeem, 2010). A co-evolutionary model of vitamin D may exist given the biocultural determinants in variation and function. In the presence of specific cultural practices as agriculture, modifying modes of nutritional production, and clothing in expression or ritual, determine how much vitamin D is getting into the system.
It has been hypothesized and criticized that vitamin D may have co-evolved under selective cultural practices resulting in lactose tolerance (DeLuca, 2008; Flatz and Rotthauwe, 1973). Being able to tolerate greater dairy intake may be advantageous in environments with poor solar or nutritional vitamin D supply. Such environmental settings include high altitudes, high latitudes, or ecological contexts poor vitamin D production or sources. While sufficient vitamin D cannot be produced at higher latitudes, this would not have been a challenge for pre-Neolithic European hunter-gatherers, with a robust marine diet, while agriculturalists may have suffered (Gerbault et al., 2011; Richards et al., 2003). The evolutionary gambit of vitamin D in humans remains understudied and unclear at best.

**Vitamin D Synthesis and Production**

Vitamin D is the only nutrient that is made by the body and obtained through food. The evolutionary diet, or publicly perceived natural diet, of humans contains little vitamin D (Naeem, 2010). Some food sources of vitamin D include egg yolk, fatty fish, and fortified products (Heldenberg et al., 1992; Ovesen et al., 2003). While plants are also able to produce a form of vitamin D (D₂), it does not satisfy the same optima in human health and does not achieve the same health benefits as D3 which is produced by animals (Armas et al., 2004; Houghton and Vieth, 2006). In its traditionally known roles as a regulator in calcium and phosphorus absorption and homeostasis, vitamin D is a central controller in making new bone and maintaining bone health. Once obtained, vitamin D must pass through several organs before activation and requires a lipid rich environment when obtained through diet. Vitamin D has a high bonding affinity with fat molecules called chylomicrons and other classes of lipid-proteins (Brannon and Fleet, 2011; Haddad et al., 1993). This is why vitamin D is sometimes described as a lipid soluble micronutrient in
nutrition and medicine. For optimal efficacy and benefit the most reliable form of vitamin D for humans to obtain is D3 (Trang et al., 1998; Heaney et al., 2010). For every 100 international units (IU) of vitamin D ingested, blood levels D increases by 1 ng/ml (Holick, 2010). While D3 is more appropriate for human health, additional research is needed to examine the metabolics of vitamin D and the effects across age, sex, and nationality (Calberg, 2014; Tripkovic et al., 2012). Regardless of how vitamin D is acquired it must pass through both the liver and kidney in order to have any biological effect (Christakos et al., 2010).

The body’s conversion of cholesterol into vitamin D from UV light is not a linear relationship. It is determined by doses of light, frequency of light and the presence of other compounds in the skin (Olds, et al., 2008). This means directly increasing exposure to sunlight does not result in a proportional increase in available vitamin D. Excessive exposure to UV radiation results in the formation of many cancers, particularly cancer of the skin and retina (Grant, 2003; Narayanan et al., 2010). Vitamin D is made in the skin by reacting a form of cholesterol with UV radiation while nutritional sources are consumed or supplemented (Chen et al., 2007; Holick, 2011). In skin cells, UV-B light (290-320 nm) help convert cholesterol to pre-vitamin D, becomes vitamin D and then bind to a chylomicron and transported to the liver for its first step in activation (Brannon and Fleet, 2011). The body’s conversion of cholesterol into vitamin D from light however is not a linear relationship. It is determined by doses of light, frequency of light and the presence of other compounds in the skin (Olds, et al., 2008). Prepared in the skin, previtamin D3 is rapidly converted to vitamin D3 by a temperature dependent process. It undergoes a
temperature-dependent thermal isomerization that takes at least 3 days to complete (Holick, 1980).

**Variation in Vitamin D**

Despite the clear importance in human health, differences in vitamin D levels and their impacts are enigmatic (Bidulescu et al., 2014; Kaneko et al., 2015; Tripkovic et al., 2012). In determining individual vitamin D levels it is important to note geographic location as levels respond to the natural UV exposure and seasonal periodicity of the sun (Heidari and Mirghassemi, 2012; Kimlin, 2008). While 50 to 90 percent of vitamin D requirements are satisfied by sun exposure there still persists an overwhelming presence of global deficiency (Heidari and Mirghassemi, 2012; Naeem, 2010; Smith et al., 2010). Populations in the greater Pacific Northwest region of the U.S. experience intense ecological vitamin D deprivation (Johnson, 2010). Producing vitamin D from sunlight is initiated by UV-B rays with a wavelength of 295-315 nanometers (nm), but production occurs optimally at 297 nm (Stinson, Bogin and O’Rourke, 2012).

Dimorphic and ethnic differences exist in vitamin D levels with women being more likely to be deficient or in states of hypovitaminosis (Bidulesecu et al., 2014; Smith, 2010; Zadshir et al., 2005). Sex-specific variations in vitamin D also appear to be influenced by age (Dam et al., 2009; Jungert and Neuhäuser-Berthold, 2015). In general, young people have higher levels of vitamin D than older people and males have higher levels than females and leaner individuals have greater vitamin D levels than heavier individuals (Yetley, 2008). The strong sex and age specific factors must be considered when designing interventional or therapy responses (Nesby-O’Dell et al., 2002). Prison populations also suffer from chronically low levels of vitamin D with severity of
incarceration as a dramatic determinant (Nwosu et al., 2014). Alcohol consumption and smoking tobacco also affect vitamin D levels and the extent to which is sex-specific (Brot et al., 1999; Lee, 2012).

The observed lifestyles, diets and livelihoods in most Western countries often place groups in natural or artificial context that are not sufficient for maintaining a safe vitamin D status or its associated benefits. Time of day, season of the year, latitude, aging, lotions and sunscreens and melanin concentration can all have a dramatic influence on the cutaneous production of vitamin D (Heidari and Mirghassemi, 2012; Holick, 2011; Kimlin, 2008; Webb and Holick, 1988). Vitamin D production does not occur before 9 AM and ceases after 4 PM, even in the summer (Holick, 2011). Exposure to sunlight through glass will not produce any vitamin D because glass absorbs UV radiation (Holick, 2011; Reichrath and Nürnberg, 2009). Vitamin D concentrations are affected by high fat diets as well (Lee, 2012; Park et al., 2015; Pinto and Cooper, 2014).

**Vitamin D Physiology and Metabolism**

Vitamin D is critically important for the development, growth, and maintenance of a healthy skeleton from birth until death (Gennari, 2001; Weaver, 2007). A central function of vitamin D is to maintain calcium and phosphorous levels homeostasis by increasing intestinal absorption of both (Bouillon and Suda, 2014; DeLuca, 1980). Vitamin D communicates to bone cells to dissolve calcium from their stores when there is inadequate calcium to satisfy bodily requirements (Holick, 2003b). Vitamin D’s effects go far beyond just bone and nutrition health though.

Vitamin D it is more accurately classified as an active steroid-hormone or prohormone (Cutolo et al., 2014; DeLuca, 2004; Verstuyf et al., 2010; Wang et al., 2005).
Steroid hormones exert a wide range of physiological responses, including functions in the immune system, protein and carbohydrate metabolism, water and salt balance, reproductive system and development of sexual characteristics (Lundqvist, 2014). Vitamin D as a steroid-hormone has demonstrated many important pleiotropic effects in the body (Pike et al., 2014; van de Peppel and van Leeuwen, 2014; Verstuyf et al., 2010). As a steroid-hormone itself vitamin D modifies production and function of other hormones in the body including sex hormones and steroids (Lundqvist, 2014). Regulating many gene networks, behavior-mediating hormones and other environmental signals, vitamin D appears to have heavy behavioral implications; in form, acquisition and function.

Vitamin D is transported in the circulatory system and throughout the body using vitamin D binding proteins (VDBP) (Christakos et al., 2010; Haddad et al., 1993). Relative to albumin, one of the most abundant blood proteins, VDBPs are only slightly smaller (Holick, 2010). Once vitamin D is made in the skin or ingested from diet, it is transported to the liver and kidney to complete the final activation steps. First, removal of a hydroxyl group by enzyme CYP27A1 in the liver and removal of a second hydroxyl by CYP27B1 in the kidney produces hormonally active vitamin D (Bochud, 2011; Holick, 2007a). Activated vitamin D can also be produced outside of the kidneys and can act locally in the tissues which produce it (Dusso and Slatopolsky, 1994). While some of vitamin D’s effects can occur in the cellular membrane it predominately acts by binding to the vitamin D receptor (VDR) and other receptors inside of the cell (Khanal and Nemere, 2007; Mizwicki and Norman, 2009). Vitamin D dissociates from the VDBP, enters the cell and then binds to and activates the VDR. The vitamin D-VDR complex enters the nucleus joining with retinoid x receptor (RXR), to form vitamin D response elements (VDRE). The VDRE
complex binds to select DNA sequences in genes and modulates their genetic expression (Carlberg, 2014; Mizwicki and Norman, 2009).

**Vitamin D and its Nuclear Receptor**

Vitamin D is pleiotropic in its function. It adjusts and modifies genetic activity and many gene networks (Pike et al., 2014; van de Peppel and van Leeuwen, 2014; Verstuyf et al., 2010). Vitamin D works in a lock-and-key manner and binds to the vitamin D receptor (VDR). This nuclear receptor is found widespread throughout the body. Variation in the VDR gene are associated with inflammation and obesity (Al-Daghri et al., 2014). The vitamin D-VDR complex then enters the nucleus, sometimes binding with additional proteins, influencing genetic activity and regulation (Davis and Milner, 2011; Haussler et al., 1998; Kamen and Tangpricha, 2010; Wood, 2008). Vitamin D is known to regulate more than 1,000 different genes (Calberg, 2014; Ramagopalan et al., 2010). Gene networks influenced by vitamin D are involved in chronic, inflammatory, immunological and metabolic diseases. These nutrigenetic capacities influence and possibly enhance individual abilities to manage ones genetic health (Chattopadhyay et al., 2003; Davis and Milner, 2011). This means dietary intake of vitamin D may be shaping the expression of human genetic traits and ultimately phenotypes (Fenech et al., 2011).

