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Use of an animal model to explore prenatal predictors of insulin and glucose metabolism in Southwestern Alaskan Yupit

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USE OF AN ANIMAL MODEL TO EXPLORE PRENATAL PREDICTORS OF
INSULIN AND GLUCOSE METABOLISM IN SOUTHWESTERN ALASKAN

YUPIIT

by

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Bachelor of Arts
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2000

Master of Arts
University of Nevada, Las Vegas
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A thesis submitted in partial fulfillment
of the requirements for the

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ABSTRACT

Use of an Animal Model to Explore Prenatal Predictors of Insulin and Glucose Metabolism in Southwestern Alaskan Yupiit

by

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Compared to other North American indigenous populations, Southwest Alaskan Yupiit exhibit very low rates of type 2 diabetes despite the occurrence of common risk factors. Contemporary Yupiit obtain a substantial portion of their calories from traditional foods, which contain high amounts of omega-3 polyunsaturated fatty acids. Epidemiological and experimental animal research has linked glucose and insulin homeostasis with a diet high in omega-3s. This study used an experimental animal model to explore potential diabetes protective effects (for adult offspring) of prenatal maternal nutrition modeled on traditional locally-obtained Yupiit diets. The results of this study showed that the adult offspring whose mothers consumed a diet modeled on traditional Yup'ik foods during pregnancy were more insulin sensitive (less prone to diabetes) than adult offspring whose mothers received a Western diet prenatally. These findings provide further insight into our understanding of the role that specific maternal nutrients play in programming adult metabolism and have significant implications for dietary intervention strategies aimed at preventing type 2 diabetes.

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CHAPTER 1

INTRODUCTION

Statement of the Problem

Type 2 Diabetes (T2D) is a metabolic disorder characterized by chronically elevated blood glucose due to insulin resistance, and eventually, insulin insufficiency. The World Health Organization estimates that the global prevalence of T2D in 2000 will nearly double from 2.8% to 4.4% by 2030 (Wild et al., 2004). Epidemiological evidence suggests that this disease was rare in the beginning of the 20th century, but after World War II rates began to increase rapidly. In the 50 years following the war prevalence continued to climb and incidence rates among children, which were once exceedingly low, became an international health concern.

Over the last decade education and awareness about T2D has significantly grown in both public and private domains. Furthermore, a significant amount of interdisciplinary research has been undertaken to understand the etiology of T2D, and to control the disorder's rapid increase. Despite these efforts, annual global prevalence continues to grow.

The present study offers a novel approach towards a better understanding of T2D origins by examining a unique *low* prevalence, but high risk population – Alaskan Eskimos of the Yukon-Kuskokwim delta area. An experimental animal study was used to explore whether or not elements of traditional Eskimo diets might provide “protection” against the development of T2D.

Epidemiology of Type 2 Diabetes in Alaska and the Lower 48 States

Among the highest prevalence populations around the world the development of T2D is linked to transitions from an indigenous, traditional subsistence economy to a wage economy (i.e., sedentism and consumption of high caloric diets which lead to the development of central adiposity). In general, such transitions (especially in a historical ‘colonial’ context) are highly correlated with T2D risk (i.e. hyperinsulinaemia, hypertryglyceridaemia, insulin resistance, glucose intolerance). “High” (>30%) prevalence populations (i.e., Pima: 50%, Oji-Cree: 40%, Havasupai: 38%, Pacific Nauruans: 28.1%, Australian Aborigines: 29.6%) are all characteristically obese – particularly carrying adipose tissue abdominally, consume a high caloric diet that is also high in saturated fats, and tend to lead a sedentary lifestyle characterized by low amounts physical activity (Benyshek et al., 2001). Some scholars have coined T2D, and other chronic disorders, “diseases of modernization,” in part because of the increase in risk observed when aboriginal populations transition to the dietary and daily physical activity level associated with a “modern Western” lifestyle. Ironically, T2D prevalence for Europeans and White Americans are anywhere from 3-10%; much lower than those for aboriginal populations living similar “Western” lifestyles (King and Rewers 1993). In 2007 it was estimated that the prevalence of diabetes in the U.S. was 7.8% (Centers for Disease Control 2007). While prevalence data for type of diabetes was not available, prevalence of T2D was likely to be around 7.0% as T2D constitutes over 90% of all diabetes cases worldwide.

In contrast to the many high prevalence Native American populations in the U.S. (e.g., Pima - ~50%, Havasupai – 38%) the most recent estimated overall T2D age

adjusted prevalence for all aboriginal Alaskan Eskimo populations in 2008 remain low – 6.9% (Centers for Disease Control 2008). While differences exist among the varying ethnic groups that inhabit Alaska (with higher percentages observed in the Eskimo’s Native counterparts, Athapaskan Indians – 10% over the age of 40 in 1987 - and Aleuts – 5.8% over the age of 35 in 1993) the prevalence of T2D among southwest Alaskan Yupiit is 3.3% - considerably lower compared to their indigenous neighbors (Mohatt et al., 2007). Two lines of evidence, however, including presumed “thrifty “ genotypic predispositions and the presence of obesity and sedentary lifestyles associated with a recent transition from a traditional high protein/high fat diet to a typical high caloric “Western” diet, place southwest Alaskan Yupiit in the highest population risk category for T2D. Despite this, southwest Alaskan Yupiit have a lower adult prevalence of T2D than another low prevalence ethnic group, White Americans (5.5% among males and 5.0% among females) (CDC 2007).

Type 2 Diabetes Etiology

Thrifty Genotype Hypothesis

When examined cross-culturally, aboriginal groups (e.g., Native Americans, Australian Aborigines) tend to be disproportionately affected by T2D. Prevalence for these groups are anywhere from two to five times higher than the overall U.S. rate of 7% (Centers for Disease Control 2007). These disparities in prevalence have prompted researchers to investigate the possible underlying mechanisms contributing to differences in disease susceptibility. The “Thrifty Genotype” hypothesis, proposed by James Neel in 1962, was one of the first etiological models developed to account for the origins of T2D.

In its original formulation, Neel suggests that the foraging ancestors of modern aboriginal populations underwent frequent periods of feast and famine (Neel 1962). Over time these periodic cycles selected for those individuals with a quick “insulin trigger”, which rapidly converted the energy obtained during periods of feasting to fat. These fat stores would then serve as an energy store in times of famine. Eventually, these same metabolism-regulating genes that offered a survival advantage during periods of energy restriction in the distant past, would become disease promoting in food secure contemporary indigenous populations living a modern “Western” lifestyle.

In the nearly five decades following Neel’s publication of the “Thrifty Genotype” hypothesis, extensive genetics research has been carried out in search of the hypothesized “thrifty genes”. While researchers were able to identify specific genes related to the monogenic forms of diabetes, (e.g., maturity onset diabetes of the young and maternally inherited diabetes and deafness), identification of the T2D susceptibility genes capable of accounting for large prevalence differences between high and low prevalence populations remains inconclusive. Of those possible genetic factors related specifically to T2D (e.g., a variant allele of the promoter region involved in insulin transcription, paternally transmitted class III alleles of the variable region upstream of the insulin gene) these factors were only found in some ethnic groups and/or they were also associated with other phenotypes (Velho and Frogul 2001). Today most researchers believe that T2D is polygenic (involving many loci), multigenic (involving a set of related genes), and pleiotropic (genes which affect several aspects of the phenotype). Despite the lack of progress in clearly identifying the population-clustered “thrifty genes”, hypothesized to

exist by Neel, the “Thrifty Genotype” hypothesis remains a firmly entrenched model accounting for population based differences in T2D prevalence.

Thrifty Phenotype Hypothesis (The Developmental Origins of Health and Disease [DOHaD])

A separate etiological model, originally advanced by Hales and Barker in 1992, offered an alternative approach to the classic genetic predisposition hypotheses and may help better explain T2D origins, especially in the highest prevalence populations. These authors posit a “Thrifty Phenotype”, which points to the causal role early life environments (e.g., intra-uterine) play in “programming” adult glucose metabolism. Importantly prenatal developmental programming is often mediated through maternal nutritional pathways. In a study that included 370 men from England, the authors found a negative correlation between the incidence of T2D (and impaired glucose tolerance) in adulthood and weight at birth (a general marker of maternal nutrition during pregnancy), suggesting that developmental processes occurring in utero may have permanent metabolic effects for the phenotype (Hales and Barker 1992). The “thrifty phenotype” model has been further substantiated in many other epidemiological and experimental animal studies. Several human and rat studies have shown that low birth weight due to prenatal exposure to famine and/or maternal malnutrition is associated with increased insulin resistance and T2D when a high caloric and high saturated fat diet is consumed post-natally (Ravelli et al., 1998; Rossetti et al., 1989; Benyshek et al., 2004). Additionally, fetal programming can lead to impaired growth and development in subsequent generations. A study published in *Diabetologia* in 2008 showed that insulin/glucose metabolism was altered in the F1, F2, and F3 generations (all receiving

nutritionally adequate diets) of offspring protein malnourished during pregnancy (Benyshek et al., 2006). Research following publication of the “thrifty phenotype” hypothesis has linked other chronic health conditions (i.e. cardiovascular disease, hypertension) with low birth weight and decreased birth length, which further substantiates the role of fetal nutrition on the development of disease in adulthood (Godfrey and Barker 2000). Today, thrifty phenotype etiological models are likely to be referred to as “developmental” because processes occurring during development appear to permanently “program” certain metabolic aspects of the phenotype.

Patterns in Aboriginal Subsistence Transitions

Several of the aforementioned clinical and experimental animal studies (and subsequent fetal growth patterns) model dietary transitions that were occurring in the late 19th and early 20th centuries in the United States. The U.S. government’s attempt to assimilate Native Americans - through the forced relocation and/or concentration of these groups onto reservations - prevented these populations from practicing and maintaining their traditional lifestyle, both in terms of subsistence and cultural beliefs and practices. Although many of these groups were given food in the early reservation era (in the form of government rations) these supplements were often inadequate in terms of daily caloric and nutrient needs, which led to starvation and famine for many indigenous communities (Benyshek et al., 2001; Benyshek and Watson 2006). Subsequent generations gestated under these malnourished conditions, then, were predisposed to developing T2D once convenience and grocery stores were established on the reservations, access to nutritionally poor food became readily available, and levels of physical activity were

substantially reduced. These combined circumstances (i.e., tribal relocation/concentration followed by bouts of undernourishment and the subsequent abundance of Western foods) represent a common theme observed in high T2D prevalence Native American communities in the U.S.

The differences observed in prevalence between what would be considered two classic “thrifty genotype” populations, Native Americans and Alaskan Eskimos, warrant further investigation. Current macronutrient intakes, genetic relatedness, shared prehistoric arctic/subarctic migration patterns, historic reliance on hunted/gathered foods, and contemporary behavioral and anthropometric disease risk factors are shared between the two groups, yet southwest Alaskan Yupiit have a much lower prevalence of T2D. Unlike high prevalence Native American groups (e.g., Pima), Yupiit diets were heavily supplemented with traditional foods until late in the 20th century (Heller and Scott 1967; Barker 1993) and in many contemporary communities still are (Parkinson et al., 1994; Ballew et al., 2004). A combination of geographical barriers and/or climatic conditions, in addition to political forces prior, during, and after the Alaskan purchase by the U.S., dramatically influenced which subsistence items would comprise and/or significantly supplement the Native Alaskan diet. Yupiit of the Yukon/Kuskokwim were not forcibly relocated out of their traditional territories where traditional food economies had to be abandoned, but instead remained in their local territory where they continue to exploit the area’s natural resources (Barker 1993). Another important difference between Alaskan Eskimo and Native American populations is the nutrient composition of the respective traditional diets. Traditional Eskimo diets were much higher in protein and fat (particularly omega-3 fatty acids commonly found in fish oils) and significantly lower in

carbohydrate content compared to the traditional diet of U.S. indigenous groups (Draper 1977).

Dietary Transitions

Contemporary dietary analyses in the Yukon/Kuskokwim rivers areas suggests that seal oil and salmon are still eaten on a regular basis and in significant amounts, although undoubtedly less compared to pre-contact periods (Adler et al., 1994; Parkinson et al., 1994). These two subsistence items, seal and salmon, are high in omega-3 fatty acids which have been implicated in the protection against several chronic disorders, including cardiovascular disease, hypertension, and hypercholesterolemia in both experimental animal studies and epidemiological findings (Das 2001; Nettleton and Katz 2005). Furthermore, studies have shown that a high omega-3/omega-6 ratio can significantly alter insulin binding and cellular glucose uptake (Luo et al., 1996). This ratio is much higher in the diets of contemporary Southwest Eskimo populations compared to “Western” diets, which suggests that this dietary component may be protecting against TD even when other risk factors are present (Parkinson et al., 1994). While high intakes of omega-3 PUFA’s have been reported in many Alaskan regions, incorporating and addressing the dietary diversity, historical events and political forces that occurred across the region is beyond the scope of this study. Instead, the current research focuses on one region of southwest Alaska for which substantial data has been gathered on diet - both spatially and temporally – and for which current prevalence and risk factors for T2D are well known – the Yukon-Kuskokwim region of southwest Alaska.

General Aims, Research Questions and Study Design

There has been extensive research examining the developmental effects of pre-natal maternal malnutrition on adult offspring metabolic health, and thereby providing an experimental animal model of high prevalence of T2D among Native American groups. Currently, however, there are no experimental animal studies that have attempted to model the potential developmental effects of a pre-natal maternal diet that reflects southwestern Eskimo dietary intakes to determine its effect on adult offspring insulin-glucose metabolism post-natally. The reasons for modeling these particular nutritional intakes are multiple: “prevalence data for which diabetes screening meets current diagnostic standards and thus is more likely to represent accurate percentages” (Murphy et al., 1995) risk factors data; and documented dietary changes, all encompassing temporal and spatial changes over time. It is not known whether a pre-natal southwestern Yup’ik diet with a high omega-3/omega-6 ratio provides an added “protective” effect over a post-natal diet with the same ratio. If maternal diet plays a pivotal role in the fetal programming of adult metabolism, then it is possible that the traditional southwestern Yup’ik diet consumed by women during pregnancy provides a “protective” effect against the development of insulin resistance/T2D in the offspring of these women, and that this effect explains the relatively low rates of T2D among Alaskan Eskimos, despite a constellation of other high risk factors. This study used an animal model to examine the role of pre and post-natal nutrition in the development of insulin sensitivity in a high risk human population with a very low prevalence of insulin resistance and T2D – Southwest Alaskan Yupiit.

