EVALUATING THE IMPACT OF DIETARY MACRONUTRIENT RATIOS ON CARDIOVASCULAR BLOOD BIOMARKERS

By

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EVALUATING THE IMPACT OF DIETARY MACRONUTRIENT RATIOS

ON

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ABSTRACT

Background: Cardiovascular disease is the number one killer in the United States leading to 647,000 deaths in 2017 alone. Not restricted to the United States, cardiovascular disease is also the number one cause of death worldwide accounting for 9 million annual deaths. The prevention of cardiovascular disease presents not only an opportunity to save lives, but also billions of dollars in healthcare costs. Lifestyle modification through diet presents an effective means of reducing the incidence of cardiovascular morbidity and mortality. Dietary approaches aimed at reducing cardiovascular risk have focused on the reduction of saturated fat limiting its potential for negatively impacting cardiovascular risk blood parameters. Recent evidence suggests, however, that while reducing saturated fat may be of benefit, the replacement nutrient selected is an equally important consideration. Dietary approaches aimed at replacing saturated fat with mono and polyunsaturated fat have shown promise as a means of improving cardiovascular biomarkers and possibly reducing the mortality and morbidity of cardiovascular disease.

Objective: The objective of this study is to determine the associations between macronutrient ratios and cardiovascular lipid biomarkers and try to determine the appropriate recommendations for beneficial biomarker outcomes.

Methods: This retrospective correlational analysis used data obtained from the 2005-2016 NHANES dataset. Data included nutritional variables, patient demographic information, and patient laboratory examinations. After cleaning and processing correlational analysis, simple and multivariate regression analysis, and ANOVA testing were performed between the independent nutritional variables and the dependent laboratory variables.

Results: There were a total of 20,007 cases to be considered from the data set. 3089 samples met the inclusion criteria for further consideration. Statistically significant correlations were noted between the percent of the polyunsaturated to saturated fat ratio and total cholesterol (r=-.055), LDL cholesterol (r=-.038), HDL cholesterol (r=.043), triglycerides (r=-.08), apolipoprotein B (r=-.065), and the total cholesterol to HDL ratio (r=-.08). Statistically significant correlations were noted between the percent of carbohydrate consumption and total cholesterol (r=-.045), HDL cholesterol (r=-.10), and the total cholesterol to HDL ratio (r=.04). Statistically significant correlations were noted between the percent of sugar consumption and HDL cholesterol (r=-.11), and the total cholesterol to HDL ratio (r=.074). Statistically significant correlations were noted between the percent of fiber consumption and HDL cholesterol (r=.061), and the total

cholesterol to HDL ratio (r=-.059). Statistically significant correlations were noted between the carbohydrate to total fat consumption and HDL cholesterol (r=-.070), triglycerides (r=.041), and the total cholesterol to HDL ratio (r=.044). ANOVA analysis showed a statistically significant impact of differing ratios of polyunsaturated to saturated fatty acids and total cholesterol (p=.0076), triglycerides (p=.0002), apolipoprotein B (p=.0042), and the total cholesterol to HDL ratio (p=.0005). Statistically significance was seen between the impact of differing ratios of monounsaturated to saturated fatty acids and triglycerides (p=.0015). Significant impact of differing ratios of carbohydrate to total fat and HDL cholesterol (p=.0035). Statistically significant impact of differing dietary fat concentrations HDL cholesterol (p<.0001).

Conclusions: Some, but not all, of the ratios suggested by this study were consistent with the expectations following the review of literature. No significant relationship exists between saturated fat and cardiovascular biomarker data. The ratio of polyunsaturated to saturated fat showed a relationship with positive cardiovascular biomarker outcomes. Diets should follow a >1.0 polyunsaturated to saturated fat ratio. There is a small inverse relationship between the ratio of monounsaturated fat to saturated fat and diet should be kept to a < 2.0 ratio. The ratio of carbohydrate consumption to saturated fat shows a small positive correlation. The ratio of carbohydrate to total fat shows a strong association between higher ratios and negative biomarker data. A diet should be less than a 1.5 ratio of carbohydrate to total fat and contain 45% or less of its calories from carbohydrates. Differences between biomarker outcome data and individual fatty acids suggest that food choice needs to consider specific fatty acid makeup.