**Vitamin D: Deficiency, Insufficiency and Disease**

Malnutrition exist in both excess and deficiency (Westerterp, 2013). Clinical practice roughly defines 3 forms of malnutrition: starvation related, acute disease related, injury related, and chronic disease related (Mauldin and O’Leary-Kelley, 2015). Malnutrition is a common symptom of obesity which results in in deficiencies of critical micronutrients connected to MetS, especially vitamin D deficiency (Foss, 2009; Via,
2012). Some estimates place the global prevalence of vitamin D deficiency for all adults and children between 30 and 50% (Gordon et al., 2004; Holick, 2008; Marwaha et al., 2005; McGrath et al., 2001; Tangpricha et al., 2002). More than 50% of the greater U.S. population is living with chronic vitamin D deficiency as well as populations in Canada, Mexico, Europe, Asia, New Zealand and Australia (Holick, 2007a). Other risk factors of vitamin D deficiency include premature birth, melanin expression, low sun exposure, sedentary behaviors, obesity, malabsorption from diet and old age (Holick, 2007b; Lips et al., 2006). The general window of acceptable vitamin D is minimally between 20-29 ng/mL with levels ≥30 ng/mL being optimal (Sadat-Ali and Allq, 2006). Blood levels of ~150-200 ng/ml approach toxic levels (Holick, 2011).

UV exposure is insufficient to produce the required quantities of vitamin D in people living at extreme latitudes for much of the year (Gerbault et al., 2011). It is expected that dietary sources satisfy bodily demands for vitamin D when light proves insufficient due to seasonality, geography, or lifestyle. The greatest source of vitamin D in the US is fortified foods, however, consumption of both conventional and fortified vitamin D food sources have declined (Del Valle et al., 2011). It is generally observed that men have higher vitamin D intake than females (Yetley, 2008).

Where food and sun fail, supplements most commonly employed in treating vitamin D inadequacy (Cianferotti et al., 2012). But, in 2008, Qato et al. estimated the prevalence and use of over-the-counter medications and dietary supplements in a sample of adults 57-85 yrs (n = 3,005). Findings showed only 4.5% of participants used vitamin D supplements (Qato et al., 2008). The U.S. and Canadian populations are largely dependent on fortified foods and dietary supplements to meet their vitamin D needs. Fortified milks
and cereals are the greatest source of vitamin D in the U.S. and fortified milk and margarine in Canada (Calvo et al., 2004). Vitamin D deficiency has been linked to obesity, as dysfunctional vitamin D levels are directly associated with many chronic and metabolic diseases found in obesity as well (Edita et al., 2014; Foss, 2009; Pereira-Santos et al., 2015; Vanlint, 2013).

**Vitamin D in Adipose Tissue and Adipogenesis**

Central to this thesis are the direct interactions vitamin D has with adipose tissue. Through its genetic or steroid-hormone abilities, vitamin D is able to changes the expression of fat genes, effect adipose tissue communication and adipose cell health (Mutt et al., 2014; Ding et al., 2012; Shi et al., 2001) Inflammation caused by adipose tissue is the key component to metabolic and chronic disease which vitamin D may potentially mediate (Cheng et al., 2010; Ding et al., 2010; Mutt et al., 2014). Vitamin D is frequently observed as a reliable predictor of adipose variation even after considering the effects of physical activity, smoking, drinking, sex and age (Cheng et al., 2010; Jamal-Allial et al., 2014; Snijder et al., 2005). Direct relationships between vitamin D and adipose health are also observed in healthy individuals otherwise not considered deficient in vitamin D (Cheng et al., 2010). Vitamin D deficiency has since been linked to obesity, possible causing extreme adipose phenotypes (Edita et al., 2014; Foss, 2009; Pereira-Santos et al., 2015). In the context of adipogenesis, vitamin D and its nuclear receptor directly affects the production of new fat cells (Ji and Hill, 2015; Ricciardi et al., 2014; Wood, 2008).
Adipose Tissue

Humans are born with the greatest volumes of body fat or adipose than other mammals, believed to support normal and healthy brain development as well as protect against times of low or deficient energy in the environment (Leonard et al., 2003).

Evolution of the large human brain has critical implications for the nutritional biology of our species; brain tissue is very energetically and metabolically expensive (Leonard et al., 2007). For decades it was believed that body fat was just a passive and inert storage space for excess calories. It is now recognized as an active component of the endocrine system, releasing hormone signals which regulate energy homeostasis (Kershaw and Flier, 2004).

Decreased physical activity and greater nutritional consumption results in the pathological accumulation of adipose tissue, thus disturbing normal hormone balance and energy regulation.

Evolution of Adipose

The anatomical complexity of adipose tissue morphology and function is not frequently explored from an evolutionary stand point (Ottaviani et al., 2011). Far more complex within an inclusive context of all organisms, isolated to mammals, adipose tissue serves as a central organ in reproduction, survival and health. While some mammals have developed tolerances or adaptations for excessive adipose tissue similar observations in humans and other primates are neither simple nor linear (Leonard et al., 2003; Pond, 2012). Conclusions rest now at the interface between brain and adipose tissue as they appear to be evolutionarily connected to each other (Grabowski, 2016; McGill, 2014; Navarrete and Isler, 2011). While adipose genes are clearly implicated in many biomedically relevant pathways, the ultimate expression of adipose phenotypes varies
dramatically by age, sex, nationality, social status, environmental endocrine blockers, nutritional deficiencies and poor food diversity (Grün and Blumberg, 2009; Karastergiou et al., 2012; Schug et al., 2011; Wells, 2012).

Hypotheses for the evolution and persistence of obesity include the thrifty gene or ‘genetic mismatch’ hypothesis stating chronic conditions like obesity and MetS result from our hunter-gatherer genetics being at odds with modern nutrition and food production (Neel, 1962). Alternatively, the drifty gene hypothesis postulates that after humans removed themselves from predation, we unintentionally removed the upper limits of our body sizes (Prentice et al., 2005; Speakman, 2008). Both hypotheses are likely acting in tandem and not alone. The explanatory mechanisms behind obesity are likely epigenetic, where genes and their networks are changed according to environmental signals (Chakravarthy and Booth, 2004; Herrera et al., 2011; Youngson and Morris, 2013). The entirety of the obesity crisis cannot be explained through genetics alone. Nutritional deficiencies such as vitamin D deficiency are identified as causative in obesity and metabolic disease (Edita et al., 2014; Foss, 2009; Pereira-Santos et al., 2015). Managing adipose tissue and the precarious balance between health and disease is a function of both culture and biology. Changes in energy balance, including energy availability, storage and expenditure over the course of life history, are central to the development of obesity.
Figure 2. The major physiological and metabolic processes adipose tissue is active in. These processes are guided by adipose derived hormones known as adipokines, including adiponectin and leptin.

Adipose Tissue

Adipose tissue should be more accurately conceived as a hormone organ rather than an inert storage tissue (Adamczak and Więcek, 2013; Scherer, 2006; Siiteri, 1987; Więcek et al., 2002). It has been discovered to perform complex functions influencing numerous physiological systems, even considered a significant organ in maintaining immunological health (Grant and Dixit, 2015; Kershaw and Flier, 2004). Finding the “obese gene” and its product, leptin, was one of the first empirical observations of a fat cell derived pleiotropic molecule, helping establish an important role for adipose tissue among the other endocrine organs (Galic et al., 2010; Gorden and Gavrilova, 2003). Placement of adipose tissue in the endocrine system requires reexamining the relationships between adipose and other major organ systems (Scherer, 2006). While many clinical and epidemiological studies of adipose have focused on the suffering induced by excess adipose, disease may arise with zero body fat as well (Handelsman et al., 2013; Herranz et al., 2008; Moitra et al., 1998). Given the existing abundance, frequent cosmetic removal and natural plasticity of adipose tissue, it
has garnered interest among tissue engineers and stem cell scientists (Cawthorn et al., 2012; Francis et al., 2010; Zuk et al., 2001; Zuk et al., 2002). A greater understanding of adipose variation will be beneficial when reevaluating preexisting knowledges of hormone biology and adipose related pathologies.

The relationships between this endocrine organ and the human environment are complex. Adipose tissue exists in different forms in order to meet select environmental and bodily demand (Giralt and Villorroya, 2013). White adipose tissue is the form most studies for its implications in the pathophysiology of many chronic and lifestyle diseases (Chudek et al., 2006; Trayhurn and Wood, 2004). Brown adipose tissue has been identified and is distinct from white adipose in anatomical location, morphological structure, function, and regulation (Cypess et al., 2009; Park et al., 2014). This form of adipose possesses chemical properties distinct from white adipose which make it an exciting target for novel metabolic therapies. Brown adipose tissue is both more abundant in females than males and is an appealing in chronic and metabolic disease research for its ability to increase energy expenditure by transforming chemical energy into heat energy (Cypess et al., 2009; Wu et al., 2013). Beige adipose as the third form of adipose tissue is relatively new in discovery (Lidell et al., 2013). Both brown and beige adipose tissue can suppress weight gain and metabolic disease through the action of their specialized, heat-producing adipocytes (Harms and Seale, 2013). Distinction of beige adipose from adipose is its formation within white adipose itself, earning the technical nickname of brite (brown-like-in-white) adipose (Giralt and Villorroya, 2013; Sanchez-Gurmaches et al., 2016). The variety of forms of adipose tissue increases the complexity between environmental stress and ultimately
phenotypic expression. Variation in individual adiposity appears to be a function of genetic and non-genetic factors (Grundberg et al., 2013).

**Adipocytes**

Adipocytes are fat cells. These cells are the primary constituent within the milieu of adipose tissue producing adiponectin and leptin. There are many different kinds of fat cells found local to the specific variety of fat tissue in the body (Giralt and Villorroya, 2013). Adipose tissue may contain few large adipocytes (hypertrophy) or many small adipocytes (hyperplasia) (Arner et al., 2010; Rutkowski et al., 2015). Individual contributions of these two responses varies depending upon genetic background, modifying behavior, diet, biological factors and adiposity pattern (Berry et al., 2013). Size of fat cells have shown to directly influence the levels of secreted adipokines (Skurk et al., 2007). In case of obesity it is the expression of adipose genes responsible for a rapid production of new adipocytes (Drolet et al., 2007; Jo et al., 2009; Lönnqvist et al., 1995; Lundgren et al., 2007).