Research Questions

The central research questions of the current experimental animal study are:
What are the effects of varying prenatal and postnatal/postweaning diets on insulin sensitivity and blood lipid levels in adult offspring? Can observed effects of varying prenatal and postnatal/postweaning diets on insulin sensitivity/blood lipid levels of adult offspring help explain the epidemiology of obesity-related disorders such as T2D and Metabolic Syndrome among Yup'ik Eskimos in southwestern Alaska today? Does a “Traditional” Yup'ik diet consumed during pregnancy offer protection from insulin resistance and dyslipidemia of offspring in adulthood?

Nine different maternal (prenatal)/offspring postweaning diet lines were used in the study. The maternal prenatal and offspring postweaning diets were designed to model: 1) “Traditional” southwest Alaskan diet, 2) “Western”; and 3) a Control diets. Female dams consumed the prenatal diets for one week prior to mating and during pregnancy and lactation. Offspring of females fed each of these diets during the prenatal and nursing period from each of the three prenatal diets in this study consumed either a “Traditional”, “Western”, or “Transitional” diet after weaning. Blood glucose, insulin, and lipid levels were then measured in adult offspring at 120 days.

Experimental animal research over the last twenty-five years in the area of glucose and insulin metabolism is highly correlated with epidemiological and intervention studies in humans. The use of a rat model was chosen for this study for several reasons: (i) the rat's ability to digest and metabolize types and quantities of macro and micronutrients is similar to humans, (ii) the presence of a mammary gland and placenta in the rat ensures that the types and quantities of specific maternal dietary nutrients will be transferred to

the offspring, (iii) rat pups undergo a period of suckling and postnatal development like humans, and this period can be affected by dietary variables, (iv) it has been shown in rats, as in humans, that dietary components are used by the body to make up components of cellular membranes and to mount certain immunological responses.

Significance of the Study

In 2009 T2D ranked 5th among the leading diseases causing death in the United States. According to the American Diabetes Association health care costs amounted to \$174 billion in November 2009, an increase of 32% since 2002. Not only do T2D complications – blindness, amputation, impotence, kidney failure – contribute to rising health care costs, but an individual possesses a two to four times risk of dying from cardiovascular disease when coupled with T2D. Once a rare disease, the Center for Disease Control estimates that T2D prevalence in the U.S. in 2006 among all ethnic groups is 7.2% and there are 150 million cases reported worldwide (WHO 2002). As incidence rates and prevalence continue to climb, T2D disease etiology and prevention become even more critical. Examining the effects of high protein/high PUFA diets *in utero* on adult offspring insulin sensitivity increases our understanding of the role of maternal prenatal/nursing diets on offspring adult metabolism, and may even point to “protective” health effects of specific prenatal dietary components found in typical low prevalence T2D populations. These findings are potentially beneficial for contributing to the development of new maternal prenatal diet recommendations and/or encouraging the maintenance of existing traditional prenatal diets that “optimize” maternal and offspring adult health.

CHAPTER 2

SUBSISTENCE PATTERNS IN SOUTHWEST ALASKA AND THEIR CHANGES OVER TIME

Russian and American Contact in the Alaskan-Yukon Delta and its Influences on Yup'ik Subsistence Practices and Health

Scholars have demonstrated that colonial influences during the late 19th and early 20th centuries had a significant impact on subsistence practices among Native American populations with high prevalence of obesity related health disorders. An extended period of malnutrition followed by a rapid transition to a diet that is high in saturated fat and refined carbohydrates has been associated with the development and perpetuation of T2D in both Pima and Havasupai populations, among others (Benyshek et al., 2001). Colonial impacts, in the Southwest region of Alaska (especially with respect to subsistence food economies) were less pronounced compared to those for the Pima, Havasupai, and other Native American groups in the lower 48 states (Zagoskin 1967; Barker 1993). In fact, many early Russian explorers made concerted efforts to maintain peaceful negotiations with the locals and to reduce intertribal feuds, while often intermarrying with native women in the delta.

Russian Period

Contact between non-natives and Natives of Alaska began in the mid 1700s when Russian settlers established forts and trading posts along the Aleutian chain and on Kodiak Island and Sitka for the purposes of obtaining beaver pelts and sea otter furs for trade with China (Barker 1993; Naske and Slotnick 1987). While a substantial amount of

exploration and business occurred along Alaskan's southeastern and northern coasts, the Yukon-Kuskokwim delta remained relatively unexplored until the beginning of the 1800s. One of the reasons for this may have been the shallow waters along the Bering Sea coast which prevented whales from migrating through these waters. Consequently, Russian navigators were forced further north in search of exploitable sea mammals (Barker 1993). Nevertheless, by 1830 an abundance of beaver was discovered along the Kuskokwim catalyzing the development of Kolmakovskiy Redoubt on the Kuskokwim and two other Russian posts along the Yukon. In exchange for tobacco, firearms, sable, beads, metal pots, knives, lances and even Russian clothing, Alaskan Natives traded their highly coveted beaver pelts, as well as otter and sometimes muskrat.

The progressive nature of Russia's capitalistic pursuits in Alaska during this time has been described as a bourgeois business enterprise fueled by a working class opposed to the feudal serfdom system in Russia. Long term Russian influence, authority, and permanence in the area, however, was limited. Russian serfdom during the early period limited the number of bodies available for the purposes of exploration and of those that managed to become a part of these voyages many were inexperienced, illiterate, or ex-convicts, all of which led to an increase in the number of lost ships and/or unproductive journeys (Naske and Slotnick 1987). Furthermore, due to the enormous expenses associated with shipbuilding and sailing from Siberia to Alaska, Russians were forced to depend on British and American agents for food and supplies once arriving on the mainland. The obstacles faced by Russian entities involved in Alaskan affairs were many. An unstable political regime combined with a constant change in leadership often meant that objectives for the Russia were in continual flux. A significant amount of

effort was placed on developing ways to emulate their European neighbors to the West and not on largely unchartered and relatively “underdeveloped” Alaskan territory. Russian trading posts were fraught with instability as evidenced by the post manager’s voiced frustrations over tardy and sometimes obsolete supplies as promised by the Russian American company (an enterprise established by Russia designed to explore the Northwest coast of America and West coast of Alaska). Even by the time Russian interest in the fur trade had strengthened, their naval powers were unable to compete with the very successful and efficient British navy occupying the territory, and without the backing of the imperial regime, the private Russian entities in the area were never able to gain substantial control. Economic dependence on the other two major forces in Alaska at the time, the U.S. and England, would eventually lead to the demise of Russia’s colonization efforts.

Despite many of the barriers and struggles faced by the early explorers, scientific exploration was encouraged by the Russian-American commanders and a handful of later explorers made significant contributions to Alaska’s zoology, geography, and ethnography (Zagoskin 1967). The first written report of any customs observed in the Kuskokwim area, was documented by Peter Korsakovskiy in 1818 and several explorers thereafter collected data on tribal location. Most of what is known by outsiders about the delta’s geography, Native people, their customs and language at the time is attributed to the Russian naval officer, Laventry Zagoskin, whose travels encompassed some 3300 miles. In an effort to expand their potential business opportunities in Alaska’s interior, to gain an understanding of routes of communication between the two major rivers, and to ascertain how furs were being transported out of the interior for trade with the Chuchki,

Russia sent Zagoskin on a two year expedition to the delta in 1843. A child raised among the feudal peasantry, Zagoskin was educated in a private school and later trained in the Cadet Corps. He served in the Navy and passed time on the ships reading books published by his Alaskan exploring predecessors, a past time which contributed to his desires to join the Russian-American enterprise. The combination of his literary breadth and astute navigational skills and experience were the foundations upon which Zagoskin successfully traversed the harsh landscape and accurately mapped geological phenomena in Alaska's interior.

By kayak in the summertime and on snowshoe in the winter, Zagoskin and his crew collected mineral, astrological, and ecological data while trading, living among, and participating in the Native way of life with the tribes along the rivers and their tributaries. Their journey consisted of a loop that began at Fort St. Michael. The crew traveled up the coast as far as Unalaklik post and then east into Indian territory. From Nulato they followed the Yukon south to Ikogmyut and across the tundra to the Kuskokwim, which they mapped as far north as the headwaters to the Tochothno River. The party subsisted primarily on the Native diet of birds, wild fowl, deer meat, sea mammals and fish and secondarily on traditional Russian food items like tea and biscuits. Due to the fact that posts and therefore, Russian presence, had been established along key trading routes and because Russian dominance had been undermined by political and economic factors, the majority of Zagoskin's encounters with the natives were peaceful and the Russian objectives were realized and tolerated.

In 1854, Russian Mission (previously known as Ikogmyut) was built upriver from the town of Marshall on the Yukon with the objective of converting the locals to the

Orthodox faith, and after the United States purchased Alaska in 1867, Catholic and Moravian churches were erected along the lower Kuskokwim. One church would be erected in every major village thereafter.

American Period

While commercial interests in salmon fishing and timber exploitation took place in the early era of American rule, it was mining that led to the first major population increase in Alaska – nearly doubling in the decade after the discovery of gold (Naske and Slotnick 1987). According to economist Richard Cooley, salmon fisheries were isolated and the labor and supplies involved with running the canneries, coupled with a small population density, mitigated American involvement in Alaska's fishing industry – at least in Central Alaska (Naske and Slotnick 1987). Mining, as compared to salmon fishing or the timber industry, produced a larger benefit to cost ratio for those prospectors involved as the value of gold was much higher than the value of salmon. The modern day towns/cities of Southwest Alaska including Bethel, Iditarod, McGrath, Flat and Ophir were born from the mining camps that had been established in the area in the late 1800s. Congress, at least in the early phases of American involvement in Alaska, emphasized great respect for the rights of Natives and demanded that their occupancy and use of their land remain undisturbed. (Interestingly, when significant amounts of oil were discovered on the North Slope in the 1960s concern over Native land rights became a politicized agenda rather than genuine consideration for the local's rights [Naske and Slotnick 1987]). Following the discovery of gold in Alaska, the U.S. government appropriated funds to the U.S. Geological Survey to be used for survey and exploration, and by 1920 nearly 4,890 miles of roads, a quarter of which were wagon roads, and trails had been

constructed which linked many – previously isolated - delta communities together.

Railroad construction was also initiated but coal field closings (due to President Theodore Roosevelt's interest in resource conservation), rugged terrain, and severe climate halted its progress.

In many ways the combination of Alaska's geographical location and resulting climate insulated the Yup'ik living in the interior from the American economic and political dominance of Native peoples so common in the lower 48 states. Population decline continued as the events of World War I took many American workers in Alaska and the post war economic boom in the U.S. gave those same workers and/or other American citizens no incentive to return. The lack of self government, an inefficient road system and a small population in continual turnover gave the inhabitants of Alaska minimal self control. Any decisions regulating Alaska's resources were to be handled by Congress who, at the time, prioritized their interests in the lower 48 states. Moreover, a decreased demand for copper once the war ended - in addition to the replacement of human labor with mechanization - decreased employment opportunities in mining which further reduced American involvement in the Alaskan economy. An already unstable economy was exacerbated with the onset of the Great Depression in the 1930s and when funds were cut for building and/or improvements that had begun in the more urban areas of Alaska. The events surrounding Alaska in World War II, however, initiated interest by the U.S. Army, and Alaska became a critical component in the security of the lower 48 states. What was once considered an unforgiving and harsh landscape, Alaska now proved to be a tremendous asset in the protection of American lands as the circumpolar weather patterns around the state played a major role in defeating Japanese military

forces. Acting as a half way point between Alaska and Japan, the Aleutian Islands became both a desirable defensive and offensive location for the U.S. Army and the Japanese Imperial High Command. Fearing the Japanese might attack the West Coast from the islands, the U.S. Army stationed 45,000 men in Alaska to counter attacks aimed at the Americans.

These events played a large role in shaping Alaska's future as a state, but the major instrument of change was the discovery and exploitation of oil on the North Slope. As pressure mounted from shareholders and pipeline developers to begin construction of the Trans-Alaska line, President Richard Nixon signed the Alaska Native Claims Settlement Act in 1971, offering \$962.5 million in compensation for use of the land and 148,500,000 acres, or 1/9th of Alaska (once controlled by the government) to the Natives after what took many years of negotiations. It was imperative that Congress pass the Act as pipeline construction could not commence until disputes over land had been settled (many Natives would not allow construction of the pipe on their land). Construction of the Trans-Alaska pipeline began in 1974 and the events which transpired significantly changed Alaska's economy and subsistence practices. Twelve regions – and later a thirteenth – representing a "Native association" and managed by corporations were born out of the act. Alaskan Natives who owned private shares of the corporations, which were obtained by enrolling in a region, managed the regional corporations and their village equivalents. Thus, corporate ownership and decision making was a collective effort based on an individual's share in the land which was ultimately rooted in Native identity. Some aspects of land management on Indian reservations in the lower 48 are similar – most reservations are owned by the tribes, and reservations possess tribal sovereignty enabling

members of the tribe to self govern. Indian reservation lands today are held in trust by the government and individual Native Indians do not own 'shares' of their land. Furthermore, when Europeans settled the lower 48 states Native Americans were often forcibly relocated to pieces of land considered less desirable to white settlers and government officials. This is in contrast to Alaskan Natives who maintained possession of their traditional land (albeit ultimately at a fraction of their traditional territories). While Alaskan Natives continued a relatively traditional subsistence lifestyle on familiar territory, many American Indians in the lower 48 states were forced to adjust to their lands which were often poor in subsistence resources and inadequate for pursuing traditional subsistence practices (Benyshek et al., 2001).

With the passing of ANCSA more Alaskan Natives began pursuing positions in hotel and fisheries management. A cash economy based on commercial fishing, fish processing and other jobs provided by the U.S. Bureau of Indian Affairs enabled village residents to purchase new items used for hunting in addition to fuel, fish nets, ammunition and common household items (Klein 1966). Out of the five southwestern villages studied by Scott and Heller in 1956, all generated income from welfare assistance and some type of wage economy (Heller and Scott 1967). Technological influences began permeating Yupik hunting activities by the early 1960s, and outboard motors, snowmobiles, and CB radios were reported as the predominant methods of transportation and communication among and between the villages some 30 years later in the 1990s (Barker 1993). One scholar suggests that the reliance on these modes of transportation is a result of the centralization of Native land and use patterns. When once previously dispersed populations settled in towns situated around schools and churches,

and subsequently adopted a more market like economy, changes in hunting patterns ensued. With a higher population density, Natives were forced to expand their hunting territory to meet their metabolic requirements and some reports show common travel in excess of 100 miles (Ellanna and Wheeler 1989).