**Adipose Tissue Signaling and Biochemistry**

Adipose health is managed similarly to any other organ which means cellular communication to and from adipose is very important. Adipose cells and their signaling molecules commonly fulfill endocrine, autocrine, paracrine and juxtacrine roles (Dozio et al., 2011; Kim and Moustaid-Moussa, 2000; Sweeney, 2011). The adipose gene leptin produces an exemplary adipose signaler or adipokine which takes advantage of both endocrine and nervous systems to communicate to the body (Mainardi et al., 2013; Nalini et al., 2015; Trayhurn and Wood, 2004). Adipose signals are active in monitoring processes and behaviors as nutritional intake, blood glucose regulation, and inflammation. Integrating the dynamic functions of adipose tissue is aided by the several different tissue
Adipokines: Signaling Molecules from Adipose

Adipose secretes many adipokines, notably leptin and adiponectin, and a diverse range of other signaling factors. The term 'adipokine' is used to describe a protein that is secreted from and synthesized by adipocytes (Trayhurn and Wood, 2004). Interestingly a positive correlation has been observed between adipocyte size and secretion of adipokines with greater adiponectin and leptin production with increasing cell size (Skurk et al., 2007). Adipokine proteins are active in lipid metabolism, insulin and vascular hemostasis as well as regulation of blood and fluid pressure. Production of these proteins by adipose tissue is increased in obesity such that irregular levels of adipokines like adiponectin and leptin are characteristic of many chronic diseases (Deng and Schere, 2010; Fontana et al., 2007; Trayhurn and Wood, 2004). Declared as novel markers in chronic disease research, adipokines are critical in the enhancement of MetS diagnostics as well as enriching individualized health (Deng and Sherer, 2010; Tilg and Moschen, 2006; Van de Voorde et al., 2013). Adipokines are implicated in the pathogenesis of obesity and MetS and associated cancers (Booth et al., 2015). Applying understanding of adipokines to chronic diseases typically associated with Western Civilization may prove beneficial.

While maintaining high potential in designing novel therapeutic strategies and biomedical interventions, standardizing individual adipokines levels is tricky due to multifactorial mediators in genetic expression of these proteins but also in population level studies (Lee et al., 2007). Several factors influencing individual levels of these hormones
include vitamin D, adiposity, smoking and drinking (Al Mutairi et al., 2008). Additionally, individual vitamin D levels may also be affected by adiposity and adipokines (Deng et al., 2010). Further discussion of adipokines can be found in a separate chapter. Focus has been given to adipokines due to their causal links to metabolic diseases such as obesity and diabetes (Skurk et al., 2007; Hara et al., 2005; Rasouli and Kern, 2008. A greater understanding of adipokines and their relationships with adipose and the environment will hopefully help in addressing adipose-related chronic disease (Alvarez-Llamas et al., 2007; Pardo et al., 2012; Young and Morris, 2013). Before adipokines may be used in interventional models a greater understanding of their variation and environmental interactions is required.

**Adipogenesis: Production of the Adipose Phenotype**

Adipogenesis is the development of new fat cells or adipocytes (Namwanje et al., 2016; Rosen and Spiegelman, 2000). The process is exceptionally complex and not well understood in totality (Parks et al., 2014; Sanchez-Gurmaches et al., 2016). Fetal development of adipose tissue is controlled by the interactions between adipogenic transcription factors, nutrients and adipokines (Kiess et al., 2008). Maternal and endocrine factors also influence specific changes in angiogenesis, adipogenesis, and metabolism. Adipogenesis is believed to begin between the 14th and 16th week of fetal development (Han et al., 2011; Poissonnet et al., 1983; Poissonnet et al., 1984). Approximately ten percent of adipocytes renewed annually at all adult ages and levels of body mass index (Spalding et al., 2008). While existing in three forms, all adipocytes of each tissue type are derived from the same starting cell (see figure 2). The ease of modifying adipocytes to make stem cells make them ideal for personalized cell therapies
for chronic and metabolic disease (Cawthorn et al., 2012; Francis et al., 2010; Zuk et al., 2001).

Figure 3. Differentiation of mesenchymal cells into a white, brown, beige or brite adipocyte or muscle cell. Brown adipose tissue is more closely related to muscle than other adipose type since muscle cells and brown adipocytes come from the same stem cell (Park et al., 2014 pg. 37, reproduced with permission).

Abnormal gene expression resulting in an abundance of adipose tissue is in part due to environment like diet and behavior but also dysfunctional transcription factors. Transcription factors work by controlling gene expression. Important adipose transcription factors include CCAAT/enhancer binding protein (C/EBP), peroxisome proliferator-activated receptor (PPAR) and the vitamin D receptor (VDR) (Fu et al., 2005; Ji et al., 2015; Lefterova et al., 2008; Ricciardi et al., 2014; Zhao et al., 2015). Natural variation in some transcription factors can be effected by age. The increased production of anti-adipogenic C/EBP is possibly due to cellular stress seen with aging (Kirkland et al., 2002;
Transcription factors C/EBP and PPAR are known to act together in adipogenesis (Rosen et al., 2002; Rosen et al., 2005; Wu et al., 1999). The expression of both PPAR and C/EBP may be regulated by nutrients and hormones (Chapin et al., 1994; Jump and Clarke, 1999; Roesler, 2001; Vidal-Puig et al., 1996). All three transcription factors however, C/EBP, PPAR and VDR, regulate other physiologies beyond adipose tissue (Dhawan et al., 2005; Sertznig et al., 2009a; Sertznig et al., 2009b; Song et al., 2006). These three proteins, along with the appropriate ligands or activators may be instrumental in addressing chronic and metabolic disease (Al-Daghri et al., 2014; Olofsson et al., 2008; Robitaille et al., 2004; Tanaka et al., 2003; Wong et al., 2009). For instance, C/EBPs work together with VDR to regulate an enzyme central to vitamin D metabolism (Dhawan et al., 2005). PPAR-alpha is one form PPAR which serves as an important environmental communicator for metabolic adaptations (Contreras et al., 2013). The functional processes shared between vitamin D and adipogenesis may be connected and responsible for both disease and nutritional epidemics.

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*Table 2.* The major adipogenic transcription factors, peroxisome proliferator-activated receptor (PPAR) and CCAAT-enhancer binding protein (C/EBP), and their isoforms. PPAR and C/EBP both guide the cellular production of adipocytes.
Vitamin D in Adipogenesis

Nutrients interact directly with subcutaneous and visceral adipose tissue in the body by differentiating gene expression patterns during periods of fasting and eating, thereby changing the response of adipose tissue to environmental signals (Einstein et al., 2005). It has been empirically demonstrated that nutritional manipulations can also greatly alter adipose tissue phenotypes (Choi et al., 2016a; Choi et al., 2016b; Ferguson et al., 2016; Kao et al., 2016; Li et al., 2016; Poulos et al., 2010). A select form of micronutrients known as lipophilic nutrients require lipid rich environments in activation and biological function. These nutrients are strong modulators of adipose physiology and biochemistry (Landrier et al., 2012). Since vitamin D is fat soluble after ingestion or production from light it get incorporated and absorbed into body fat. Vitamin D and its nuclear receptor have the capacity to directly alter the expression of adipose transcription factors and adipokines (Blumberg et al., 2006; Ding et al., 2012; Gysemans et al., 2005; Ishida et al., 1988; Jamal-Allial et al., 2014; Ji et al., 2015; Kong et al., 2013; Mutt et al., 2014; Ricciardi et al., 2014; Stokić et al., 2015; Wood, 2008). Other lipophilic nutrients are also able to interfere with the genetic production of adipose (Jamal-Allial et al., 2014; Ji et al., 2015; Schwarz et al., 1997; Sun and Zemel, 2008). These nutrigenetic mechanisms that allow components of food to modify disease risk at a genomic level provide a profound direction for epidemiological research into chronic and metabolic disease.
Obesity

Obesity is a multifactorial chronic disease resulting from complications in energy use created by genetic and environmental factors (Koszowska et al., 2014). In 2010 overweight and obesity were responsible for over 3.4 million deaths worldwide (Ng et al., 2014). Apart from bariatric surgery, which is costly and not without risk, there are currently no successful long-term treatment options for obesity (Tam et al., 2011). Chronic disease management of dysfunctional adipose tissue is estimated to cost the U.S. upwards of 140 billion dollars annually (Zamosky, 2013). Clinical obesity and surgical bariatric procedures also drive reproductively significant epigenetic variation in men (Donkin et al., 2016). In spite of its several limitations, body mass index (BMI), a measure of individual adiposity or body fat, is the most widely used and accepted means for classifying overweight and obesity status (Bennasar-Veny et al., 2013; Freedman and Sherry, 2009; Oud, 2013; Wright et al., 2015).
While obesity is clinically defined as the pathological accumulation of adipose tissue, individuals vary significantly in body fat patterning. By the turn of the century, obesity replaced undernutrition and infectious diseases as leading contributor to ill health (Kopelman, 2000). Within U.S. populations, an association exists between obesity status and cancer mortalities, with rates of obesity doubling since 1980 (Calle et al., 2003; Belardi et al., 2013; WHO, 2014). This June, Flegal and colleagues (2016) found obesity, the single greatest risk factor for MetS, to be over 35% 40% in U.S. males and females respectively (2016). Despite community, political and medical interventions and research, chronic and metabolic disease prevalence soars.

Adiposity patterns may determine which comorbidities one experiences from excess adipose including cardiovascular disease and MetS (Bennasar-Veny et al., 2013). MetS is characterized by the simultaneous presence of multiple chronic and age related conditions with obesity as the leading risk factor (Rogers et al., 2003; Scaglione et al., 2010). Methods attentive to cohort variation in obesity prevalence and age variation in obesity's effect on mortality risk suggest that obesity significantly shapes US mortality levels, placing it at the forefront of concern for public health action (Masters et al., 2013). Based on comparable risk assessment methods, poor health behavior such as lifestyle-related risk factors are the foremost causes of death and disability in the United States and in the world (Go et al., 2014).

**Vitamin D, Obesity and MetS**

Vitamin D deficiency and obesity are both major public health problems worldwide, and there is mounting evidence and data that they are connected (Earthman et al., 2012). Global obesity has more than doubled since 1980, with more than 600 million
adults and 42 million children under the age of 5 obese (WHO, 2014). The US and global levels of vitamin D have been declining and obesity rates continuously escalating (Del Valle et al., 2011; WHO, 2014). Obesity-associated vitamin D insufficiency is likely due to the decreased bioavailability of vitamin D3 from cutaneous and dietary sources because of its deposition in body fat compartments (Worstman et al., 2000). Hypotheses of vitamin D deficiency have suggested the rates of vitamin D deficiency that are observed in obese patients is also a question of sequestration in adipose tissue due and a dilution of concentrations by mass (Drincic et al., 2012).