Traditional and 'Transitional' Yup'ik Subsistence

Archaeological and biological anthropologists estimate that Yup'ik Eskimo populations have inhabited Alaska for around 5,000 years, and their unique cultural and physiological adaptations enabled them to survive the harsh Alaskan environment during that time (Szathmary 1984). In addition to developing both cultural and biological strategies for maintaining normal body temperatures in this cold environment, the Eskimo adopted specialized subsistence patterns to meet their metabolic demands (Draper 1977). Both early explorers and contemporary researchers in the region noted an annual seasonal shift in the pursuit of game animals, fishing, and wild plant collection. This was most likely a result of the polar climatic conditions in the area characterized by the freezeup and breakup of the river ice, thereby enabling or inhibiting subsistence activities which centered around fishing (Barker 1993).

The majority of the hunting and subsistence practices took place during the spring and summer months, with some fishing and gathering in the fall and winter depending on the village (Heller and Scott 1967; Barker 1993). The Natives moved to their winter houses once freeze-up commenced, usually by mid to late September, and survived on their stores of dried fish, seal oil, and/or fish eggs that were cached from the spring and summer months (Zagoskin 1967). In general the summer and winter houses were built

in the same locality along the Kuskokwim and lower Yukon, but they were separated by some distance. Life, therefore, was semi-nomadic with families establishing camp closer to the rivers during hunting season and moving back to the villages during the winter.

During the colder months the community spent a significant amount of time in ceremonial practice. These rituals emphasized the connection to the natural world and great care was taken to ensure that the souls of the animals that gave them nourishment were treated with respect, thereby increasing the likelihood of successful hunting and fishing endeavors in the future (Barker 1993). These rituals took place in the community *kazhim*, which served multiple purposes. It was a guesthouse, a bathhouse and the men's sleeping quarters, a location for tanning hides, weaving fishnets, dining and dancing, and a discussion room for community matters. Other gendered activities like parka manufacture and maintenance and summer hunting and trapping preparation took place in the *kazhim*. The activities which took place in the *kazhim* underscore the traditional reliance on subsistence foods and the connection of these items to both cultural and individual sustainability.

Early spring marked a period of lower caloric intake as fish stores, in general, were significantly reduced. By March temperatures began to rise but travel by water was limited due to incomplete thawing of the river ice. The partial thaw also prevented extensive travel by land as slushy ground impeded efficient sled movement – the predominant means of travel over the ice and snow. Village inhabitants thus hunted migratory birds, when available, and survived on any animals obtained by trapping.

A traditional Yupik diet consisted primarily of fish and/or seal oil and secondarily on animals such as moose, wildfowl – like ptarmigan, geese, pintails, and mallards -

snowshoe and arctic hare, muskrat, and berries depending both on the village and the season (Klein 1966; Heller and Scott 1967). The extent and variety of fish in the diet varied depending on the tribes' proximity to the coast and/or their location along the 2 rivers. Zagoskin noted varying quantities of silver, humpback, king, and Chinook salmon along the Yukon as well as larger quantities as compared to the Kuskowim. In Ikogmyut and other non-coastal towns fish was the staple, but beluga fat was considered a delicacy and was traded through succession from the coastal peoples. Other subsistence items included partridge, grouse, whitefish, yukola (split/dried fish) and tolkusha (fat and dried meat or fish made with roots or berries). Zagoskin also commented on the feasibility and sustainability of agriculture in the interior, but the practice only became successful among certain Alaskan Indians (Zagoskin 1967). Based on the predominance of these food items, nutritional researchers estimate that the majority of calories in the traditional diet, approximately 85-90%, were supplied by game animals and wildfowl, both high in structural fat and low in saturated fat, and/or marine mammals and fish (Heller and Scott 1967; Mann et al., 1962; Barker 1993). While seasonal berry collection did occur it is estimated that all sources of carbohydrates (also from wild greens, "mouse food", Eskimo potato, and glycogen from the meat consumed) provided only 10-15% of the total dietary calories (Heller and Scott 1967).

The inability of Russian, English, and American explorers and/or businessmen to exert overt control over indigenous Yupitt, combined with the environmental and economical constraints preventing access, until fairly recently, to more "Western" foods, have had several effects on the traditional practices of Alaskan Eskimos in the delta (Zagoskin 1967; Heller and Scott 1967). Delta Yupiit did not experience the same

extended periods of chronic food shortage and starvation common to many reservation-dwelling U.S. Native Americans. Additionally Yup'ik diets were heavily supplemented with traditional foods until the end of the 20th century (Heller and Scott 1967; Knapp and Panruk 1978; Nobmann et al., 2005; Ebbesson et al., 1999; Barker 1993; Mohatt et al., 2007). The combination of political, historical, and economic forces beginning at contact and outlined above, helped insure that many indigenous practices – especially those associated with subsistence activities and dietary practices - remained strong up through the 20th century, unlike the changes that occurred for their Native neighbors on the north coast of Alaska (Barker 1993).

Recent Southwestern Yup'ik Diet (1950s to beginning of 21st Century)

Significant dietary changes in the region, in terms of major macronutrients, began occurring in the 20th century with the first recorded analysis reported in the mid 1950's. In the Alaska Dietary Survey, conducted from 1956-1961, Heller and Scott provide a detailed analysis of macro and micronutrient composition in five villages – Akiak, Napaskiak, Kasigluk, Hooper Bay, and Newtok (Heller and Scott 1967). Intakes over the span of the study and across the hunting seasons were averaged, but percentages likely fluctuated depending on the season. Carbohydrate consumption increased to 33 percent of total calories due to the influence of “Western” foods and included items such as sugar, syrup, soft drinks, breads and cereals. Local carbohydrate sources represented less than one percent of all carbohydrate intakes. Protein constituted ~30% of total calories with the majority of the calories coming from local sources, especially fish. The remainder of calories came from fat which was primarily from seal, seal oil and fish with

some supplementation of butter, margarine, and hydrogenated fat. As a result of these “Western” fat items, the saturated fat content of Eskimo diets began to increase. The nutritionists noted that out of all Eskimo villages studied, southwest Alaska was the last region to be significantly affected by Western food items. Nevertheless, traditional food items remained a significant part of the Native diet and represented nearly half of caloric intakes.

A separate study conducted by the Interdepartmental Committee on Nutrition for National Defense (ICNND) found similar results. These researchers observed that protein intake, in grams, by Native men working in the National Guard were comparable to intakes by men in American Army training camps (Mann et al., 1962). It was noted that among men of all ages, 29.3 % of calories came from protein, 35.4% from fat, and 35.3% from carbohydrate. In their mixed diet of traditional and store bought foods, the majority of the latter were cereals and sugars. By 1978, macronutrient compositions of the native diet paralleled intakes by populations in the U.S. (Knapp and Panruk 1978). Five villages representing differing geographical locations in the delta were studied; Kwigillingok (Southern Coastal), Akiak (Kuskokwim River), Mountain Village (Yukon), Hooper Bay (Northern Coastal), and Kasigluk (Tundra Area). Macronutrient percentages equaled 17% protein, 35% fat, and 50% carbohydrate, but fish and indigenous protein were still major components of the diet.

A survey conducted by the Alaska Department of Fish and Game in the 1980s observed that of the 700 pounds of wild food procured per person per year, 518 pounds were fish, and 66 pounds were sea mammals, while greens, waterfowl, and berries comprised the remaining 41 pounds (Wolfe and Walker 1987). By 1990 this number had

decreased to 454 pounds per person yet is still relatively higher compared to more urban towns such Juneau, Anchorage, and Fairbanks with less than 50 pounds per person. High seal oil (95%) and fish consumption (99%) was also reported among 556 Yup'ik Eskimos >40 years of age from 15 villages in the Yukon/Kuskokwim Rivers delta studied by Adler et al. (Adler et al., 1994). Although data was collected on the frequencies of traditional and nontraditional food items as well as information about physical activity, this data was not presented in their report. These data clearly point to the continued reliance on traditional subsistence food items in the contemporary diet (Barker 1993).

Not every study, however, has reported the same increased consumption in Western food items. During July and August of 1984, an analysis of coastal versus inland village subsistence was performed on 80 participants between the ages of 4 and 40 (Parkinson et al., 1994). The inland village was located 20 miles east of Bethel, Alaska and the other village on the Bering Sea coast, 100 miles west of Bethel. The researchers noted that while Western foods were available in stores they were infrequently consumed and thus, an analysis of nontraditional foods was excluded from the study. Compared to river village residents, coastal village residents reported eating traditional foods more frequently and consumed significantly more marine fish, birds, and marine mammals. These traditional foods were also eaten with more seal oil. River village residents, on the other hand, consumed significantly more salmon. Significant for this study, however, are the results from their analysis. Both groups of village residents had significantly higher concentrations of plasma omega-3 (w-3) fatty acids compared to nonnative controls from the University of Oregon Family Heart Study, which appears to be consistent with a traditional diet high in marine mammals and fish. There were no significant differences

in plasma w-6 fatty acids. Limited intakes of more Western food could have been the result of village proximity and, therefore, access to convenience foods, the higher costs associated with food items that are transported into remote villages, a reliance towards a cultural identity centered around traditional subsistence items and the means of obtaining them, and/or a distaste for “Western” foods. While later studies explored the correlation between these variables and current subsistence behaviors, this study did not provide an explanation for the limited intakes of Western food items observed in this study.

In 2001 a large scale study was conducted on women of child bearing age in 1 urban and five rural regions in Alaska. Researchers collected data using a one-day 24-hour dietary recall and the Block Brief Food Frequency tool (BBFFQ). In both the urban and rural regions, sweetened beverages (soda, fruit juices, and sweetened beverages) accounted for 33 percent of the total caloric intake. The majority of the carbohydrates were high in sucrose as opposed to complex grains, but indigenous plants and berries continued to be eaten in large amounts when available. Meat and fish were prepared by boiling versus frying and, similar to other studies, use of traditional subsistence foods was high (Smith et al., 2008). The following summer The Alaska Traditional Diet Survey was conducted in villages of five Regional Health Corporations using an interviewer administered food frequency questionnaire. Among the top 50 most frequently mentioned foods reported by 224 participants in the Yukon-Kuskokwim Health Corporation Region, Hi-C, sugared soda-pop, and fruit juice ranked first, second, and third. One third of the top 50 foods were traditional food items out of which half were fish and seal oil. The most recent dietary data has been collected by researchers working with the Center for Alaska Native Health Research (CANHR), formed in 2002.

Funded by the Centers of Biomedical Research Excellence at the National Center for Research Resources of the National Institutes of Health, CANHR researchers developed a tri-part program designed to investigate how the combination of genetics, nutrition and cultural-behavioral factors influenced the development of chronic disease in southwest Yupiit. These researchers were particularly interested in Southwestern Yupiit who, out of all Eskimo groups in Alaska, had remained the most intact culturally (using the extent of Native language use as a measure) yet possessed equal overweight/obesity prevalence (32.5% and 32%) to that observed in the lower 48 states. These Yupiit, while supplementing their diet with Western food items, continue to consume significant amounts of traditional subsistence items (Mohatt et al., 2007).

Physical Activity

Researchers (Adler et al., 1995) analyzing food consumption in the Yukon/Kuskowkim rivers delta also collected information on the participation in “traditional” (i.e. walking, rowing a boat, carrying water by hand, washing clothes by hand, using a dog sled or handsaw) vs. “modern” (i.e. using a vehicle or motorboat, transport of water by vehicle, and using a chainsaw) physical activities during a 13 month period between 1987 and 1988. The researchers also observed that approximately 65% of the population was engaged in moderate to high physical activity, while only 35% reported low physical activity. The effects of an increasingly sedentary lifestyle, however, are evident in growing prevalence of obesity in these communities. This may, in part, be due to a shift towards a wage economy, which reduces time spent actively pursuing traditional activities like hunting and fishing (Heller and Scott 1967).

Type 2 Diabetes and its Associated Risk Factors among Delta Yupiit

Approximately twenty thousand people living in 50 villages now reside in the Alaskan Southwest, a flat delta the size of Kansas (Barker 1993). Studies in the 1950's and 1960's suggest that diabetes was rare - if not absent- in the Native Alaskan population. In fact, a study undertaken by the ICNND in 1958 showed that infectious disease, specifically tuberculosis, and poor dental health were two of the most common health problems in the state (Mann et al., 1962). This does not mean, however, that T2D was as low as the initial estimate suggest, as early studies were limited by several factors including, consistent definitions of T2D, screening methods and/or laboratory techniques. The first few confirmed cases of T2D in the delta were reported in 1962. A survey conducted in the Yukon-Kuskowkwim region during that year suggested that 1.1% of the population over the age of 20 "probably" had diabetes (Mouratoff et al., 1967). Twenty-five years later, the same 15 villages studied in 1962 were reassessed and it was found that 1.73% of village residents over the age of 20 had T2D (Murphy et al., 1995). This data was obtained from interpreting oral glucose tolerance tests (OGTT) according to World Health Organization (WHO) criteria.

By the 1980's the number of cases of T2D had increased, but were still very low relative to Native American populations in the lower 48 states. In contrast to the many high prevalence Native American populations there (e.g., Pima - ~50%, Havasupai - 38%) the estimated overall T2D prevalence rates for indigenous Southwest Alaskan Eskimo populations remain low, 3.4% (Mohatt et al., 2007). T2D prevalence exists along a continuum (.08% to 15%) in Alaska, increasing with contact/exposure to Euro/American communities and Western foods. Those Yup'ik populations with the

highest prevalence tend to have had the earliest and most extensive contact with explorers. Athabascan Indians and Aleuts, for example, have slightly higher prevalence, 6.46%-11.54% respectively- over the age of 35, as a result of intense salmon exploitation by whites (Naylor et al., 2003).

Overweight, Obesity, and Fat Patterning

The ICNND in 1958 reported that obesity was rare in a group of Southwestern Alaska Yup'ik men working for the National Guard (Mann et al., 1962). However, the same study found that in a group of 70 women, 15.7% were considered obese ("Obesity" taken as >120% of standard weight using the U.S. Medico-Actuarial Tables). As with other cardio-metabolic risk factors, obesity has increased in the delta. From January 1987 to February 1988, a village based health fair screening study was conducted among residents ≥ 20 years old from 15 villages along the Yukon and Kuskokwim rivers in Southwestern Alaska (Murphy et al., 1995). The average BMI for Yup'ik males and females ≥ 20 -39 years of age was 25.1 and 27.4 respectively, while the average BMI among males and females ≥ 40 years of age were 25.7 and 29 respectively. According to the National Center for Health Statistics, BMI's $< 25 \text{ kg/m}^2$ are considered low, 25-30 kg/m^2 medium/overweight, and $>30 \text{ kg/m}^2$ are high/obese. Thus, twenty-seven percent of Yup'ik men and 51% of women over the age of 20 were overweight. Interestingly, in this study, Yup'ik women had a significantly higher BMI than Indian women ($P < .001$), yet prevalence of T2D among Indian women was higher, 3.37% vs. 1.73%. Statistics gathered by the State of Alaska in 1999 suggest that 38% of rural Alaskans near the Bethel census area were overweight (The Alaska Bureau of Vital Statistics). These statistics mirror rates observed in the U.S. where, interestingly, T2D

prevalence is higher. According to the 1999 National Health and Nutrition Examination Survey (NHANES), 27% of Americans between the ages of 20 and 74 were considered obese and 34% were considered overweight (Alaska Department of Health and Social Services). Some researchers have questioned the validity of using BMI calculations to assess overweight and obesity among Eskimos who are characteristically shorter and wider (Young 2007). This phenotype, which has the effect of reducing overall body surface area, limits body heat loss and is generally considered a physiological adaptation to cold environments (Bergmann 1847). Because BMI calculations can increase with reduced stature, some Eskimos could be erroneously defined as overweight.

In most high prevalence populations, T2D has been positively correlated with being overweight or obese as measured by waist: hip ratios or waist circumference. While an increase in obesity has been observed in the delta over the past 50 years - similar to the increase in T2D prevalence - it is especially noteworthy that there does not appear to be a corresponding increase in other metabolic markers (increased triglycerides, total and LDL cholesterol) commonly associated with obesity. The Center for Alaska Native Health Research studying rural Southwestern Alaskans has coined this phenomenon Metabolically Healthy but Obese.

Current epidemiological data suggests that Alaskan Yupiit in certain regions of the Southwest are becoming more overweight, engaging in less physical activity, and are increasing their consumption of non-traditional foods. As a result, one would expect to find similar T2D prevalence among Alaskan Yupiit and Native Indians in the U.S. with comparable diet, activity level and anthropometric risk factors. Surprisingly, the opposite is seen; current prevalence among Alaskan Yup'ik populations is not only considerably

lower than their Native American counterparts in the U.S., T2D prevalence among Yupiit is among the lowest reported for T2D by ethnicity in the U.S. (Boyer et al., 2007). This difference in prevalence, between what would be considered two classic “thrifty genotype” populations, Native Americans and Alaskan Yupiit, provided the inspiration for the current study.

CHAPTER 3

POLYUNSATURATED FATTY ACIDS

Macronutrient Composition of Traditional Southwest Alaskan Yup'ik Diet

The low carbohydrate content of the traditional Yup'ik diet is significant when considering that the human adult brain alone requires around 100 grams of glucose per day and the traditional high protein diet probably only provided 10 of these (Draper 1977). Furthermore, ample supplies of glucose are critical for the developing fetus during pregnancy and after birth for lactation. Draper suggests that the high protein concentration of the traditional diet was critical since "...extra protein was necessary to furnish the amino acids required for glucose synthesis beyond those required for body protein synthesis" (Draper 1977). That is, the body might have improved its efficiency for gluconeogenesis, a physiological process whereby the body creates glucose from protein substrates. It may also be possible that Yupiit consuming a diet made up solely of locally-obtained food possess sufficient levels of circulating glucose as a result of the body's especially efficient response to a high protein diet. When protein is consumed the liver produces glucagon, a hormone which causes cells in the body to release their stored contents of glucose. Other scholars have proposed that Eskimo populations adapted metabolically to a low carbohydrate diet via acquisition of an insulin resistant genotype (Colagiuri and Brand Miller 1997). Skeletal muscle cells that were resistant to the action of insulin would allow for higher circulating levels of blood glucose which could then be utilized for the brain and the developing fetus. If an insulin resistant genotype characterized Eskimo populations in the recent past, then this insulin-resistant, "thrifty

genotype” should characterize contemporary Eskimo populations. Furthermore, an insulin resistant genotype - in conjunction with the insulin resistance associated with high caloric/high carbohydrate diets and obesity - should dramatically increase the prevalence of T2D. As discussed in Chapter 2, this is not the case among Yupiit in Alaska today. When Neel originally proposed his “thrifty genotype” he did not take into account how circulating levels of insulin are affected by ratios of the 3 energy yielding macronutrients in the diet and/or the source of the macronutrients. Insulin production and metabolism in the body is complex. It affects and is affected by prenatal conditions, other circulating hormones and cellular constituents, as well as exogenous nutrients that enter the body. In fact, research has shown that when relatively healthy individuals are placed on a more “traditional hunter-gatherer” diet (the one characterized in Neel’s model) overall biological markers of health (i.e. plasma insulin, triglycerides, LDLs, total cholesterol) improve (Frassetto et al., 2009). Unless these periods of feasting included a universal consumption of large amounts of simple sugars (which cause a spike in insulin) the conversion to fat that Neel attributes to an “insulin trigger” may in fact be nothing more than the body’s reaction to convert excess nutrients to fat when caloric intake has exceeded its metabolic requirements. Southwestern Alaskan Yupiit appear to be insulin sensitive, not insulin resistant. Recent research suggests that Yup’ik insulin sensitivity is associated with high intakes of omega-3 fatty acids (Ebbesson et al., 1999). Likely candidates for this metabolic observation are the polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) contained in traditional marine subsistence items and a lower saturated/polyunsaturated ratio.

Polyunsaturated Fatty Acids and their Physiological Roles

Polyunsaturated fatty acids exist as both short chain (<20 carbon atoms long) and long chain (>20 carbon atoms long) fatty acids. Unlike monounsaturated fatty acids, which contain one unsaturation and one double bond, or saturated fatty acids, whose carbon atoms are all saturated with hydrogen atoms, polyunsaturated fatty acids possess multiple double bonds. Polyunsaturated fatty acids (PUFA's) are classified into three main families, omega 9, omega 6, and omega 3 depending on the position of the first double bond counting from the methyl (i.e., the 'omega') end. While omega 9 fatty acids play a lesser role in human nutrition (are synthesized by the body when omega 3 or 6 fatty acids are unavailable), omega 3 and omega 6 are essential, both as structural components within cells and as biological regulators. These essential fatty acids comprise a substantial portion of the lipids in cellular membranes and are also precursors for chemical mediators the body creates for specific metabolic functions. Research using cell lines has shown that the fatty acid profile of a cell membrane (i.e. different amounts of saturated, monounsaturated, and polyunsaturated fatty acids) can influence the function of certain membrane proteins like receptors, transporters, and enzymes (Spector and Yorek 1985). Furthermore, alterations in membrane lipid composition have been shown to affect phagocytosis, endocytosis, exocytosis, cytotoxicity, prostaglandin production, and cell growth (Spector and Yorek 1985). Table 1 lists the types of short chain and long chain polyunsaturates within each lipid family in addition to food sources in which they are found. (The Fish Foundation).

Table 1. Polyunsaturated Fatty Acids and their Sources.

<u>Lipid</u>	<u>Name</u>	<u>Number of carbons and number of unsaturations</u>	<u>Source(s)</u>
n-9	Oleic acid	18:1	
		18:2	
		20:2	
	Eicosatrienoic acid	20:3	
		22:3	
n-6	Linoleic acid (LA)	18:2	Vegetable oils such as sunflower, corn, cottonseed sesame and safflower Evening primrose oil, Blackcurrant seed
	Gamma-linolenic acid (GLA)	18:3	
	Arachidonic acid (AA)	20:3	Found in small amounts in meat (Chicken, beef, Pork, lamb, and turkey) egg yolk, liver, kidney
		20:4	
		22:4	
n-3	Alpha-linolenic acid (LNA)	22:5	From linseed, rapeseed, flaxseed and soybean oils
		18:3	
	Eicosapentaenoic acid (EPA)	18:4	Only significant source is oil rich fish
		20:4	
		20:5	
	Docosapentaenoic acid	22:5	Major source is oil-rich fish, small amounts in in meats and eggs
		24:5	
24:6			
	Docosahexaenoic acid(DHA)	22:6	

PUFA's have several different metabolic fates in the body. They can be elongated, desaturated, shortened, or converted to other bio-active molecules like prostaglandins or leukotrienes. They can also direct glucose towards glycogen storage and reduce

triglyceride synthesis from fatty acids, increase fatty acid oxidation and suppress hepatic lipogenesis (Clarke 2000). Prostaglandins and leukotrienes represent two of the four major classes of eicosanoids or signaling molecules. They have a range of biological activities from influencing the contraction of smooth muscle and the aggregation of platelets to the participation in the pain and inflammatory responses (Sears 2005).

Prostaglandins and leukotrienes are primarily pro-inflammatory eicosanoids that are derived from arachidonic acid (AA), an omega-6 fatty acid. Fat cells, primarily in the abdominal region, sequester AA which over time leads to the production of pro-inflammatory eicosanoids as well as cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF). Both IL-6 and TNF enter the bloodstream and mount inflammatory responses. It had been suggested that there is a correlation between insulin resistance and an increase in TNF (Sears 2005). In one experimental animal study, rats were fed a standard or high fat (cafeteria) diet. In a subgroup of cafeteria fed rats, EPA – an anti-inflammatory precursor - was administered for 5 weeks. Not only was a marginally lower body weight gain observed in the EPA subgroup, but EPA administration prevented the rise in TNF alpha observed in the cafeteria fed diet group not receiving EPA (Perez-Matute et al., 2007).

Excess insulin can also increase the production of AA and IL-6. Because omega-6 fatty acids tend to be consumed to a greater extent than omega-3 fatty acids (in the U.S) most eicosanoids in the body are of the omega-6 type (Broadhurst 1997; Sears 2005). Since long chain omega-3 PUFA's (>20 carbon atoms long) are essential, the body converts dietary short chain fatty acids into long chain fatty acids. For instance, when linseed oil or rapeseed oil is consumed the alpha-linolenic acid within these oils can be

converted to EPA or DHA. Epidemiological evidence in the U.S. suggests, however, that saturated fats and omega-6 PUFA's from oil seeds (soybean, corn, sunflower, and safflower) are increasing and omega-3 are decreasing, which further reduces the amount of omega-3's in the body's cellular structures (Korotkova et al., 2002). Furthermore, the short chain to long chain conversion may not be efficient enough to provide the necessary amounts of long chain fatty acids the body needs, which is especially true for infants in the early stages of life (Pella et al., 2004).

Marine sources of non-white flesh, primarily pelagic fish which feed more in the surface layers of the ocean, contain higher levels of lipid (of the omega-3 type) in the flesh. "White" fish such as cod, haddock, plaice, and whiting, for example, have lower lipid levels (0-2%) compared to the non-white fish such as herring, mackerel, sardines, tuna, salmon, and trout (5-15%). These higher lipid levels in the non-white fish is the reason that they are referred to or characterized as "fatty fish", "oily fish", or "oil-rich fish". It is currently thought that microscopic algae, plankton, and planktonic crustacean, residing in the surface layers of the ocean, can form the long chain omega 3 polyunsaturates, which are then passed up through the food chain (Innis and Kuhnlein 1987). A study published in 1987 investigated the fatty acid composition of marine mammals, polar bear, and caribou. They discovered that marine mammals and polar bear contained substantial quantities of polyunsaturated fatty acids, which supports the food chain hypothesis (Innis and Kuhnlein 1987).

EPA is the major omega-3 polyunsaturated fatty acid in most seafoods followed by DHA. The remainder of the polyunsaturates, 22:5, 20:4, 18:3, and 18:4 are minor components of most fish. EPA increases the production of anti-inflammatory eicosanoids

and partially inhibits the enzyme delta-5-desaturase which makes AA. While DHA cannot directly be metabolized to eicosanoids, it can be retroconverted to EPA and is, therefore, indirectly related to eicosanoid balance in the body. DHA is a major structural component of the brain, nerve, and retinal membranes where it can form up to 60% of the polyunsaturates present. It can also alter a cell's sensitivity to insulin by binding to transcription elements on DNA (Sears 2005).

The critical periods for PUFA incorporation in brain tissue are likely the last trimester of gestation through infancy (around 2 years of age) and some scholars have postulated that an inadequate supply of these fatty acids during these periods, "...may cause a defect in the expression or function of insulin receptors resulting in type 2 diabetes" (Pella et al., 2004). Breast milk can be comprised of .1-.4% of fatty acids as DHA, which can be altered by dietary intakes of fish and fish oils. The levels of PUFAs in maternal milk are nearly identical to maternal dietary PUFAs (Korotkova et al., 2002). EPA, however, is virtually absent in breast milk.

Clinical, Epidemiological and Experimental Animal Studies and Polyunsaturated Fatty Acid Diets

A large body of clinical, nutritional, and experimental animal research has reported on the health benefits associated with a diet rich in omega-3 fatty acids. In 2005 researchers Nettleton et al., compiled the data pertaining to omega-3 studies and reported that high intakes tend to be linked with lower incidences of CVD, hypertension, and T2D (Nettleton et al., 2005). The researchers commented on health statistics in Iceland, in terms of coronary heart disease and T2D, and found that the data mirror the trend

observed in Southwest Alaska. Despite the presence of T2D risk factors (including a high prevalence of overweight and obesity) in Iceland, disease prevalence was low. Researchers there noted a correlation between low T2D and high consumption of milk in Iceland, which contains more omega 3 PUFA's than in other Nordic countries due to the presence of fish meal in the animal fodder (Vilbergsson et al., 1997). The milk is also lower in omega 6 PUFA's which increases the omega-3/omega-6 ratio. In the review Nettleton et al. observed that the majority of these studies found a daily consumption of ~3g/day of fish oils reduced triglyceride levels, increased high density lipoprotein levels, lowered blood pressure, and improved endothelial function (Nettleton et al., 2005). Endothelial function plays an important role in glucose balance. This barrier between the bloodstream and the organs can increase or decrease the efficiency of insulin transport across the endothelium, thereby enabling or inhibiting insulin to interact with cellular receptors (Sears 2005). It is possible that n-3 PUFA's play an important role in reducing CVD by lowering RLPs (remnant lipoproteins are highly atherogenic) and reducing coronary artery narrowing (Nettleton et al., 2005).