The shared pathways of vitamin D deficiency and dysfunctional adipose tissue lead to the development of many cardiometabolic risks chronic diseases. Edita and colleagues (2014) suggest a negative correlation between vitamin D levels and leptin while finding an intensive positive correlation between adiponectin and vitamin D. Some studies show an inverse association between vitamin D levels and insulin resistance and diabetes while improving vitamin D status shows a positive correlation with insulin sensitivity (Ford et al., 2005; Scragg et al., 2004). Vitamin D deficiencies have also been shown to reduce insulin secretion in a population of East London Asians with 95% of at-risk persons were vitamin D deficient (Boucher et al., 1995). The gene considered to be central in developing new fat cells (PPARδ) is directly targets and influenced by vitamin D, potentially swaying the associations between vitamin D and adipose tissue (Dunlop et al., 2005; Matsuda and Kitagishi, 2013; Pike and Meyer, 2010). These data suggest a possible connection between vitamin D to the prevalence and risk of MetS.
Adipokines

The hormones leptin and adiponectin are two examples of adipokines which play central roles in managing the energy acquisition and use within the human body. Larger implications of adiponectin and leptin include novel roles in modulating lipid phenotypes, metabolism and treating chronic disease (Deng and Scherer, 2010; Lago et al., 2009). Single nucleotide polymorphisms (SNPs) of the adiponectin or leptin gene may explain variation in health and lifespan (Arai et al., 2006; Khabour et al., 2010; Kolovou et al., 2011). It should be noted that in both men and women, adiponectin and leptin are susceptible to environmental silencing in adipose tissue and blood cells depending on individual BMI, waist measures and LDL lipids (Houde et al., 2015). These two adipokines will be the focus of this chapter.

Adiponectin and leptin are present at significant concentrations in developing tissues during embryogenesis and fetal development (Kiess et al., 2008). Adiponectin and leptin are both made within fat cells (Cammisotto et al., 2006; Chandran et al., 2003; Koh et al., 2007). The genes for adiponectin and leptin can sometimes be turned off by environmental signals (Fasshauer et al., 2002; Stöger, 2006). These hormones help control bodily use of energy and body composition (Havel, 2002; Schoppen et al., 2010; Smith et al., 2006; Sweeney, 2011). Complications from having high or low levels of these hormone often requires clinical intervention and eventually leads to chronic and metabolic disease if left unaddressed or undiagnosed (Meirer and Gressner, 2004). There is significant variation between individuals in these two hormones which is heavily determined by sex (Ahmed et al., 1999; Bidulescu et al., 2014; Lee et al., 2007; Ronconi et al., 2009). Adiponectin and
leptin levels are also influenced by nutritional intake (Edita et al., 2014; Spreadbury et al., 2012).

**Adiponectin**

Discovered in 1995, the adipokine adiponectin is directly implicated in many chronic and metabolic diseases in both adults and young children (Kardas et al., 2013; Trujillo and Scherer, 2005; Wiecek et al., 2007; Yamauchi and Kadowaki, 2013). Adiponectin functions as a hormone in bodily energy homeostasis as well as mediates inflammation signaling pathways as an anti-inflammatory (Ouchi and Walsh, 2007; Shehzad et al., 2012; Sweeney, 2011). Adiponectin’s additional role as an anti-inflammatory may explain some of its large implications within MetS and other major chronic illnesses (Cai et al., 2015; Matsuzawa et al., 2004; Ohashi et al., 2004). Chan and colleagues (2012) report finding adiponectin to be a potential therapy in treating the neurotoxicity of Alzheimer’s disease. Possessing many active roles, adiponectin has become an important part of many metabolic therapies and chronic disease treatments.

As adiponectin is most actively produced by adipocytes it quickly enters the bloodstream and to then onto the local organ and cells of interest. Tissues requiring regular adiponectin homeostasis possess several endothelial cells and layer, requiring specialized active transport. Predicated upon oligomer size adiponectin requires differential trans-endothelial transport. (Rutkowski et al., 2014). The pathological accumulation of adipose tissue characteristic of metabolic phenotypes is directly due growth and development of new adipocytes rather than simply the expansion of existing ones (Jo et al., 2009). When circulating in the body adiponectin operates as a globular protein, binding to other adiponectin hormones forming larger adiponectin molecules. Decreased levels of
adipokines like adiponectin may be pathological and can be caused by interactions with specific alleles or environment factors like sleep or even a high-fat diet (Hara et al., 2005; Nakagawa et al., 2008).

Many metabolic pathways of adiponectin interact or utilize vitamin D in order to change and regulate many physiological processes, complicated by age, sex and individual adiposity (Bidulescu et al., 2014; Kardas et al., 2013). A mutual site of interest in adiponectin and vitamin D metabolism is the adipocyte. While adiponectin is synthesized within adipocytes, vitamin D is stored in them and aids in adipocyte energy homeostasis (Ding et al., 2012; Edita et al., 2014; Mutt et al., 2014). Enhancing understanding of adipokines and nutritional intake may prove beneficial in designing individualized and economically open interventions to MetS using diet and adipokines like adiponectin.

Leptin

Leptin’s name is derived from the Greek word “leptos” generally meaning thin (Kelesidis et al., 2010). With the discovery of leptin and its production by adipose helped pioneer the concept that adipose tissue was not just an inert storage organ but rather an active component to the endocrine organ system. Leptin is another important adipokine made in adipose and other tissues including placenta, ovaries, mammary epithelium, bone marrow, and lymphoid tissues (Cammisotto et al., 2006; Margetic et al., 2002). This implies leptin’s involvement in other physiological system, including reproductive, immunological, and cellular growth (Fantuzzi, 2006; Gat-Yablonski and Phillip, 2008; Hill et al., 2008). Leptin is important in maintaining many health systems particularly in regulating and maintaining adipose tissue, weight and energy balance (Chehab, 2000; Friedman and Halaas, 1998l Mantzoros et al., 2011; Nalini et al., 2015; Frühbeck and
Pentice, 1998). Identified in 1994, leptin was believed to be the treatment to many chronic and metabolic diseases; especially obesity (Gautron and Elmquist, 2011). Leptin is capable of indicating nutritional and energy deficits as well as predict future feeding and food intake (Chin-Chance et al., 2000; Margetic et al., 2002; Vinyard et al., 2012).

Total leptin in the body includes free floating and protein bound leptin (Brabant et al., 2000). The leptin receptor and its concentrations are dependent upon individual sex hormones, adiposity, sleep cycle and present levels of leptin (Chan et al., 2002). Unlike the manner in which adiponectin moves through body, leptin uses receptor-mediated transcytosis (Cammisotto et al., 2010). Unique to the leptin hormone is its ability to also function as a neurotransmitter, fulfilling roles in maintaining healthy neural development and plasticity (Mainardi et al., 2013; Paz-Filho et al., 2008). Since leptin communicates to the body using both nervous and endocrine systems it may be more accurately described as a neuro-hormone (Ahima et al., 2000; Wauters et al., 2000). One of the sources of interest in leptin is its ability to directly control feeding behavior using neural circuits (Bouret et al., 2004; Crespi and Unkefer, 2014). Highlighting its nervous and metabolic roles, leptin is responsible in regulating where adipose tissue is stored on the body (Schoppen et al., 2010; Smith et al., 2006).

Minocci et al. (2000) find that an individual’s leptin concentrations are heavily dependent upon not just amount of adipose but also its distribution on the body. Between visceral and subcutaneous adipose, leptin production and secretion appears to be anywhere from two to four times greater in subcutaneous adipose in women, both lean and obese (Van Harmelen et al., 1998). Exogenous leptin administration in normal weight samples, leptin responsive inhibits food intake and reduces the size of body fat (Harris, 2014). When
fat mass falls, plasma leptin concentrations fall too, stimulating appetite and suppressing energy expenditure until fat mass is restored while an increase in fat mass results in an increase in leptin levels, suppressing appetite until weight is lost (Friedman, 2010). Leptin is thereby conveying nutritional information to the brain, which in turn regulates most, perhaps all, other physiological systems. The homeostatic functions of maintaining energy use, acquisition and individual adiposity are central to leptin’s role as a neuro-hormone (Blundell et al., 2015; Schoppen et al., 2010; Smith et al., 2006; Wynee et al., 2005).

Leptin is a powerful environmental signaler provided its importance in navigating both nervous and endocrine systems. Leptin is understood to be protective against periods of ecological famine and feast by signaling the periods of environmental stress to the body (Crespi and Unkefer, 2014; Unger, 2005). This neurohormone enables humans and other animals to maintain levels of stored energy under many different environmental conditions. Due to its large role as an environmental signaling molecule, leptin is susceptible to environmental silencing, meaning the leptin gene may be tuned off or not expressed (Stöger, 2006). Leptin’s modulation of eating behavior, satiety and adipose tissue are arguably the leading considerations for its novel applications in aging and metabolic disease discussions (Pelleymounter et al., 1995; Tam et al., 2011).
Figure 5. Illustration of the adipose-leptin-neural circuit. The principle effects of leptin are on feeding behavior, metabolism and fat mass (adapted from Friedman et al., 2010 pg. 1103).

Clinical Significance of Adipokines

Adiponectin dysfunction is a functional means to diagnose chronic illnesses like MetS and obesity (Hu et al., 1996; Kohara et al., 2014; Goldstein and Scalia, 2004). For this reason, concentrations of adipokines like adiponectin and leptin have been used in diagnosing and management of many different forms of metabolic disease in humans (Deng and Schere, 2010). Adipokines execute mediating homeostatic functions in maintaining adipose tissue health and communication to other tissue systems (Lago et al., 2007; Tilg and Moschen, 2006; Xu and Vanhoutte, 2012). Adipokines leptin and adiponectin have demonstrated novel pharmacological potentials in treating and diagnosing chronic disease (Deng and Scherer, 2010; Van de Voorde et al., 2013). In fact, collection of biological samples or “biomarkers” along with nutritional information should become routine (Bingham, 2002; Jenab et al., 2009; Ocké and Kaas, 1997).
Methods

Nutritional and epidemiological investigations must go to great lengths in identifying ideal sample populations, gaining access, and establishing well defined parameters prior to data collection. While prevalence of vitamin D deficiency in the general population is alarmingly high, action is warranted and should be focused upon the population level rather the risk group level (Hyppönen and Power, 2007). The Kansas Mennonite are socio-culturally distinct in religious practice, migration history and cultural origins. Because of this relative isolation working with the Anabaptist communities is an opportunity to conduct a comparative and population level study on nutrition and markers of disease risk. Details and rationale of data collection methodologies are documented in Mosher et al. 2016. The objective of this research was to determine any sex-specific relationships between nutritional intake and concentrations of adiponectin and leptin in this Mennonite population.