Other studies not included in Nettleton's review have reported on the correlation between omega-3s and certain biomarkers of disease. A study conducted in the 1980s examined the relationship between the types and quantities of nutrients consumed with the occurrence of impaired glucose tolerance (IGT), a "pre-diabetic" condition. According to the WHO IGT is defined as two hour glucose levels of 140 to 199 mg/dl after a 75 gram glucose load. A diagnosis of IGT carries an increased risk for the future development of T2D. After adjusting for age, BMI, and sex they found that moderate and high levels of physical activity reduced the prevalence of glucose intolerance. Daily

salmon consumption also provided protection against glucose intolerance (Adler et al., 1994).

In 1994 researchers Adler et al., published a study conducted among 556 Eskimos and 110 Athabaskans over the age of 40 from 15 villages situated along either the Yukon or Kuskokwim rivers. After reviewing self-administered questionnaires it was observed that daily seal oil and salmon consumption were associated with a lower prevalence of glucose intolerance after controlling for age, ethnicity, BMI, and sex. Consumption of seal oil once a week or less significantly increased the chances of developing glucose intolerance as compared to individuals who ate it at least five times per week. (Adler et al., 1994).

In a separate study published one year later Murphy et al. administered a food frequency questionnaire to Eskimo and Indian residents > 20 years old during the winter months. Fifteen villages were studied and the majority of the villages were located along either the Yukon or Kuskokwim rivers. Among Eskimos younger than 30 years of age, nonindigenous protein, low-nutrient density carbohydrate, and non-indigenous fat (i.e., processed snack foods) was more frequently consumed compared to Eskimos that were > 60 years of age. In addition, “Subjects with IGT reported a significantly more frequent use of nonindigenous protein and less seal oil, and they had a significantly higher prevalence of overweight” (Murphy et al., 1995).

A separate clinical study observed that, “Forty-four Alaskan Inuit [Eskimo] with impaired glucose tolerance, excess weight, or obesity were counseled to eat fewer foods high in saturated fats, palmitic acid, and trans fatty acids, and more traditional foods,

especially fish and marine animals [and]...after 4 years, no participants developed type 2 diabetes, despite not losing weight” (Ebbesson 2002).

In 1994 the Alaska Siberia project reported an association between normal glucose tolerance, impaired glucose tolerance, and T2D with serum fatty acid balance. Those individuals with IGT and T2D had lower levels of plasma omega-3 fatty acids found in traditional foods and higher concentrations of fatty acids found in non-traditional foods like butter and bacon (Ebbesson et al., 1999). In 2005 the same researchers measured plasma fatty acids in 447 Norton Sound Eskimos and found that they were highly correlated with increased intakes of dietary omega-3s. Additionally, omega-3 concentrations were highly correlated with high density lipoproteins (HDLs or “good cholesterol”) and inversely correlated with 2 hour insulin and glucose, triglycerides (TG) and diastolic blood pressure (Ebbesson et al., 2005). Consistent with these findings, researchers examined the fatty acid profile in the Inuit population of Nunavik in northern Quebec. Plasma omega-3s were positively associated with HDLs and inversely associated with TGs, but they did not find an association with blood pressure or plasma insulin (Dewailly et al., 2001).

In early 2002 Barry Sears working with Princeton Medical Resources, administered a low-glycemic load diet and 1.6 grams per day of EPA and DHA to 68 T2D patients. After only 6 weeks, insulin, TG, HDL, glycated hemoglobin (HbA1c – a measure of blood glucose levels over approximately 3 months), and fat mass significantly (<.0001) improved.

Similarly a 2007 CANHR study reported that the prevalence of metabolic syndrome in southwest Alaskan Eskimos was 14.7%, which is lower compared to NHANES III

findings of 23.9% for the general U.S. population with similar risk factors. According to the International Diabetes Foundation, IDF, metabolic syndrome is a cluster of traits including abdominal obesity (an index of insulin resistance/glucose intolerance), dyslipidemia, and hypertension. The presence of one or more of these traits can increase a person's risk for developing T2D. Boyer and researchers also noted that compared to the general U.S. population, HDLs in Eskimos were significantly higher, triglycerides significantly lower and fasting glucose levels were also lower, $p=.065$, levels that may be explained, at least in part by the effects of traditional Eskimo diets on these cardio-metabolic markers. Metabolic syndrome was higher in Yup'ik women compared to men, which may be explained by their larger waist circumferences.

Experimental animal studies have complimented these epidemiological and clinical findings in humans. Several studies have shown that a balanced fish oil diet compared to an olive oil or vegetable oil diet can improve levels of plasma triglycerides, cholesterol, insulin, and adipocyte insulin stimulated glucose transport in insulin resistant or diabetic rats (Fickova et al., 1998; Luo et al., 1996; Peyron-Caso et al., 2002). It has also been shown that when rats consume a high fish oil diet they do not exhibit the sucrose-induced hyperinsulinemia and hypertriglyceridemia present in rats on olive oil and standard oil diets (Peyron-Caso et al., 2002). In a study published in 1997, rats fed an n-3 diet - identical in macronutrient percentages to a comparable to an n-6 diet, showed an increase in lipolysis with diminished lipogenesis (Fickova et al., 1997). Furthermore, smaller increments in weight gain over one week were observed in the n-3 diet. In other words, the metabolic conditions associated with insulin resistance/T2D (i.e., hyperinsulinemia)

seem to improve when high levels fish oil, rather than olive oil or mixed oil diets, are consumed.

In another study control and diabetic induced rats were fed a 27% (w/w) casein protein, 38% carbohydrate, and 35% fat (w-3 1% w/w) diet that either contained a high (2.0) or low (0.2) P/S content (ratios based on consumption by segments of the North American population). While control animals showed an improvement in cardio-metabolic function (i.e., insulin binding, and a significant increase in the rate of insulin-stimulated glucose transport and lipogenesis) on the high P/S diet, diabetic animals did not show the same improvements in the amount of insulin working effectively as the rates of insulin-stimulated glucose transport and lipogenesis were significantly lower ($p < .05$) in diabetic animals compared to control animals. Noteworthy, however, is that the high P/S diet for diabetic animals significantly improved ($p < .05$) the rates for all three functions. It is possible that marked improvements in these rates for diabetic animals over what was observed could be manifested by a diet that contained higher amounts of w-3s (Field et al., 1990).

There appears to be a strong correlation between dietary polyunsaturated fatty acids and adipocyte membrane composition, function, and fluidity, and these effects of dietary lipids are likely responsible for some of the metabolic (glucose and lipid) improvements mentioned above (Field et al., 1990; Luo et al., 1996). The findings presented here suggest that metabolic changes can occur within the lifespan (and changes can be observed in as little as 1 week) in experimental animals and that dietary supplementation of n-3 fatty acids can improve insulin sensitivity and glucose transport.

While many studies have found a strong correlation between improved insulin sensitivity and glucose uptake, decreased plasma triglycerides, and lower incidences of CVD and hypertension with large consumptions of omega-3s from fish oil, other studies have reported no such effect(s). Several of the studies reviewed by Nettleton et al. did not support the correlation between omega-3 consumption and lower incidences of CVD and/or hypertension described in the literature. In addition, a study conducted by the Alaska Siberia project in 1994 found that plasma omega-3s did not significantly differ among those Alaskan's with CHD and those without. While researchers noted that Alaskans, both with and without CHD, continued to consume high amounts of omega-3s these PUFAs did not protect against CHD when other risk factors were present. These observations may, in part, be explained by other biological and nutritional phenomena occurring during prenatal life.

CHAPTER 4

DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

The Role of the Intra-Uterine Environment on Post-natal Metabolism

The Developmental Origins of Health and Disease (DOHaD) is a relatively new area of medical research that seeks to define the various role(s) of early life variables (e.g., maternal prenatal nutrition) in the developmental programming of disease. For metabolic disorders, the idea that processes occurring in-utero can program post-natal metabolic profiles and shape certain phenotypic characteristics such as insulin resistance later in life is well established by research (Hales and Barker 1992; Benyshek et al., 2004; Ravelli et al., 1998).

Retrospective Epidemiological Studies

A study published in *Diabetologia* in 1992 by researchers Hales and Barker reported that among 5,654 men from Hertfordshire, England those with low birth weight and weight at one year of age possessed a three times higher death rate from ischaemic heart disease compared to normal birth weight babies. These low birth weight infants, who were at such an increased risk of dying from CVD, were referred to as “thrifty-phenotypes”. The same researchers took 370 of those same men and administered a 75 gram oral glucose tolerance test. Those with relatively higher birth weights (>5.5 pounds) and weights at one year of age (>18 pounds) tended to have fewer cases of impaired glucose tolerance and type 2 diabetes. Similarly, low birth weight, but not lower weight at one year, was associated with hypertension in adulthood (Hales and Barker 1992). This suggests that the development of certain conditions or types of

chronic disease in adulthood may arise according to when a certain “insult” occurred during development – a “critical window” – during gestational development or early infancy. The potential for environmental triggers occurring during a critical window of development to influence or ‘program’ fetal or infant physiology and/or metabolism and predispose to a large array of diseases in adulthood has been examined extensively.

A similar study to the one conducted by Hales and Barker was published in 1998. During the Dutch famine of 1944 to 1945, the western part of the Netherlands experienced a widespread famine. Several hospitals in Amsterdam during this time collected and maintained detailed prenatal records and birth weights of the babies born in the facilities. Researchers traced 5,425 people born in these Amsterdam hospitals and administered an OGTT to 702 of these men and women either conceived prior to, during, or after the famine. Compared to their cohorts who were conceived prior to and after the famine, those individuals exposed to the famine prenatally (via their mother’s malnourished state) were more glucose intolerant, and glucose intolerance was even more pronounced in famine exposed babies who became obese in adult life. In addition, glucose levels 2 hours after the OGTT were higher in men and women exposed to famine during mid to late gestation compared to early gestation (Ravelli et al., 1998). While the specific environmental trigger(s) responsible for causing the observed effects in these individuals is not completely understood, these findings implicate an important role for maternal nutrition during pregnancy on metabolic processes in adulthood. Dutch mothers who received rations from only 400 to 800 calories a day gave birth to babies that were unable to maintain glucose homeostasis later in life when substantially more calories became available. While these and other retrospective human studies can illuminate the

importance of relative nutrient availability on fetal programming of adult metabolism, they do not address which nutrients are most critical and/or to what extent they can shape adult health outcomes.

Several epidemiological DOHaD studies, however, have further refined which maternal nutrients consumed during pregnancy and lactation might influence early developmental programming and have also broadened the scope of inquiry to include the role of nutrient provisioning following birth (Gluckman and Hanson 2005).

DOHaD Experimental Animal Studies on Obesity- Related Disorders

Experimental animal studies have modeled the dietary transitions (i.e., maternal malnourishment/undernourishment during pregnancy followed by a relatively adequate caloric diet for the offspring post weaning) that occurred in certain human populations like the Pima Indians and the Dutch population in Amsterdam during the early to mid 1900s. Similar to the epidemiological findings in humans, rats that are undernourished (calorically low diets) prenatally and are weaned onto control diets (calorically adequate) tend to exhibit increased adiposity in adult life (Vickers et al., 2003). Other studies have shown that rats undernourished during gestation had low birth weights, and a subsequent 'catch' up in growth occurring the first 6 weeks was associated with obesity and glucose intolerance by six months of age (Jimenez-Chillaron et al., 2006).

Maternal diet during pregnancy has also been associated with hypertension, hyperglycemia, and insulin resistance in the adult offspring. Liang et al. (2009) demonstrated that mice fed a high fat ("fast food") prenatal diet and a control diet post weaning, exhibited hypertension, hyperglycemia, and insulin resistance in adult life.

These and other studies have illuminated the importance of maternal diet during pregnancy on the developmental programming of metabolic traits.

Intergenerational Transmission of Developmentally Programmed Traits

Experimental animal studies have also been extended to understand the intergenerational transmission of fetally programmed traits. Researchers from the University of Nevada in Las Vegas and Arizona State University used a rat model to examine the effects of isocaloric low protein diets (8%) during gestation on birthweight and insulin metabolism. The offspring of rats undernourished in utero received a nutritionally adequate or high fat diet (overnourished) postweaning. Birthweights and weights in adulthood for the experimental animals were significantly lower compared to control animals. Fetally malnourished offspring consuming an adequate diet postweaning had significantly greater fasting insulin compared to control animals. Second generation (F2) experimental rats received the same diets as their parent and both adequate and high fat fed F2 generation rats had markedly high levels of insulin compared to controls and F1 generation rats. In other words maternal diet (low protein) can program metabolism in such a way that when offspring are placed on a standard, or a high fat diet, they are insulin resistant in the second generation. A second set of pups were over-nourished in utero, maintained on a high fat diet postweaning, and their offspring F2 received the same high fat diet. All offspring over-nourished post weaning, regardless of prenatal diet, had significantly reduced fasting insulin sensitivity compared to controls (Benyshek et al., 2004).

These same researchers used a similar experimental approach in a second study but extended it to include a third generation. In this study female dams were protein

malnourished during pregnancy and lactation. The first, second, and third generations from these protein malnourished dams all consumed an unrestricted nutritionally adequate diet postweaning, and during pregnancy and lactation. The first generation showed reduced insulin secretion; the second generation was insulin resistant; and the third generation also showed impaired (although improved) insulin sensitivity (Benyshek et al., 2006). Taken together, these results point to some intergenerational transmission of developmentally programmed traits, especially with respect to glucose/insulin metabolism.

DOHaD and PUFA Intake

As discussed in Chapter 3, DHA and AA are major components of developing brain and retinal tissues. Babies fed formula made with vegetable oils tend to have delayed neural development and significantly less DHA and total long chain polyunsaturated fatty acids (LCPUFAs) in the lipid bilayer of skeletal muscle compared to breast fed babies (Baur et al., 1998).

DOHaD Experimental Animal Studies with Polyunsaturated Fatty Acids

The consequences of the maternal nutritional milieu on fetal growth and development in humans have been modeled in animal studies. Manipulation of dietary variables in highly controlled environments during pregnancy and lactation has broadened our understanding of the role maternal nutrition during these critical periods plays in shaping offspring metabolism. In one study animals were given DHA immediately after birth during the perinatal period. Compared to cohorts supplemented with DHA during the perinatal period, animals fed DHA after the perinatal period, or at weaning, had raised blood pressure (Weisinger et al., 2001).

Other animal studies have explored how pre-natal diets differing in amounts and/or ratios of PUFA's affect insulin and glucose metabolism post-natally. One study explored the effects on offspring of three maternal prenatal diets: 1) prenatal diets high in n-3 (linseed oil); prenatal diets high in n-6 (sunflower oil), and 3) prenatal diets high in n-3 and n-6 (soybean oil) (mixed diets). Researchers then measured glucose, protein, cholesterol, serum leptin, and triacylglycerol levels of offspring at one and 3 weeks of age (Korotkova et al., 2005). Leptin, a hormone produced in adipose tissue that controls food intake and energy expenditure, was lowest among animals whose mothers were fed the n-3 diet during pregnancy and lactation and highest in the "mixed" prenatal diet group. There were no significant differences in serum glucose, triglycerides, protein or cholesterol among the 3 groups, although the n-3 group tended to have lower levels of triglycerides. Adipocyte size, fat depots, body weight and length were significantly reduced in the n-3 group during the suckling period.

In a later study researchers used these same methods but extended the project to include an analysis of long term effects. At 28 weeks of age there were no differences in protein, glucose, or leptin, but triglycerides were higher in male rats fed a mixed diet. Fasting insulin was significantly higher in the mixed diet for both males and females. The researchers, however, did not use fish oil which altered the composition of EPA and DHA fatty acids in the diet. As mentioned previously, it is thought that these fatty acids are critical factors in the potential programming of insulin metabolism.

In another study Chapman et al. (2000) designed two diets, a 5g/100g fish oil diet and a 5g/100g mixed oil diet - meant to represent a typical UK diet - and pair fed them to rats during the last 2 weeks of gestation, lactation, and up to 5 weeks of age. Animals were

given a mixed oil test meal at five and ten weeks of age to test triglycerides, cholesterol, and GIP (glucose-dependent insulintrophic polypeptide) concentrations. Results revealed more differences between age groups as opposed to between the two diet line groups suggesting that the age of the animal has more effect on the handling of a test meal challenge. Animals gestated on the fish oil diet also had higher oxidative stress, which continued into adulthood as measured by catalase (an endogenous antioxidant enzyme) activity.

While these experimental studies address the effects of fish oil diets during gestation, lactation, and early post-weaning life on adult metabolism, they do not account for other necessary fatty acids, specifically arachidonic acid, that when present with other PUFAs help to achieve metabolic homeostasis. Prenatal diets that are low in arachidonic acid lead to decreased fetal growth and development (Amusquivar et al., 2000). As previously mentioned, normal glucose and insulin homeostasis are thought to require both omega-3s from fish oils and omega-6s, like arachidonic acid dietary intake. An excess of one or the other is not optimal for fetal growth and development or for metabolic processes in adulthood. The ideal dietary concentrations appear to be reflected by the relative ratios of the fatty acids.

Researchers from the University of San Pablo in Spain analyzed the consequences of low arachidonic acid and compared the effects of fish oil versus olive oil during pregnancy and lactation on offspring development, fatty acid profile, and vitamin E concentration. Offspring whose mothers received the fish oil diet during pregnancy had a lower postnatal increase in body weight and body length, and delayed body and psychomotor maturation. These results were also observed in the pups gestated on olive

oil diet but cross fostered with a dam that received the fish oil diet during pregnancy. The fish oil fed group had higher levels of EPA and DHA in fetal plasma and liver contents and lower concentrations of arachidonic acid. Researchers suggested that the lower levels of arachidonic acid were responsible for the delayed growth (Amusquivar et al., 2000). The effects of the fish oils on these phenotypic traits may be related to dose. Fish oil was administered at 10% of the diet which is relatively high when compared to the high range of intake observed in humans.

Several other studies have addressed the effects of fish oils on rats rendered diabetic during pregnancy. Female rats were given a vegetable oil or EPAX diet (containing EPA and DHA -2.1% of the total diet) 15 days before mating and some of the female rats were subsequently made diabetic on the fifth day of pregnancy. All of the macrosomic offspring of diabetic dams were hyperglycemic at birth regardless of maternal diet. The n-3 diet did, however, significantly reduce the incidence of macrosomic pups, attenuated hyperlipidemia in macrosomic pups, and improved antioxidant status in both mothers and offspring. Oxidative stress has been associated with diabetic patients and infants from mothers with gestational diabetes which is, in part, thought to be the result of high blood glucose.

Postnatal High Fat Diets

Experimental human and animal research has shown that serum insulin levels can change as a result of diet during the course of a lifetime. More specifically, humans and rats that were otherwise normoglycemic and relatively insulin sensitive become insulin resistant and hyperinsulinaemic - when placed on a high saturated fat and high caloric diet as compared to diets that were low fat and complex in carbohydrates (Barnard et al.,

1998). Additionally, insulin sensitivity can be improved in insulin resistant, obese, type 2 diabetic subjects/rats through a hypocaloric high protein diet (Belobradjic 2004; Farnsworth et al., 2003; Piatti et al., 1994; Zhao et al., 2000; Boden et al., 2005). Interestingly, contemporary Yup'ik Eskimos whose diet is high in refined sugars and relatively higher in saturated fatty acids do not appear to be hyperinsulinaemic which suggests that some other variable, perhaps the n-3s from sea mammals and fish, is protective against glucose and/or insulin imbalances.

If nutrient composition and availability during gestation can program metabolism, and the ratio of omega-6/omega-3 in the diet can substantially affect components of lipid, glucose, and insulin metabolism then it is possible that a prenatal diet high in omega-3s could prevent particular imbalances in the offspring consuming a different post natal diet. The reason for the relatively healthy metabolic profile in Alaskan Yup'ik Eskimos may in part be attributed to their high intakes of omega-3s from fish oils. Not only are omega-3s beneficial for glucose and insulin homeostasis when a high caloric and saturated fat is consumed post-natally, but the nutrients themselves and the corresponding effects on the endocrine system *during pregnancy* may provide an added protective effect for the developing fetus even if a substantial amount of omega-3 PUFAs are not consumed in its post-natal life.

CHAPTER 5

METHODS

Experimental Animals and Protocol Description

Six adult female and two adult male Sprague-Dawley rats were obtained from Simonsen Laboratories, Inc. and housed in the University of Nevada, Las Vegas Animal Care Facility. Males and females were housed separately in plastic cages (2 females per cage, and one male per cage) and were maintained on a control chow diet for 10 days while acclimating to the new environment (Table 2). On the 10th day animals were randomly assigned to one of three test diets: “Alaskan”, “Western”, or Control. The Alaskan diet was custom formulated to model the ‘traditional’ Alaskan Yup’ik Eskimo dietary intakes. Western diets were formulated to model the highly processed, ‘fast food’ diet associated with the ‘nutritional transition’ around the world. Control diets were standard rat chows included for comparative purposes (See Table 3). Two females were placed on each of the test diets; males consumed the same diet as their female cage-mates.

Table 2. Experimental Design

Maternal Diet Lines:	F1 Generation: Post-weaningdiets	# of offspring
Alaskan	F1: Alaskan Western Transitional	(n = 5) (n = 5) (n = 5)
Western	F1: Alaskan Western Transitional	(n = 6) (n = 6) (n = 6)
Control	F1: Alaskan Western Transitional	(n = 6) (n = 6) (n = 6)

Table 3. Diet Composition

Component	Control	Alaskan	Transitional	Western
Calories provided by:				
Protein	28.507%	30.3%	16.9%	17.8%
Fat	13.496%	59.8%	33.9%	29.8%
Carbohydrate	57.996%	10.0%	49.2%	52.3%
P/S	1	2.01	1.25	.5
N6:N3	6.4	1.4	4	9.1

Females were maintained on their assigned diet for seven days. During this time, both food and water were supplied ad libitum. On day 17, males and females on the same diet were combined and transferred to larger plastic cages to begin breeding. The two dams consuming a Control diet were placed with a male breeder after the other 4 experimental dams became pregnant. Litter size was standardized for size and sex within

each prenatal diet group to nine pups per litter (Western prenatal: m=11, f=6; Control: m=7, f=11) using cross-fostering and culling techniques when the pups were 10 days of age. (The two Alaskan-Prenatal diet litters were ‘standardized’ to two litters consisting of 7 [m=5, f=2] and 8 [m=4, f=4] pups, respectively). Pups received ear tags immediately prior to weaning at 21 days and were randomly assigned to an Alaskan, Western, or Transitional (i.e., mixed Alaskan-Western) postnatal diets at weaning (Table 2). Both food and water were supplied ad libitum throughout the study. Weights were recorded approximately every three days for 120 days. On the 119th day animals were fasted overnight. The following morning animals were restrained in a breathable tube, and lidocaine was applied (approximately one cm in length) to the end of the tail. A very small piece of tail (~2mm) was cut off the end of the tail with a surgical scalpel and blood collected by “milking” the tail. 100ul of blood was collected in heparin coated capillary tubes for the glucose analysis, 600ul was collected in non-heparin capillary tubes for insulin analysis, and 2ul for HbA1c. Blood collected for insulin analysis was spun at 4000g at 4 degrees Celsius. Plasma was removed and transferred to Eppendorf tubes and stored at -40 degrees Celsius until insulin analysis was performed. The study was approved by the UNLV Institutional Animal Care and Use Committee (IACUC).

Diets

Experimental diets differed in macronutrient composition, fat sources, and ratios of polyunsaturated to saturated fatty acids (Table 3). While the obvious limitation of the diet compositions was the ability to exactly duplicate the fat sources characterizing the Yup’ik diets being modeled, careful consideration was made to choose sources that most closely approximated them. The Alaskan diet (Purina Testdiet, Greenfield, Indiana) differed slightly in mineral content for the breeders versus weanlings, but both contained

the same percentages of macronutrients, 30% protein, 60% fat, and 10% carbohydrate (Table 3). The Alaskan diet for breeders contained slightly more selenium, calcium, iron and zinc as recommended by the commercial vendor nutritionist. The sources of fat for the Alaskan diet were fish and soybean oil (Table 3). The Transitional diet (Purina Testdiet, Greenfield, Indiana) contained 33.9% fat, 16.9% protein, and 49.2% carbohydrate with a mix of lard, fish and soybean oil. A mix of corn, canola, soybean, safflower, coconut and fish oils, lard, beef tallow, milkfat, and cocoa butter were the fat sources for the Western diet (Harlan Teklad Madison, Wisconsin). All diets contained a standardized AIN-93 vitamin and mineral mixture except for the noted modification mentioned above. Western and Transitional diets were kept at 4°C, Control diets at room temperature, and Alaskan diets at -40°C. At the beginning of each week, a week's allotment of Alaskan food was transferred to 4°C refrigeration. To prevent oxidation of the Alaskan diet animals were provided with fresh food every two days. Animals receiving the Western and Transitional diets were supplied with fresh food ~every three days and animals consuming the standard diet had a constant supply of food.

Diets reflect traditional or Alaskan, Transitional, and Western intakes in terms of macronutrients (i.e. carbohydrate, protein, and fat) and fatty acid composition. The percentage of macronutrients used for the Alaskan diets are based on dietary surveys conducted in southwest Alaska during 1956-1961 (Heller and Scott 1967), 1978 (Knapp and Panruk 1978), and 1994 (Parkinson et al., 1994). Western intakes are based on the USDA's 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII). Polyunsaturated/saturated fatty acid and omega-6/omega-3 ratios in the two Yup'ik diets (Alaskan and Transitional) were adopted from data collected from a dietary survey

conducted in a coastal and inland village of southwest Alaska. Ratios for the Western diet are based on the USDA's 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII).

Biomarker analyses

HbA1c analysis was performed to determine average plasma glucose levels during the preceding six to eight weeks. HbA1c is reported as percent glycosylated hemoglobin. Blood collected for HbA1c was analyzed using the CLIA-waived Bayer DCA 2000 Analyzer. Glucose concentrations were measured using the CLIA-waived Abaxis Piccolo Blood Chemistry Analyzer. ZRT laboratories in Beaverton, Oregon analyzed insulin by ELISA.

Statistical Approach.

Differences between mean fasting glucose levels, HOMA (insulin resistance), HbA1c, and BMI were tested using a one-way ANOVA after testing for normality. Differences between mean fasting insulin levels were tested using Kruskal-Wallis. Any outliers beyond 2 standard deviations were excluded from analysis. A Levene's test for homogeneity was conducted to determine if the spreads or variances of the populations were approximately equal. LSD post hoc tests were performed to determine which groups differed from each other. SPSS 16.0 software was used to analyze the data. Data are reported as means \pm SD. Statistical significance is defined as $p < .05$ with higher significance noted.

CHAPTER 6

RESULTS

Analysis of Data

Using an experimental animal model, this study aimed to explore the potential protective metabolic effects of a prenatal diet modeled on traditional Yup'ik nutritional intakes, on blood glucose, insulin, and lipid levels in adult offspring.

Effects of an Alaskan Prenatal Diet on Insulin Sensitivity

Among rats consuming a Western diet postweaning, those whose mothers consumed an Alaskan diet during pregnancy and nursing, were significantly less insulin resistant (i.e. more insulin sensitive) as measured by Homeostatic Model of Insulin Resistance (HOMA) ($p < .001$) than animals whose mothers consumed a Western diet during pregnancy and while nursing (Figure 1).

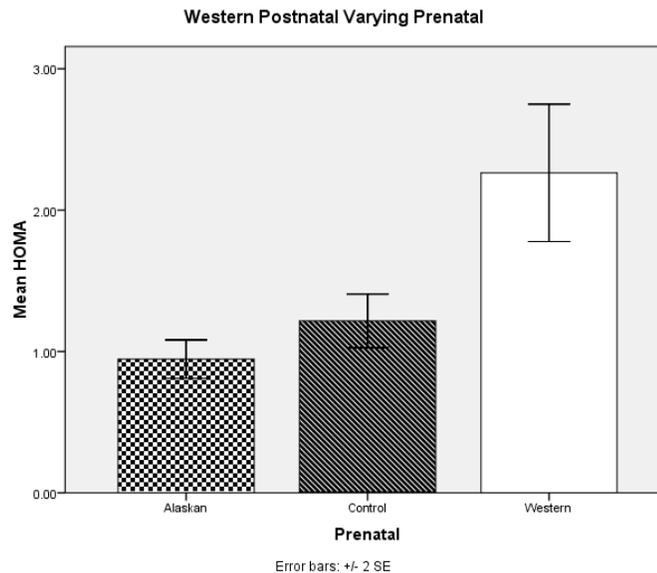


Figure 1. Insulin Resistance as Measured by HOMA at 120 days.

Table 4 shows the details of the metabolic markers of offspring who consumed a Western diet postweaning, but whose mothers consumed an Alaskan, Western, or Control diet during pregnancy.