Population and Participants Studied

The Anabaptist faith goes back to the Protestant Reformation in 1517 started by Martin Luther in present-day Wittenberg, Germany (Collinson, 2007; MacCulloch, 2004). Around 1525 formal Anabaptist sects were recognized in Germany, Switzerland and the Netherlands (Rogers and Rogers, 2000). Under the larger religion of Protestantism, Anabaptism divides into three primary denominations: Mennonite, Amish and Hutterite. Anabaptist populations have experienced many migratory occasions which have contributed to their social and biological isolation (see figure 7) (Crawford et al., 1989; McKusick, 1973; Pichler et al., 2010; Puffenberger, 2003). Upon the crumbling of Polish royal power in the 17th century, Poland was partitioned among Russia, Prussia and Austria.
Because of this, much of the Mennonite population came under Prussian rule. The Mennonite way of life and religion that developed under Polish rule was now threatened and in danger. This cultural persecution and change in politics paired with the propositions of free land provided significant motivation for Mennonite families to move to Russia beginning in 1788 (Friesen, 1989).

The Kansas population in this study has undergone several migrations as a result of social and religious persecution, figure 8 illustrates the fusion and splitting along congressional lines and the sharing of common migration histories (Crawford, 2000). The act of immigrating is an instance of forsaking cultural and linguistic heritage for the purpose of adapting to entirely different environments, surroundings and people (Buchheit, 2009). Moving to locations with differences in climate, food chain, social and economic factors can have consequences for individual health and wellbeing (Mosher, 2012). Coming into contact with intricate cultural strategies in managing environmental change can lead non-assimilation after migrating, instead forming religious, cultural and linguistic isolate communities to maintain cultural homogeneity and identity preservation. The settling of the Kansas Mennonite in the 19th century, along with other Anabaptist groups, are such cases (Buchheit, 2009).
Figure 6. Map illustrating several Mennonite migrations across Central and East European countries from Germany, city of Danzig, Poland (contemporary Gdańsk), Ukraine and Sweden (Crawford, 2000 pg. 39; reproduced with permission).

The Mennonite successfully established autonomous peoplehood characterized by nonconformity enforced by the exclusive use of German and spatial isolation (Engbrecht, 1985). As religious and economic refugees, Mennonite groups emigrated across Europe and into parts of the U.S. in 1874, Canada in the 1870’s as well and Mexico in the 1920’s (Allen, 1988; Keel, 2012; Rogers and Rogers, 2000; Stevenson and Everson, 2000). Immigration to the U.S. in 1874 is a demonstration of efforts to retain ethno-religious distinctiveness and rights. The Mennonite sought complete society autonomy in the U.S. but instead were offered individual self-sufficiency (Engbrecht, 1985). Upon arrival to the
U.S., the Mennonite became two separate groups with one group settling in Lincoln, Nebraska and the second near Wichita, Kansas.

![Map of Kansas, surrounding states and geographic distribution of some Mennonite congregations (from Melton, 2010 pg. 270).](image)

*Figure 7.* Map of Kansas, surrounding states and geographic distribution of some Mennonite congregations (from Melton, 2010 pg. 270).

While Amish and Hutterite communities have received considerable interest from researchers and scholars, Allen and Redekop (1967) were the first to conduct a study that focused specifically on non-Amish Mennonite. Collaboration with the Anabaptist Mennonite communities of Kansas and parts of the Midwest has been extensive (Crawford, 2000; Demarchi et al., 2005; Melton, 2012; Stevenson, Everson, and Crawford, 1989). While each Anabaptist religion has its own distinct cultural history, the Mennonite have settled across the U.S. several times in recent history. In 1858 John Halderman founded the Church of God in Christ Mennonite in Ohio (Melton, 2010). This particular group is considered a mix of Pennsylvanian Dutch, Germans and Mennonite immigrant populations from Russia (Crawford, 1989). The Halderman congregation soon split into the Meridian, Garden View and Lone Tree of Kansas. The most recent Mennonite immigrants to Kansas
are the Old Colony Mennonites in the 1990s to Garden City. This particular group traces its history to a group of Mennonite who originally immigrated to West Canada, then Cuauhtémoc, Chihuahua Mexico in the 1920’s and finally to Kansas (Allen, 1988; Allen and Redekop, 1967). In this thesis participants come from several Kansas Mennonite congregations. Specifically; Meridian, Garden View, Lone Tree and Gossel.

Figure 8. Ethnohistorical dendrogram of the Anabaptist Mennonite congregations (Martin et al., 1996 pg. 49, reproduced with permission).

Previous work has established the relatedness between some Anabaptist Mennonite congregations and families of the Midwest (Demarchi et al., 2005; Melton, 2010; Martin et al., 1996). Within the last 250 years the Mennonite of the Midwest have lived in three different locations and with distinct demographic contexts in each place (Rogers and Rogers, 2000; Stevenson and Everson, 2000; Stevenson, Everson and Grimes, 2004). The Kansas Mennonite practice largely traditional agricultural lifestyles, do not smoke or drink,
maintained extensive genealogical records and may share similar nutrition histories among them (Crawford, 2000; Rogers and Rogers, 2000). These cultural features of the Mennonite provide more reliable context in which to ask questions about nutrition and human health. Exclusion of smoking and drinking behaviors enhances data interpretations as these effect hormone and nutrient levels. Analogues to the greater U.S., the greatest cause of mortality among some Mennonite communities is chronic disease including heart disease, cancer and stroke (Melton, 2006). For these reasons the Mennonite of Kansas serve as an ideal population for studying the relationships between nutrition and phenotypes associated with disease.

This thesis uses previously collected data from the Kansas Nutrition Project (KNP) designed by Dr. M.J. Mosher and Dr. Michael Crawford at the University Kansas. The KNP sought to provide nutritional knowledge and context of Mennonite farming communities, examining relationships between nutrition, genes and chronic health and check for nutrition and health patterns within families. Researchers met with each congregation in their respective churches and explained the nature of the work. Samples of volunteers attended clinics that were held the week after community meetings. Particularly, data collected and analyzed occurred between 2003 and 2005. Sample collection included nutritional data in the form of three nonconsecutive days of food diary entries, fasting blood draws for hormone and lipid profiles and standard anthropometric and skinfold measures for body composition. Funds for the original project come from the State of Kansas Attorney General’s Settlement Fund. This thesis is covered by IRB clearance under Secondary Use of Data.
**Diet and Adipokines in Non-Human Studies**

Adipokine concentrations are used in diagnosing and treating metabolic conditions in non-human primates including Rhesus monkeys and Cynomolgus macaques (Bauer et al., 2011; Wagner et al., 2006; Hansen, 2001; Hotta et al., 2001). The effects of vitamin D on markers of metabolic health are also observed in non-human animals including murine and porcine models. Past work has shown a high fat diet resistant lean phenotype in vitamin D receptor null mutant mice suggesting a role for vitamin D in energy metabolism (Wong et al., 2011). Zeitz et al. (2003) report increased glucose levels in mice with functionally inactive vitamin D receptors while comparing diets enriched with calcium, phosphorus and lactose to both wild-type and mutant mice. Vitamin D deficiency often coexists with non-alcoholic fatty liver disease resulting in the dysfunction of this tissue central in vitamin D metabolism and activation (Eliades and Spyrou, 2015). Mice fed diets high in both vitamin D and calcium demonstrate activation of calcium mediate apoptotic protease pathways within mature adipocytes (Sergeev and Song, 2014). Vitamin D receptor null mice display a reduction in adipose mass, reduced plasma triglycerides and reduced cholesterol under normal calcemic conditions relative to wild-type counterparts (vinh quốc Luong and Hoàng Nguyễn, 2013). It has been shown that mice treated with vitamin D have increased adiponectin concentrations and mice without the vitamin D receptor have reduced leptin levels (Narvaez et al., 2009; Suarez-Martinez et al., 2014).

Vitamin D is also found to increase leptin expression in mice (Kong et al., 2013).

Adipokines like adiponectin appear to be intimately linked to obesity and inflammation in pigs and humans (Eliades and Spyrou, 2015). Ontogenetically, the expression of adiponectin and leptin increase over time in pigs (Ramsay and Caperna, 2009). Comparative dietary studies suggest nutritional regulation of adipokines
adiponectin and leptin in mini-pigs (Yang et al., 2013). Vitamin D deficiency in groups of pigs results significant changes in key metabolic and cardiac health markers including decreases in adiponectin within epicardial adipose (Gupta et al., 2012). Leptin concentrations are increased by high-energy diets as well as post-transcriptional mechanisms in pigs (Chen et al., 2006). Observed effects in non-human animals may be complicated by the recruitment of many molecular cascades within many tissue types (Suarez-Matinez et al., 2014).