Table 4. Metabolic Markers for Males and Females. Western Postweaning - Varying Prenatal Diets

	Alaskan-Western (a)	Western-Western (b)	Control-Western (c)	P
HbA1c	3.44 ± .09 (5)	3.37 ± .10 (6)	3.25 ± .05 (6)	
Glucose mmol	7.82 ± .31 (5)	8.06 ± .99 (6)	8.39 ± 1.15 (6)	
Insulin pmol	18.94 ± 2.25 (4)	41.64 ± 6.93 (5)	24.65 ± 9.89 (6)	*
HOMA	.95 ± .14 (4)	1.98 ± .76 (5)	1.22 ± .23 (6)	** (a)(b), ** (b)(c)
G/I	.41 ± .04 (4)	.18 ± .01 (4)	.41 ± .10 (6)	** (a)(b), ** (b)(c)
BMI	.60 ± .08 (5)	.60 ± .09 (6)	.54 ± .06 (6)	
Triglycerides	56.20 ± 13.29 (5)	71.17 ± 17.15 (6)	68.67 ± 13.75 (6)	

Values are means + SD with the number of rats given in parentheses

* Diet lines significantly differ p<.05

** Diet lines significantly differ p<.01

Among animals that consumed a Transitional diet postweaning, those whose mothers consumed an Alaskan diet during pregnancy and nursing were significantly (p=.01) more insulin sensitive, as measured by the glucose to insulin ratio than those whose mothers were fed a Western diet during pregnancy and nursing (Figure 2).

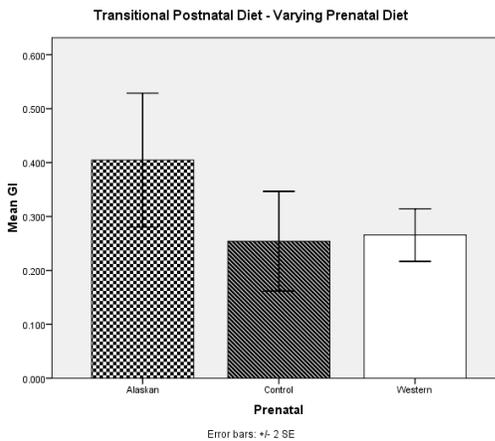


Figure 2. Insulin Sensitivity as Measured by Glucose mmol/Insulin pmol at 120 days.

Table 5 shows the details of the metabolic markers of offspring who consumed a Transitional diet postweaning, but whose mothers consumed an Alaskan, Western, or Control diet during pregnancy.

Table 5. Metabolic Markers for Males and Females. Transitional Postweaning - Varying Prenatal Diets

	Alaskan-Transitional (a)	Western-Transitional (b)	Control-Transitional (c)	P
HbA1c	3.46 ± .11 (5)	3.42 ± .12 (6)	3.32 ± .10 (6)	
Glucose mmol	8.46 ± .47 (5)	8.29 ± 11.61 (6)	8.04 ± .65 (6)	
Insulin pmol	23.44 ± 11.24 (4)	33.03 ± 10.40 (6)	36.03 ± 12.98 (6)	
HOMA	1.29 ± .63 (4)	1.81 ± .77 (6)	2.02 ± .74 (5)	
G/I	.40 ± .12 (4)	.27 ± .06 (6)	.25 ± .13 (5)	*(a)(b), *(a)(c)
BMI	.60 ± .08 (5)	.60 ± .09 (6)	.54 ± .06 (6)	
Triglycerides	65.80 ± 18.75 (5)	64.67 ± 8.64 (6)	53.00 ± 12.32 (6)	

Values are means + SD with the number of rats given in parentheses

* Diet lines significantly differ p<.05

** Diet lines significantly differ p<.01

Effects of a Western Prenatal Diet on Insulin Resistance

Among animals consuming an Alaskan diet postweaning, those whose mothers consumed a Western diet prenatally were significantly more insulin resistant than animals whose mothers consumed an Alaskan diet during pregnancy and nursing (Figure 3).

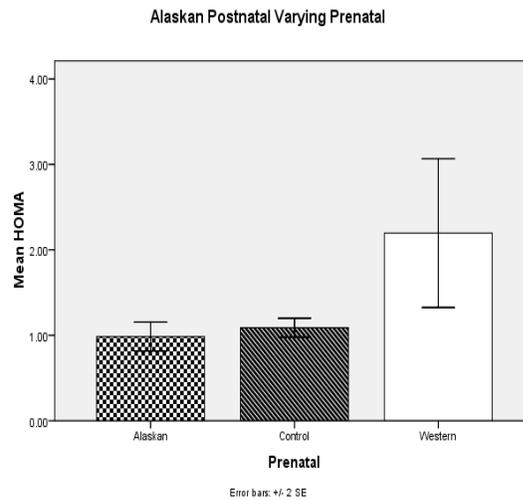


Figure 3. Insulin Resistance as Measured by HOMA at 120 days.

Table 6 shows the details of the metabolic markers of offspring who consumed an Alaskan diet postweaning, but whose mothers consumed an Alaskan, Western, or Control diet during pregnancy.

Table 6. Metabolic Markers for Males and Females. Alaskan Postweaning - Varying Prenatal Diets.

	Alaskan-Alaskan (a)	Western-Alaskan (b)	Control-Alaskan (c)	P
HbA1c	3.40 ± .19 (5)	3.33 ± .08 (6)	3.33 ± .21 (6)	
Glucosemmol	8.56 ± 1.30 (5)	9.23 ± .49 (5)	8.57 ± .91 (6)	
Insulinpmol	20.58 ± 6.18 (5)	33.18 ± 1.47 (4)	20.05 ± 3.1 (5)	
HOMA	.99 ± .17 (4)	2.20 ± .75 (3)	1.09 ± .12 (5)	** (a)(b), ** (b)(c)
G/I	.44 ± .12 (5)	.25 ± .07 (3)	.44 ± .10 (5)	** (a)(b), ** (b)(c)
BMI	.61 ± .08 (5)	.60 ± .08 (6)	.58 ± .05 (6)	
Triglycerides	58.25 ± 13.22 (5)	54.00 ± 8.67 (6)	56.83 ± 16.70 (6)	

Values are means ± SD with the number of rats given in parentheses

* Diet lines significantly differ p<.05

** Diet lines significantly differ p<.01

Lipids

Mean circulating triglycerides among animals who consumed Western diets post weaning, but whose mothers were fed an Alaskan diet while pregnant and nursing (56.2 mg/dL) were 21 percent lower than those offspring whose mothers also consumed the Western diet during pregnancy and nursing (71.2 mg/dL). Compared to the same Western prenatal/Western post-weaning diet animals (71.2 mg/dL), mean triglycerides were 18 percent lower among offspring whose mothers consumed an Alaskan diet during pregnancy and nursing, and who also consumed an Alaskan diet post weaning (58.2 mg/dL). Neither of these differences reached the .05 level of statistical significance, however.

Body weight

There were no significant differences in body weights on day 7 among offspring whose mother consumed an Alaskan, Western, or Control diet during pregnancy and nursing. By day 21, offspring whose mothers consumed an Alaskan diet during pregnancy and nursing were significantly ($p < .01$) heavier compared to offspring whose mother consumed either a Western or Control diet during pregnancy and nursing (Figure 4).

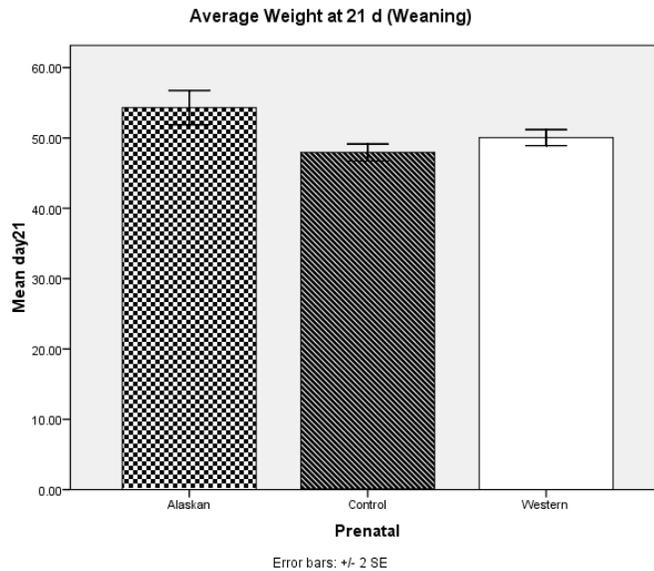


Figure 4. Average Body Weight at 21 d (Weaning) among Offspring of Mothers Consuming Alaskan, Western, and Control Diets during Pregnancy/Nursing

Table 7 shows the average body weights at 7 and 21 days among offspring whose mothers consumed an Alaskan, Western, or Control diet during pregnancy and nursing.

Table 7. Average Weight at 7 d, and 21 d (Weaning) for Males and Females among Offspring of Mothers Consuming an Alaskan, Western, or Control diet during Pregnancy/Nursing

	Alaskan	Western	Control	P
Day				
7	16.8 ± 4.37(15)	16.44 ± 2.03 (18)	17.2 ± .81(18)	
21	54.31 ± 4.72 (15)	50.04 ± 2.41 (18)	47.93 ± 2.58 (18)	** (a)(b), ** (a)(c)

Values are means ± SD with the number of rats given in parentheses

* Diet lines significantly differ p<.05

** Diet lines significantly differ p<.01

The differences observed in body weights at day 21 washed out by 25 days and older.

Relative growth over time (i.e. at 25 days and older) was similar among all three

postnatal diet groups (Figures 5, 6, and 7). Table 8 shows the average body weights

among offspring 25-120 days old that consumed an Alaskan, Western, or Transitional

postweaning diets whose mothers consumed varying prenatal diets.

Table 8. Body Weights for Offspring from 25 d to 120 d for Males and Females by Alaskan , Western and Transitional Postweaning - Varying Prenatal Diets

	Alaskan-Alaskan (a)	Western-Alaskan (b)	Control-Alaskan (c)	P
Day				
25	63.24 ± 4.78 (5)	66.35 ± 4.69 (6)	63.62 ± 2.90 (6)	
49	196.76 ± 34.86 (5)	181.95 ± 21.77 (6)	175.1 ± 18.88 (6)	
63	259.2 ± 66.14 (5)	229.52 ± 39.80 (6)	244.85 ± 55.42 (6)	
70	273.92 ± 69.74 (5)	252.00 ± 50.62 (6)	263.57 ± 57.03 (6)	
81	312.74 ± 92.80 (5)	280.00 ± 56.86 (6)	286.27 ± 68.19 (6)	
109	353.34 ± 107.96 (5)	329.6 ± 73.29 (6)	322.98 ± 80.05 (6)	
120	353.78 ± 105.85 (5)	339.52 ± 75.62 (6)	337.67 ± 75.20 (6)	
	Alaskan-Western (a)	Western-Western (b)	Control-Western (c)	P
25	64.94 ± 9.58 (5)	66.55 ± 8.13 (6)	62.78 ± 3.18 (6)	
49	207.28 ± 31.1 (5)	207.28 ± 40.33 (6)	188.95 ± 31.1 (6)	
63	267.72 ± 72.1 (5)	265.45 ± 62.76 (6)	236.18 ± 52.07 (6)	
70	291.34 ± 80.61 (5)	284.95 ± 65.91 (6)	250.08 ± 58.66 (6)	
81	316.56 ± 90.27 (5)	311.85 ± 72.07 (6)	270.18 ± 69.51 (6)	
109	351.14 ± 104.34 (5)	362.35 ± 77.73 (6)	295.62 ± 85.43 (6)	
120	354.34 ± 105.69 (5)	365.38 ± 84.14 (6)	303.48 ± 91.44 (6)	
	Alaskan –Transitional (a)	Western-Transitional (b)	Control-Transitional (c)	P
25	63.16 ± 5.58 (5)	66.55 ± 6.24 (6)	68.60 ± 3.80 (6)	
49	197.90 ± 27.23 (5)	201.00 ± 35.35 (6)	204.28 ± 34.01 (6)	
63	261.24 ± 57.75 (5)	269.35 ± 57.23 (6)	263.65 ± 51.09 (6)	
70	284.70 ± 67.51 (5)	288.20 ± 63.47 (6)	283.83 ± 56.97 (6)	
81	310.04 ± 75.46 (5)	323.75 ± 73.79 (6)	307.90 ± 60.47 (6)	
109	348.60 ± 92.46 (5)	381.68 ± 89.25 (6)	336.50 ± 76.26 (6)	
120	347.88 ± 97.14 (5)	388.15 ± 92.78 (6)	347.88 ± 80.70 (6)	

Values are means + SD with the number of rats given in parentheses

* Diet lines significantly differ p<.05

** Diet lines significantly differ p<.01

Figures 5, 6, and 7 show growth over time after weaning among offspring consuming an Alaskan, Western, or Transitional postweaning diets whose mothers consumed varying prenatal diets.

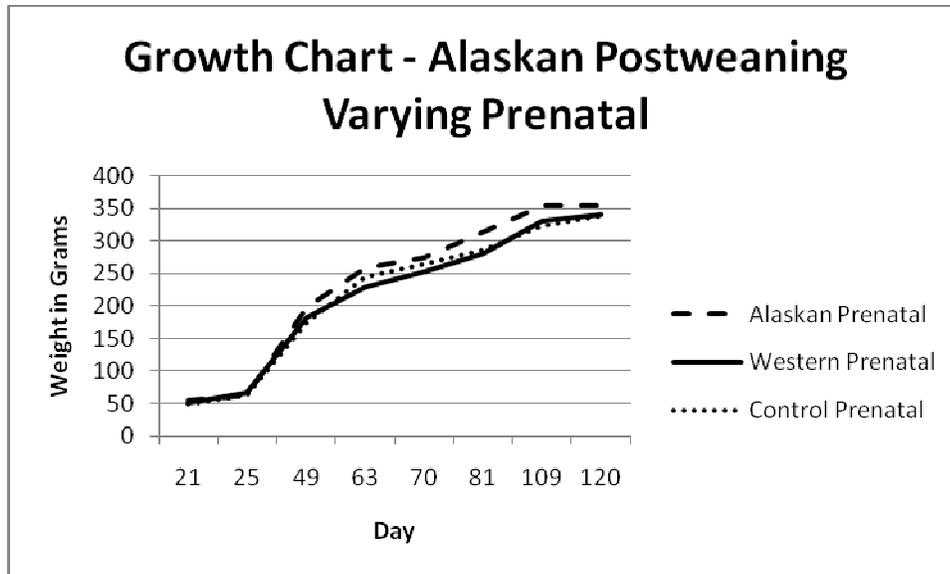


Figure 5. Growth Over Time among Males and Females Consuming an Alaskan Postweaning Diet.