**Diet and Adipokines in Humans**

Nutrients and their receptors interact with genes coding for hormones, particularly adipokines, which are central to chronic disease and aging biology (Krawczynska et al., 2014; Nimitphong et al., 2009; Xiong et al., 2014). Vitamin D may directly or indirectly influence normal levels of adipokines adiponectin and leptin (Bidulescu et al., 2014; Ghavamzadeh et al., 2014; Kong et al., 2013; Maetani et al., 2009; Menendez et al., 2001; Vaidya et al., 2012). While vitamin D has been shown to regulate the expression of some adipokines, these relationships are sensitive to age, sex, smoking, drinking, nationality and BMI (Bidulescu et al., 2014; Lee et al., 2007; Menendez et al., 2001; Sun and Zemel, 2008). Pischon et al. (2005) report modest alcohol consumption increasing adiponectin concentrations while a diet high in carbohydrates reduces concentrations, further supporting diet-adipokine interactions. Bidulescu et al. (2014) assert that associations between vitamin D and adiponectin to be strongest in females, any associations are dependent upon age, BMI and ancestry (Bidulescu et al., 2014). Cheng and colleagues (2015) find that variations in vitamin D enzyme genes are significant in determine ratios of adiponectin and leptin in a representative male Taiwanese sample. The effects of vitamin D
on adipokine phenotype is observed in both health and sick populations (Kim et al., 2013; Vilarrasa et al., 2010). The associations between vitamin D and adipokines have been examined in relation to cancer, bone protection in diabetic patients and MetS research (Cheng et al., 2015; Gannagé-Yared et al., 2009; Kasiappan et al., 2014; Lara-Castro et al., 2007; Maggi et al., 2013). Correlations between vitamin D and adipokines however may not be significant in those with additional endocrine complications such as thyroid disorders (de Luis et al., 2012). Vitamin D’s influence on adipokine concentrations is also partially determined by intake of other nutrients such as calcium, vitamin A or vitamin E (Mendez et al., 2001; Tabesh et al., 2012; Xiong et al., 2014). Jamal-Allial et al. (2014) noted that levels of vitamin D predict adiposity phenotype. Effects of vitamin D on adipokine levels are observed in healthy and sick populations (Kim et al., 2013; Vilarrasa et al., 2010).

Some investigations are criticized for producing conflicting results and suffering from poor research design. A critical look at Bidulescu’s experimental design reveals insufficient understanding of subpopulation characteristics, suboptimal sampling methods, small sample sizes and use of highly heterogeneous populations (Kuo, 2014; Lee et al., 2007). The Kansas Mennonite provide a unique opportunity to examine such gene-environment hypotheses with insulation resulting from the shared migratory histories, agricultural traditions, cultural and religious isolation of the Mennonite within greater America. Investigations in this study include the relationships of vitamin D intake with adipose and adipokine phenotypes. Adiponectin and leptin both possess high potential for treating and preventing chronic and metabolic disease (Deng and Scherer, 2010; Mattu and Randeva, 2013; Patel et al., 2008). Vitamin D is an active deciding factor in adipogenic
processes, interacting with circulating levels of adipokines and modifying genetic production of adiponectin and leptin (Bidulescu et al., 2014; Lee et al., 2007; Menendez et al., 2001; Sun and Zemel, 2008). I predict any correlations to operate within a dimorphic model with sex-specific associations between vitamin D and adipokine phenotype.

**Nutritional Data**

Nutrition is determined by many factors including evolution, food allergies and local ecosystems, individual preferences and bodily growth and development (Amarasekera et al., 2013; Oshaug, 2006; Patel et al., 2013). Diet, the manifestation of individual food preferences, is partially determined socially and psychologically (Johansson et al., 1999; Shepard, 1999). Many social variables greatly influence nutrition, individual diet as well as effecting knowledge and decision making processes about food. Food choices must be made multiple times a day and may exist between options that are realistic, preferred, sustainable and economic and geographically accessible. Socioeconomic standing, political ecology and local production-sustainability models are all major deciding factors in shaping and determining a person’s diet (Contento, 2007; Patrick and Nicklas, 2005). In nutritional studies there are three methods that can be used to determine an individual nutritional status; questionnaires, diet recalls and biomarkers (Ocké and Kaas, 1997; Willett, 2014). KNP participants were asked to keep food diaries, the gold standard and ideal method at the time for collecting individual nutritional habits and dietary patterns. Nutritional data were later entered into NutriBase™ processing software to analyze macro and micronutrient values of foods consumed. This permitted the construction of individual nutritional intake profiles of each participating Mennonite,
recoding their macro and micronutrient intake from their own described foods, recipes and conception of nutritional environments.

![Diagram of nutrient measurement methods]

**Figure 9.** Comparison of the three ways individual nutrition status can be measured in epidemiological investigation: (A) questionnaires, (B) dietary recalls and (C) molecular techniques, where p is the validity coefficient, r is sample correlation and T is true intake value. When statistically compared, differences between these methods are negligible (see Appendix A) (adapted from Ocké and Kaas, 1997 pg. 1241 and Willett, 2014 pg. 123).

In most epidemiological applications, long-term diet is the most rationale level of exposure, with studies focusing on physiological intermediates or changes interested in smaller time windows (Willett, 2014). Measuring and quantifying individual nutrition and dietary intake with high precision is challenging however (see figure 10) (Jl, 1993; Kipnis, 2002). In a contrast, a smoker can tell you how many and how frequently they smoke within an accurate average whereas asking most individuals what, how much and how often results in inaccurate or inconclusive answers. Understanding the nature of day-to-day
forces influencing variation in diet is essential in choosing an appropriate method to assess diet and to interpret data collected using various methods (Willett, 2014).

Figure 10. The process of determining the strength, weaknesses, common sources of error and objective and subjective limitations in dietary assessment. Picking the ideal method to measure or determine individual diet pattern requires navigating the different stages and levels people may construct or perceive their nutrition environment (adapted from MacIntyre, 2009 pg. 8).

Serological Samples
Central to the KNP objectives, nutritional and genetic interactions can be studied if biological samples are obtained (Bingham, 2002). Designed by researchers at the Laboratory of Biological Anthropology at the University of Kansas, the KNP included fasting blood draws for serological data including circulating blood lipid levels, serum hormone concentrations and extraction of DNA. Blood samples were collected by venipuncture from participants of each congregation. These samples were centrifuged in order to isolate important blood components such as plasma which was frozen on site (Demarchi, 2005). Centrifugation also provided DNA extraction from the buffy coat, the
layer of separated blood with the greatest number of leukocytes and platelets, using a
Quick Gene Kit (Analytical Genetic Testing Center, Denver, CO). Fasting blood draws
also provided accurate levels of adiponectin, leptin and circulating lipids including
triglycerides, high density and low density cholesterol. LDL cholesterol was calculated
using Friedewald’s calculation (Friedewald et al., 1972; Mora et al., 2009).

**Anthropometrics**

Quantifying adipose tissue with considerable accuracy is not simple (Deurenberg et
al., 2001). Calculated by taking one’s weight divided by their height squared, variation in
BMI scores is predicated on whether weight and height measures are self-reported or
empirically measured such as using a scale (Stommel and Schoenborn, 2009; WHO, 2014).
BMI limitations are many as it has no means to account for the different forms of adipose
one has, fails to discriminate between weight from muscle, bone or fat and BMI has little
to no diagnostic power (Romero-Corral et al., 2008). Despite inherent shortcomings, body
mass index (BMI) is the most widely used and accepted method in determining individual
adipose tissue (Bennasar-Veny et al., 2013; Freedman and Sherry, 2009; Oud, 2013;
Wright et al., 2015). Individuals differ not only in the amount of adipose they store, but
also in bodily distribution of it (WHO, 2015). The distribution of fat induced by weight
gain affects the risks associated with obesity and the kinds of diseases that result from it
(Canoy et al., 2007; Després et al., 1990; WHO, 2015). KNP participants had their BMI’s
calculated by taking scaled measures of weight and height as well as skinfold taken over
thin or light clothing around the triceps, subscapular, waist, wrist and hip regions. No thigh
measures were taken out of deference for the participants. Adipose measures can become
more accurate in capturing the differences in fat pattern by including measures of skinfolds
from specific body regions (Bl, 1990; Moreno, 20002; Wells, 2007). Interestingly it is also believed that measures of the leptin hormone directly reflect body mass as well (Brabant et al., 2000).

**Statistical Tests**

All statistical analyses were completed using SPSS® version 23. Univariate analyses were conducted to assess normality; any non-normally distributed data were natural log transformed including nutritional intake values, blood lipids and adipokine concentrations. All tests were run sex-stratified and adjusted for age. One-way ANOVA tests were performed to determine and statistically significant differences in average measures of each variable between males and females. Hypotheses and assumptions were checked using tests of correlation and constructing multiple linear regression models with a selected significance of $p < 0.05$. These models evaluate the relationships between leptin and adiponectin and nutritional, anthropometric and biological variables (blood lipids).

**Limitations**

Limitations to the study include exclusions of important metabolic information such as blood glucose and insulin and narratives of food preparation practices. Future research could include collection of these biomarkers ethnographic surveys on nutritional knowledge, attitudes and practices. Epigenetic information relevant to adiponectin, leptin or the vitamin D receptor would provide valuable insight to gene-environment and gene-nutrient interactions.
Results

Characterization of sample

N=160 (84 females; 76 males)

Population age range: 20 to 90

Average age for females: 57 (± 17.13)

Average age for males: 52 (± 16.09)

Anthropometrics

Anthropometric measures include weight, height and BMI score as well as body region skin folds for the measure of individual adiposity and dimorphic patterns in adipose phenotype. In males, height and weight were 178.3 cm (± 6.4) and 186.3 lbs. (± 31) respectively. The average BMI score was 26.5 (± 3.7), average hip measure 106.8 (± 7.5), waist measure 97.6 mm (± 8.7) and average wait-to-hip ratio 0.91 (± 0.91). Comparatively, the average score for every anthropometric measure was greater in males than females except in average triceps measure at 16.6 mm (± 5.8) in males and 26.4 mm ± 6.3 in females. In females, height and weight were 164.9 cm (± 7.8) and 151 lbs. (± 25.8) respectively. The average BMI score was 25.3 (± 4.3), average hip measure 105.6 (± 9.3), waist measure 79.9 mm (± 9.3) and average wait-to-hip ratio 0.7 (± 0.04).

Nutritional Intake

All micronutrient intake values were natural log transformed for normality of distribution. Individual nutrients selected for analyses were determined by solubility rules and isomerization in light. The greatest average nutrient intake was vitamin A at 8.5 IUs (± 0.6) of vitamin A and the lowest was vitamin E at 1.8 IUs (± 0.7). Average vitamin D intake scored at 3.7 IUs (± 1.2), average calcium 6.7 IUs (± 0.4) and average folate at 5.6
IUs (± 0.4). In females, the greatest average intake was vitamin A at 8.5 IUs (± 0.6) and the lowest average intake score was vitamin E at 1.7 IUs (± 0.7). Average vitamin D intake in females was 3.2 IUs (± 1.5), average calcium 6.7 IUs (± 0.4). Overall, males had the greatest average intake of every nutrient except for average intake of vitamin A which was the same in both sexes however with different standard deviations of ± 0.6 in males and ± 0.7 in females.