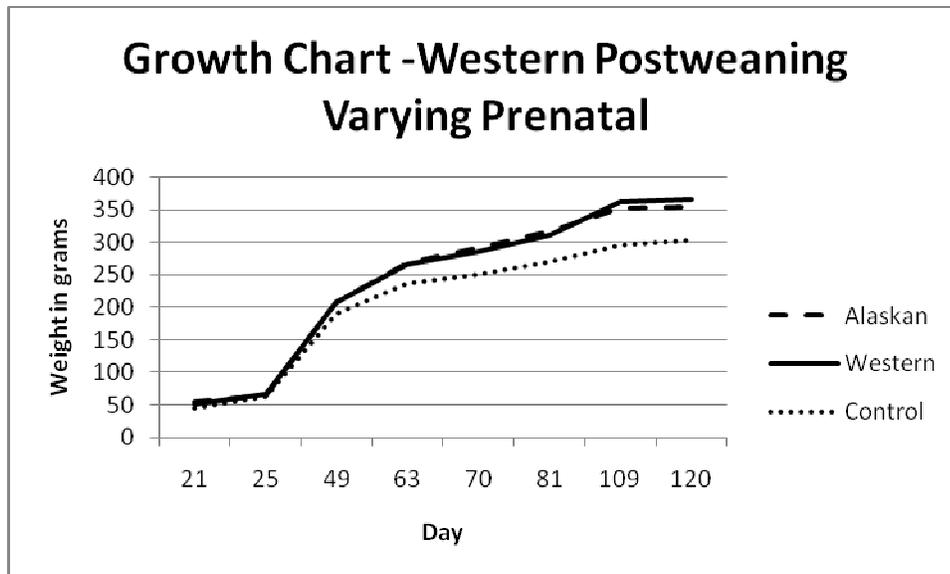


Figure 6. Growth Over Time among Males and Females Consuming a Western Postweaning diet.

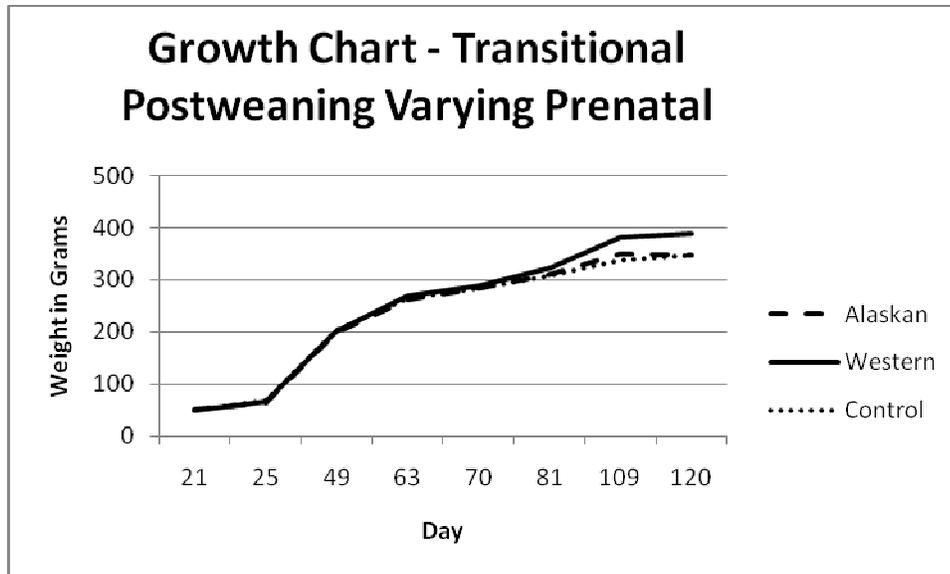


Figure 7. Growth Over Time among Males and Females Consuming a Transitional Postweaning Diet.

CHAPTER 7

DISCUSSION AND CONCLUSIONS

Discussion of Results

Maternal Diet and Insulin Resistance

Previous experimental animal studies have demonstrated that the adult offspring of mothers who consume a diet high in saturated fat during pregnancy and lactation, possess high levels of fasting insulin and are insulin resistant (Liang et al., 2009). The present study is consistent with this previous research in that adult offspring that consumed an Alaskan diet postweaning and whose mothers consumed a high (saturated) fat Western diet during pregnancy, were also insulin resistant. This suggests that even diets known to improve metabolic health in adulthood (i.e., the Alaskan diet in the present study), was unable to compensate for the developmentally programmed effects of the high-saturated fat, prenatal Western diet.

Maternal Diet and Insulin Sensitivity

This present study also suggests that offspring insulin sensitivity in adulthood may be improved by a prenatal diet that is high in omega-3 PUFAs similar to that found in the traditional Yup'ik diet. It is important to note that in the present animal study, the adult offspring whose mothers consumed Alaskan diets during pregnancy and lactation (which contained relatively *higher* percentages of fat compared to the Western diet) were not more insulin resistant than adult offspring whose mothers consumed a Western diet during pregnancy. This suggests that although the Alaskan diet was even higher in overall fat content, the type of fat it contained in the Alaskan diet (i.e., higher amounts of omega-3s from fish oils), was a critical factor in the developmental programming of

insulin sensitivity. This programming effect, likely as a result of the higher amounts of omega-3s might, in part, be responsible for maintaining blood glucose and insulin homeostasis in offspring consuming a different postweaning diet. That is, even if a postweaning diet contains higher amounts of saturated and omega-6 fats, as in the Western diet, the adult offspring are ‘protected’ from the deleterious metabolic consequences (i.e., hyperglycemia, hyperinsulinemia) generally associated with a high saturated fat diet due to the relatively higher amounts of omega-3 PUFAs received during gestation.

Findings from the present study did show a slight difference (although not statistically significant) in insulin sensitivity between adult offspring that consumed an Alaskan diet postweaning and whose mothers that consumed either an Alaskan diet or Control diet during pregnancy and lactation. The Alaskan and Control diets mirrored one another in terms of relatively high protein percentages (~30%) however, and both diets were also higher in P/S fatty acids and lower in omega-6/omega-3 PUFAs compared to the Western diet. It is possible, therefore, that the ‘protective’ effect on adult offspring insulin sensitivity observed in the Alaskan and Control prenatal diets are related to a diet that is high in omega-3s *and* protein. These effects might also be the result of some ‘threshold’ effect related to fatty acid ratios provided by the maternal diet during pregnancy and lactation. That is, once saturated fats and/or omega-6 PUFAs reach a certain percentage relative to PUFAs or omega-3s, glucose and insulin metabolic programming may shift towards insulin resistance. In fact, it has been demonstrated that a high saturated fat ‘junk food’ diet consumed during pregnancy leads to insulin resistance in adult offspring, despite postweaning diet (Liang et al., 2009).

Interestingly, the Control and Western diets also contained similar amounts of carbohydrates. Thus, mothers consuming a Control or Western diet during pregnancy might have relatively higher amounts of circulating blood glucose (to which the developing fetus is exposed) than mothers consuming an Alaskan diet during pregnancy. Previous research has shown when maternal blood sugars are high during pregnancy, insulin resistance is likely to be developmentally programmed in their adult offspring. Alternatively, if pregnant females consuming the Alaskan diet during pregnancy maintained lower (but adequate) blood sugars (without suffering any nutritional deficiencies) other animal research has shown that their offspring would be much less likely to develop insulin resistance in adulthood (Benyshek et al., 2007).

While these results do suggest that adult offspring whose mothers consumed Control diets during pregnancy were relatively insulin sensitive, they do not suggest that adult offspring whose mothers consumed Western diets were insulin sensitive. In fact, the adult offspring whose mothers received a Western diet during pregnancy were insulin resistant, regardless of postweaning diet. From a ‘thrifty-genotype’ inspired evolutionary perspective, one might expect that a low carbohydrate prenatal diet (e.g., Alaskan diet) would develop insulin resistance, as this would program a glucose-conserving metabolism that would ensure a sufficient supply of glucose (the brain’s sole energy source) in carbohydrate-poor environments (Ritenbaugh and Goodby 1989). Interestingly, results from the current study show the opposite effect. Offspring whose mothers received the Alaskan diet prenatally were relatively insulin sensitive.

Other research, however, has shown that the second generation (F2) adult offspring consuming an energy restricted diet postnatally whose mothers also consumed an energy

restricted diet during pregnancy and lactation, were insulin resistant (Benyshek et al., 2008). Given the findings in the present study, perhaps this means there is a *narrow range of maternal blood glucose* that programs for relative insulin sensitivity. If maternal glucose is too low and glucose-conserving, insulin resistance is developmentally programmed [i.e., the ‘famine’ pathway]. If, on the other hand, maternal glucose is too high, then developmentally programmed metabolic traits that ultimately lead to insulin resistance, T2D in adulthood, although via a separate route [the ‘feast’ pathway], occurs.

It is also possible that other developmental factors are contributing to these observed effects. Perhaps offspring whose mothers are receiving a low carbohydrate (i.e., Alaskan) prenatal diet are developmentally programmed to optimize gluconeogenesis (the conversion of protein to glucose signaled by the release of glucagon), rather than insulin resistance. If gluconeogenesis is developmentally optimized, then it is possible that offspring whose mothers are consuming an Alaskan diet during pregnancy are especially efficient at converting protein to glucose. If these same offspring are also consuming a (high fat, high carbohydrate) Western diet postweaning, the blood sugars in these animals should be especially high. Under these circumstances, blood glucose (and insulin resistance) should have been highest in this study’s Alaskan prenatal-Western postweaning animals. As a result, it is likely that some other factor (e.g., high amounts of prenatal dietary omega-3s, or overall higher prenatal dietary PUFAs, or an optimal maternal blood glucose level during gestation) is primarily responsible for the observed effect of developmentally programmed insulin sensitivity.

Body Weights

Similar to what has been demonstrated in other experimental animals studies, the current study suggests that prenatal diet significantly affects growth during the perinatal period, but that postweaning diet does not significantly affect growth after weaning. This is because rodents seem to have a particularly robust body weight set-point that resists efforts of researchers to intentionally overfeed. It is not known from this study, however, at what developmental stage prenatal diet affects perinatal growth as body weights were not significantly different on day 7. By day 21 pups whose mothers consumed an Alaskan diet were significantly heavier than pups whose mothers consumed either a Control or a Western diet. Interestingly, these results are inconsistent with other studies that have examined the effects of prenatal diets that are high in omega-3s on body weight. These studies have generally found that a high omega-3 diet consumed during pregnancy leads to offspring that are lower in weight compared cohorts whose mothers consume a high omega-6 diet. This suggests that some other component of an Alaskan diet might be contributing to growth differences during the perinatal period and/or that, once again, there are relatively narrow ranges of dietary intakes, in this case PUFA intakes, that program for postnatal growth trajectories.

Conclusions

The Traditional Yup'ik Diet and the Developmental Programming of Insulin Sensitivity

While other experimental animals studies have modeled dietary transitions that have occurred in high T2D prevalence populations, and thereby provided support for a developmental origins model of the disease in these populations (Martin et al., 1999;

Benyshek et al., 2007) the current study modeled the dietary continuity and the slower nutritional transition occurring that has occurred in a unique low prevalence population to understand how dietary processes occurring *in utero* might protect the adult offspring from the metabolic consequences associated with high fat Western diets and obesogenic lifestyles.

The results of this study have helped to illuminate what may be part of the reason for current health paradox among contemporary Yupiit of southwestern Alaska. This study suggests that some dietary component(s) of a traditional Yup'ik diet consumed during pregnancy and lactation, confers a protective effect on glucose and insulin metabolism when a high saturated fat, Western diet is consumed postweaning. It is possible that the low prevalence of T2D and Metabolic Syndrome among Yupiit can be explained by the continued reliance on traditional subsistence items in their current diets.

Ideas for Future Research

As evidence for the developmental origins of health and disease continues to gain strength, it becomes imperative to further refine the role that various early life exposures and conditions, interacting at different stages of development, play in the development of chronic disease – especially those in which insulin resistance has been identified as the linchpin. There are many possibilities for future experimental animal studies that might address such pressing concerns. One possibility is to use the same diet lines that were created in the current study, but to then cross foster (diet lines) at birth to determine the specific role the nursing period has on glucose, insulin, and lipid metabolism. It is also important to understand what kind of multigenerational protective effects insulin-sensitizing prenatal diets might have, especially among animals consuming high saturated

fat Western diets. In other words, to what extent do insulin-sensitizing prenatal diets protect against insulin resistance in future generations, even if they continue to consume a high fat - Western diet.

Considering that this study found a potential T2D ‘protective’ (i.e., insulin-sensitizing) effect on glucose and insulin metabolism when mothers consumed Yup’ik modeled Alaskan diet during pregnancy and nursing, the results raise another question. Does the timing matter? Does an Alaskan diet consumed during the first, second, or third trimester of pregnancy have differential effects on the developmental programming of insulin metabolism in adult offspring?

Education, Prevention, and Intervention

Western culture today plays a predominant role in shaping/changing diet and lifestyle in developing and developed countries around the world. This diffusion of cultural components (i.e., clothing, diet, leisure activities) can occur at a rapid pace and pose significant consequences for other societies, especially in economically developing countries. Those poverty stricken communities, which are less likely to have access to health care and nutritionally dense foods, and health promoting resources, are at increased risk for both the development of T2D (as a result of their own personal prenatal history) and perpetuation of T2D. As Western diet and lifestyles continue to influence countries around the world, the need for healthcare management and especially effective disease prevention programs in these countries becomes crucial.

While other studies have sought to understand how T2D and other obesity related disorders are transmitted across generations, this study sought to shed light on how prenatal diets might protect against the development of metabolic traits associated with

these diseases. While the results of the current study are preliminary and should be interpreted with caution, the current study suggests that carefully designed and monitored dietary interventions during the prenatal period may be able to offer significant protection to offspring from metabolic disorders in adult life.

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Publications:

Brendan M. Giles, Julie J. Kachinski, Svetlana N. Tchepeleva, Byron P. Croker, Laurence Morel, Susan A. Boackle. Augmentation of NZB Autoimmune Phenotypes by the *Sle1c* Murine Lupus Susceptibility Interval. *Journal of Immunology* 2007 Apr1;178(7) 4667-75.

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