Blood Lipids

Blood lipid levels are useful phenotypes in understanding changes and sex-specific variation in the nutritional environment and overall bodily health. In males, the average levels of HDL cholesterol levels of 42.2 mg/dl (± 10.3), average LDL cholesterol 111.8 mg/dl (± 29.8), average triglycerides 120.6 mg/dl (± 63.8) and total cholesterol 178.2 mg/dl (± 34.3). In females, average HDL cholesterol was 56.3 mg/dl (± 15), average LDL cholesterol was 110.8 mg/dl (± 34.5), triglycerides 123.1 (± 72.2) and total cholesterol 191.7 mg/dl (± 38.1). On average, males had lower HDL cholesterol, triglycerides and total cholesterol than females but greater average LDL cholesterol.

Adipokines

In males, average adiponectin levels were 2.1 ug/ml (± 0.5) and average leptin levels were 1.6 (± 0.6). In females, average adiponectin was at 2.5 ng/ml (± 0.5) and average leptin levels were 2.6 ng/ml (± 0.6). Both adipokines were found to be in higher concentrations on average than what was observed in males.
Table 10. Sex-stratified summary of the calculated averages and standard deviations for anthropometrics, nutrient intake, blood lipid and adipokine levels for females (n=84) and males (n=76).

<table>
<thead>
<tr>
<th>Female Average</th>
<th>Std. Dev.</th>
<th>Anthropometric</th>
<th>Male Average</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>164.9</td>
<td>7.8</td>
<td>Height (cm)*</td>
<td>178.3</td>
<td>6.4</td>
</tr>
<tr>
<td>151.1</td>
<td>25.8</td>
<td>Weight (lbs.)*</td>
<td>186.3</td>
<td>31</td>
</tr>
<tr>
<td>105.6</td>
<td>9.3</td>
<td>Hip (mm)</td>
<td>106.8</td>
<td>7.5</td>
</tr>
<tr>
<td>79.9</td>
<td>9.3</td>
<td>Waist (mm)*</td>
<td>97.6</td>
<td>8.7</td>
</tr>
<tr>
<td>16.1</td>
<td>.8</td>
<td>Wrist (mm)*</td>
<td>18.6</td>
<td>0.96</td>
</tr>
<tr>
<td>26.4</td>
<td>6.3</td>
<td>Triceps (mm)*</td>
<td>16.5</td>
<td>5.8</td>
</tr>
<tr>
<td>21.6</td>
<td>7</td>
<td>Subscapular (mm)</td>
<td>22.5</td>
<td>6.8</td>
</tr>
<tr>
<td>25.3</td>
<td>4.3</td>
<td>BMI*</td>
<td>26.5</td>
<td>3.7</td>
</tr>
<tr>
<td>0.7</td>
<td>0.04</td>
<td>WHR*</td>
<td>0.91</td>
<td>0.03</td>
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</table>

<table>
<thead>
<tr>
<th>Female Average</th>
<th>Std. Dev.</th>
<th>Nutrient (IUs)</th>
<th>Male Average</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>1.5</td>
<td>Vitamin D*</td>
<td>3.7</td>
<td>1.2</td>
</tr>
<tr>
<td>8.5</td>
<td>0.7</td>
<td>Vitamin A</td>
<td>8.5</td>
<td>0.6</td>
</tr>
<tr>
<td>1.7</td>
<td>0.9</td>
<td>Vitamin E</td>
<td>1.8</td>
<td>0.7</td>
</tr>
<tr>
<td>6.3</td>
<td>0.4</td>
<td>Calcium*</td>
<td>6.7</td>
<td>0.4</td>
</tr>
<tr>
<td>5.3</td>
<td>0.4</td>
<td>Folate*</td>
<td>5.6</td>
<td>0.4</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Female Average</th>
<th>Std. Dev.</th>
<th>Lipid (mg/dl)</th>
<th>Male Average</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.3</td>
<td>15</td>
<td>HDL*</td>
<td>42.2</td>
<td>10.3</td>
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<td>110.8</td>
<td>34.5</td>
<td>LDL</td>
<td>111.8</td>
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<tr>
<td>123.1</td>
<td>72.2</td>
<td>Triglycerides</td>
<td>120.6</td>
<td>63.8</td>
</tr>
<tr>
<td>191.7</td>
<td>38.1</td>
<td>Total cholesterol*</td>
<td>178.2</td>
<td>34.3</td>
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</table>

<table>
<thead>
<tr>
<th>Female Average</th>
<th>Std. Dev.</th>
<th>Adipokine</th>
<th>Male Average</th>
<th>Std. Dev.</th>
</tr>
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<tbody>
<tr>
<td>2.5</td>
<td>0.5</td>
<td>Adiponectin (ug/ml)*</td>
<td>2.1</td>
<td>0.5</td>
</tr>
<tr>
<td>2.6</td>
<td>0.6</td>
<td>Leptin (ng/ml)*</td>
<td>1.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Statistically significant differences between females and males (p<0.05).

The Vitamin D and Adipokine Study
Using data previously collected for the KNP, I investigated hypotheses about the interactions between adipokine phenotypes and nutritional intake in a semi-isolate group of Kansas Mennonite. Anthropometric, nutritional and serological variables were highly intercoorelated and proved to be significant predictors of both adiponectin and leptin levels in males and females. Initial tests of adipokine concentrations against vitamin D intake
exclusively were not strong or reliable. Inclusion of anthropometric and biological traits increased the strength and clarity of linear relationships between vitamin D and adipokines.

**Adiponectin in Women**

Variation in average adiponectin between females was statistically significant ($p = 0.001$). The adiponectin model in females shows age, wrist, hip and subscapular measures and HDL cholesterol were significant predictors, explaining up to 35.8% of adiponectin variation ($F = (5, 77) = 8.57$) ($p < 0.001$). In females, the strongest variables determining adiponectin levels were HDL cholesterol ($\beta = 0.344$, $p < 0.001$), wrist circumference ($\beta = -0.309$, $p = 0.002$ and age2 ($\beta = 0.237$, $p = 0.017$) with hip adiposity possibly contributing to the observed trend as well ($\beta = 0.218$, $p = 0.067$).

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
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<th>Adj R Squared</th>
<th>Std. Error of Estimate</th>
<th>Change Statistics</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td>R Square Change</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F Change</td>
</tr>
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<tr>
<td>1</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

**Adiponectin in Men**

Variation in average adiponectin between males was statistically significant ($p = 0.001$). In males, the regression model suggest age, subscapular measures, triceps folds, LDL cholesterol, triglycerides, leptin levels and vitamin D intake appear to be the strongest predictors in leptin variation. This model suggests approximately 26.5% of adiponectin variation could be explained by these variables ($F = (7, 61) = 3.14$) ($p = 0.007$). Leptin variation in men was most significantly influenced by BMI ($\beta = 0.314$, $p = 0.058$), waist adiposity ($\beta = 0.292$, $p = 0.166$), subscapular adiposity ($\beta = 0.219$, $p = 0.029$) and triglyceride lipids ($\beta = 0.196$, $p = 0.009$).
Leptin in Women

Variation in average leptin between females was statistically significant ($p = 0.001$). Results indicate age, subscapular, triceps, hip, waist, and BMI measures, triglycerides, and folate intake were the most significant variables in predicting leptin variation at 52.6% ($F = (8, 73) = 10.1$) ($p < 0.001$) in females. Female leptin variation was most significantly influenced by subscapular adiposity ($\beta = 0.384, p = 0.011$).

Leptin in Men

Variation in average leptin between males was statistically significant ($p = 0.007$). Using multiple linear regression, age, subscapular, triceps, hip, waist, wrist measures, BMI, adiponectin levels, and vitamin D intake proved to be the strongest predictors of leptin, explaining 78.2% of its variation ($F = (10, 58) = 20.8$) ($p < 0.001$). Scaled to the same level, the variables with the greatest effect on adiponectin phenotypes in men were age$^2$ ($\beta = 0.326, p = 0.013$), vitamin D ($\beta = 0.018, p = 0.018$), triglyceride lipids ($\beta = -0.308, p = 0.018$), and subscapular adiposity ($\beta = -0.358, p = 0.037$).
Discussion

There are relatively few studies examining dietary intake and adiponectin and leptin hormone levels. The goal here was to investigate the associations between nutritional intake values and measures of hormone levels relative to adipose within a representative Mennonite sample population. This study suggests a complex relationship between dietary vitamin D and adipokine concentrations in a representative sample population of Kansas Mennonite. Findings are nutrient-specific and sex-specific. Vitamin D intake was statistically significant in predicting adiponectin and leptin variation in men but not women. Folate is also a UV sensitive nutrient and its intake was statistically significant in determining leptin levels in women but not in men. Adiponectin influences leptin variation and leptin influences adiponectin variation in men but not women. Findings validate existing works supporting a hypothesis of vitamin D as a determinant of biomarkers relevant to chronic disease risk (Bidulescu et al., 2014; Lee et al., 2007; Yannakoulia et al., 2003).

Mennonite communities have traditionally observed largely agricultural lifestyles and practices of endogamy, marrying within their church groups as means to maintain degrees of cultural and political isolation. These features of the Mennonite communities increase the probability and duration of time in UV light and suggests a holistic nutritional context distinct from the nutritional and dietary habits and beliefs of the greater U.S. Through practice of endogamy, genealogical records and general isolation the communities have greater similarities on a population level than a random heterogeneous U.S. sample. Previous research on nutrition and chronic disease risk has not controlled for heterogeneous sampling, limiting the ability to make population level conclusions and
implement operational change. The communities represented here still observe strict non-smoking and non-alcohol drinking practices, both of which influence the biological variables collected for this study. These cultural features remove both confounders which are otherwise frequently seen in the general U.S. population. The church groups in this thesis are believed to have lost a degree of genetic isolation as a result of increase gene flow into these populations (Melton, 2010; Crawford, 2000).

The relationships between nutritional intake on adiponectin and leptin levels provide enhanced means to study the interactions between human health and changes in the nutritional environment. This study may highlight a need for refined nutritional quantification methodologies for epidemiological studies as well as ideal population dynamics in which to test nutritional epidemiological questions regarding intake and markers of metabolic disease risk.
Conclusions

Chronic diseases are the leading cause of death and all-cause mortality in the world (WHO, 2014). Their increasing prevalence has resulted in the decrease in overall healthy life-years experienced within developed and developing countries (Farder, 2014). Chronic diseases are often lifelong once they develop, becoming more common and persistent with time. Age-related diseases are believed to be evolutionary trade-offs of the human lifespan; the longest of all primates. These extraordinary lifespans are the results of the conscious improvement of the human environment (Finch, 2010). Through the cultural enhancement of group behaviors, food quality and production, antibiotic and viral medicines, we saw reduction and ultimate removal of many sources of early life mortality. Unique to humans is also the ubiquitous lifespan advantage of one sex, with women on average living longer than men. Instances of focused environmental stress or insult however, such as nutritional deficiencies, accelerate and cause the developmental manifestation of chronic disease.

A superficial digital survey of US medical schools’ curricula makes apparent the absence of certain topics. Evolutionary theory and its relevance for clinicians are usually not discussed. There is also little education about diet, sex-specific phenotypic diversity and how both impact pathological trajectories and health outcomes (Adams et al., 2006; Adams et al., 2010a; Adams et al., 2010b; Winick, 1993). Most unfortunate is the ignorance of the importance of diet. U.S. doctors unfortunately receive little to no nutritional training in their formative years of medical school; traditional allopathic training focuses on anatomy and physiology. (Frantz et al., 2011; Lenders et al., 2013). Doctors and health practitioners are often incapable of counselling or advising on personal health complications or management strategies in nutritional contexts, forcing patients to
seek out alternative health expertise that are otherwise socio-politically and bio-medically stigmatized. Unfortunately, physicians are overwhelmed with many demands on their time starting in medical school. There is also much emphasis on ethics, health care system navigation, being culturally competent and thinking critically during diagnosis. These curricular shortcomings are not likely to be remedied.

Despite evidence illustrating nutrition’s direct role modulating chronic disease phenotypes, proximate explanations and mechanisms lack clarity (Gibney et al., 2014). Research in nutritional management of chronic disease risk and MetS is confounded by subpopulation factors and high bio-social heterogeneity within populations (Bidulescu et al., 2014; Kuo, 2015; Liu et al., 2009). As pharmaceutical industries continue to expect leveraging information from the Human Genome Project in novel drug development, the food industry is equally able to position nutrition and functional foods to promote health and prevent disease (Gillies, 2003). It should be noted however that epigenetic and nutrigenetic responses to chronic disease and individual health are not without public and scholarly critique (Heijmans and Mill, 2012; Saukko et al., 2010).

**Nutrition in Disease Risk Management**

Most the world is malnourished, either due to overconsumption of calories or as a function of ignorance or poverty regarding nutritional deficits (Foss, 2009; Via, 2012; Westerterp, 2013). Another challenge for allopathic physicians is the shift from infectious disease to rapidly escalating rates of chronic diseases and the pathologies associated with MetS. Understanding that nutrition directly impacts one’s wellbeing and genetics it is vexing to see many U.S. doctors and healthcare professionals receive little to no nutritional education and training in their formative years of schooling (Frantz et al., 2011; Lenders et
This is likely a product of the social movement among physicians and doctors in resisting evidence based medicine; the medical practice of applying research evidence to medical practice (Pope, 2003). An analysis of this phenomena within a critical social or medical anthropological framework may prove beneficial in designing nutritional interventions and treatments to chronic disease at both the population and individual levels separately.

Nutritional counselling and therapy strategies are foreign to allopatric and Western physicians. This is likely a product of the social movement among physicians and doctors in resisting evidence based medicine; the medical practice of applying research evidence to medical practice (Pope, 2003). The neglect of nutrition is unfortunate and frustrating considering most the world is malnourished, either due to overconsumption of too many calories or as a function of either ignorance or poverty regarding nutritional deficits (Foss, 2009; Via, 2012; Westerterp, 2013). Additionally, there is a shift in disease patterns of the US with infectious disease having been replaced by chronic conditions. Poor diet and physical health lead to obesity and associated comorbidities as hypertension, cardiovascular disease, diabetes and obesity. Successful application of nutrition in health and disease management may be possible with the concept of personalized nutrition; using individual genetics to help determine individual nutritional recommendations (Konstantinidou et al., 2014; Matthys et al., 2014). Additionally, changes in behavioral routines and lifestyle have been shown to improve adipokine levels in obese and diabetic patients (Monzillo et al., 2003).

The obvious dimorphism of chronic health and individual variation in health traits make it a necessity to examine fundamental social and biological elements (Goldman et al,
2004). Investigating disease where genes and environment interact to create them requires consideration of both the social and environmental contexts in which they persist as well as the biomedical mechanisms underlying them (Chakravarthy et al., 2008; Naukkarinen et al., 2012; Neel, 1962; Odling-Smee, 2003; Prentice et al., 2005; Speakman, 2008). Gene-environment interactions between nutrition and lifestyle contribute to the differences and variations in human health outcomes and physiology (Kaput, 2016). Developing risk-benefit factors must be completed within the individual contexts of food complexity, genetic diversity, life-history experience, cultural narratives and lifestyle and variety of metabolic processes that drive health and disease (Kaput, 2016).
Glossary

**Adipocyte**: A fat cell, adipocytes are the cells which make up adipose tissue. There are several different kinds of adipocytes which determine what kind of adipose tissue is being expressed. There are white, brown and beige or brite adipocytes and therefore adipose tissue as well.

**Adipogenesis**: The biological process that produces new fat cells. This process is either promoted or inhibited by internal and external environmental signals, lifestyle, nutritional status and genetics.

**Adipokine**: A specific class of hormone that is primarily but not necessarily exclusively by adipose tissue. Adipokines are powerful signaling molecules that communicate with and respond to changes in the body. Some adipokines require both nervous and endocrine systems to work, adiponectin and leptin are two examples of these.

**Adiponectin**: A hormone that is secreted by adipose tissue which is anti-inflammatory, insulin sensitizing and neuroprotective. A person’s adiponectin levels are often lower than average in some chronic disease states.

**Adipose Tissue**: One of the organs in the endocrine system. Adipose tissue helps maintain the all other bodily systems. Adipose tissue is highly responsive to environmental stress, helping maintain energy homeostasis throughout the life. Humans, relative to all other animals, have a lot of adipose tissue.

**Adiposity**: The individual body fat content and body fat pattern made by adipose tissue expression and deposition. An individual’s adiposity determines the chronic diseases that follow after development of obesity. Adiposity works under influence from age, sex, nutrition and environmental exigencies.

**C/EBP**: Short for CCAAT enhancing binding protein. C/EBP is a specialized protein referred to as a transcription factor which regulates genetic expression and proliferation of new adipocytes.

**Chronic disease**: Chronic diseases – i.e. heart disease, stroke, obesity, diabetes, cancer- are the most common, costly and preventable of all health problems. Yet, chronic diseases are also greatest cause of death worldwide.

**Dimorphism**: Dimorphism or sexual dimorphism refers to the distinction between a male and female members of the same species. The differences in traits can include variations in size, color, shape or even structures. These differences are from the inheritance sexual pattern from one’s genetic.

**Epidemiology**: The field of medicine that is concerned with the spread, prevalence and control of disease.
**Epigenetics**: The study of how genes are turned on or off by environmental signals. These gene-environment interactions can influence health and wellbeing. Contributing factors in individual epigenetic variation includes nutrition, age, chemical that interrupt or block biological processes, climate, geography, migration, culture and language. The magnitude of influence these forces have on a person’s epigenetics is spatiotemporally relative.

**Gene**: A stretch of DNA molecule that codes for important proteins and enzymes. Some genes are larger than others and can be present in different forms (polymorphism).

**Genetic expression**: When the product of a genetic code is produced.

**Genetic Epidemiology**: The field of epidemiology that takes advantage of genetic information and methods in understanding disease risk and incidence. Particularly useful and applicable when examining complex traits and chronic disease.

**Hormone**: A molecule that carry chemical signals throughout the body which communicate stimuli from changes in internal and external environments. Organs which play significant roles in hormone homeostasis, such as adipose, are part of the endocrine system.

**Integumentary**: The one of the many body’s organ systems, is comprised of the skin and all of its adjuncts. This system includes hair, nails, hooves, feathers and scales in general biology.

**Isomerization**: A chemical process that occurs when one molecule transforms into another by rearranging the order of its elements, not by adding or removing additional ones. When vitamin D is produced in the skin, the compound that becomes vitamin D3 undergoes an isomerization processes before being transported out of the skin cell.

**Lipophilic**: A term used to describe something that binds to or dissolves in lipids. Vitamin D is lipophilic, meaning it bind to lipids when it is complete and dissolves in them when active.

**Metabolic syndrome**: Described as a constellation like pathology, the metabolic syndrome is the combined occurrence of several chronic diseases typically associated to energy homeostasis and metabolism. Obesity is the greatest risk factor.

**Nutrigenetics**: A field of epigenetics that studies how diet and genetics interact. Gene-nutrient interactions may silence or express individual genes, modify cellular behavior and homeostasis and impact bodily health and wellbeing.

**PPAR**: Short for peroxisome proliferator-activated receptor. PPAR, like C/EBP, is an important protein that regulates genetic expression and proliferation of new adipocytes.
Appendix and Supplementary

Notations used in construction of the triad in nutritional methodologies (methods chapter):

$r = \text{Sample correlation}$

$p = \text{Validity coefficient}$

$A = \text{Intake measured using questionnaire answers}$

$B = \text{Intake measured using 24-dietary memory recall}$

$C = \text{Intake measured using laboratory biochemical methods}$

$T = \text{Estimation of true intake of nutrient}$

\[
\begin{align*}
  r_{AB} &= p_{at} \cdot p_{BT} & p_{AT} &= \sqrt{\frac{(r_{AB} \cdot r_{CA})}{r_{BC}}} \\
  r_{BC} &= p_{BT} \cdot p_{CT} & p_{BT} &= \sqrt{\frac{(r_{AB} \cdot r_{BC})}{r_{CA}}} \\
  r_{CA} &= p_{AT} \cdot p_{CT} & p_{CT} &= \sqrt{\frac{(r_{CA} \cdot r_{BC})}{r_{CA}}} 
\end{align*}
\]
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