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It has been through the continued support from others that I have been able to pursue my PhD in Biomedical Informatics and complete this dissertation. I would therefore like to acknowledge the following:

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Dedication

This dissertation is dedicated to my wife Katie, daughters (Samantha, Nicole, and Vivian), my grandsons Jacob and Charles and the rest of my family. I would not have been able to pursue and finish my doctoral studies without their understanding and support. They provide me with the motivation to persevere when things get rough. Their contributions to this dissertation and my life are incalculable. Thank you and I love you.

Table of Contents

Table of Figures

CHAPTER I

INTRODUCTION

Statement of the Problem

Cardiovascular disease (CVD) and the concomitant related conditions are the number one cause of death both in the United States and worldwide. In 2016, heart disease lead to approximately 165.5 deaths per 100,000 members of the American population (see Figure 1) and accounted for approximately 647,000 deaths per year in 2017, this equates to a quarter of all deaths in the US with at least one death per minute related to heart disease. More than half of the deaths due to heart disease in the US are in men and heart disease is the leading cause of death for African Americans, Hispanics, and Caucasians. [1, 2]

Figure 1-Age-adjusted death rates for the 10 leading causes of death in 2016: United States, 2015 and 2016
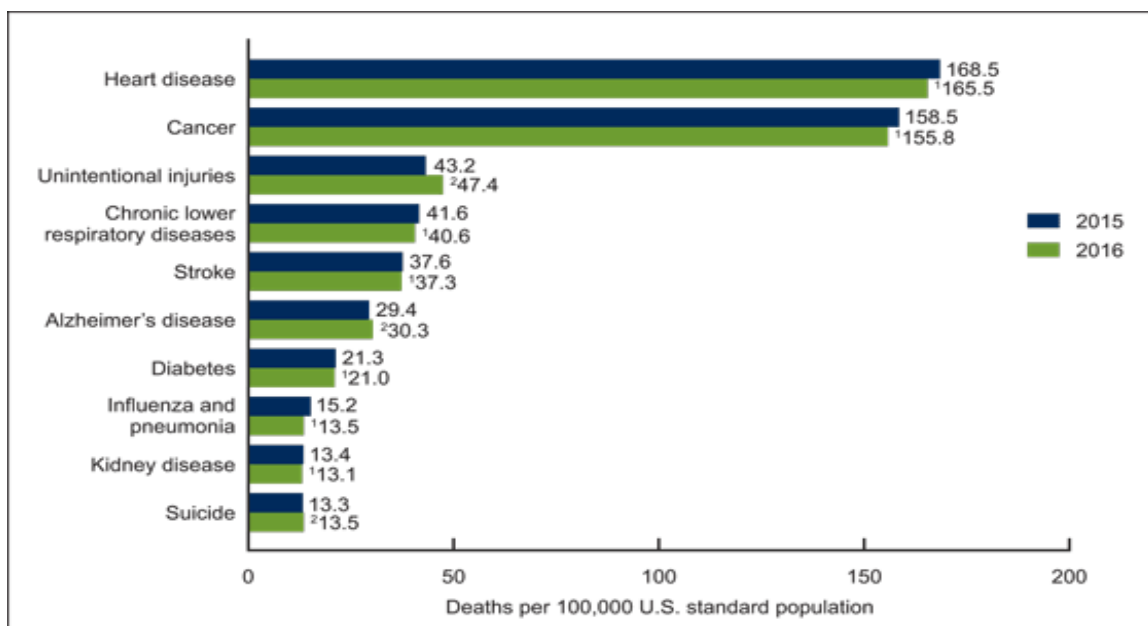
Source: CDC - National Center for Health Statistics

Heart disease is not unique to the United States however. According to the World

Health Organization (WHO), ischemic heart disease is the leading cause of death

worldwide with over 9 million deaths annually (see Figure 2). [3] Indicating that this

problem, although pervasive in the US, is a worldwide public health concern.

Additionally, the worldwide impact of heart disease has grown in the period of 2000-

2016 indicating an acceleration of this health issue (see Figure 3). [4] Heart disease, as a

whole, is a multifactorial problem with a variety of contributing risk factors ranging from

genetic predisposition to modifiable risk factors, such as smoking, inactivity, and diet.

According to the CDC, it is through affecting these modifiable risk factors that the

greatest impact on heart disease can be seen. [2]

Figure 2-Top 10 Global Causes of Death 2016
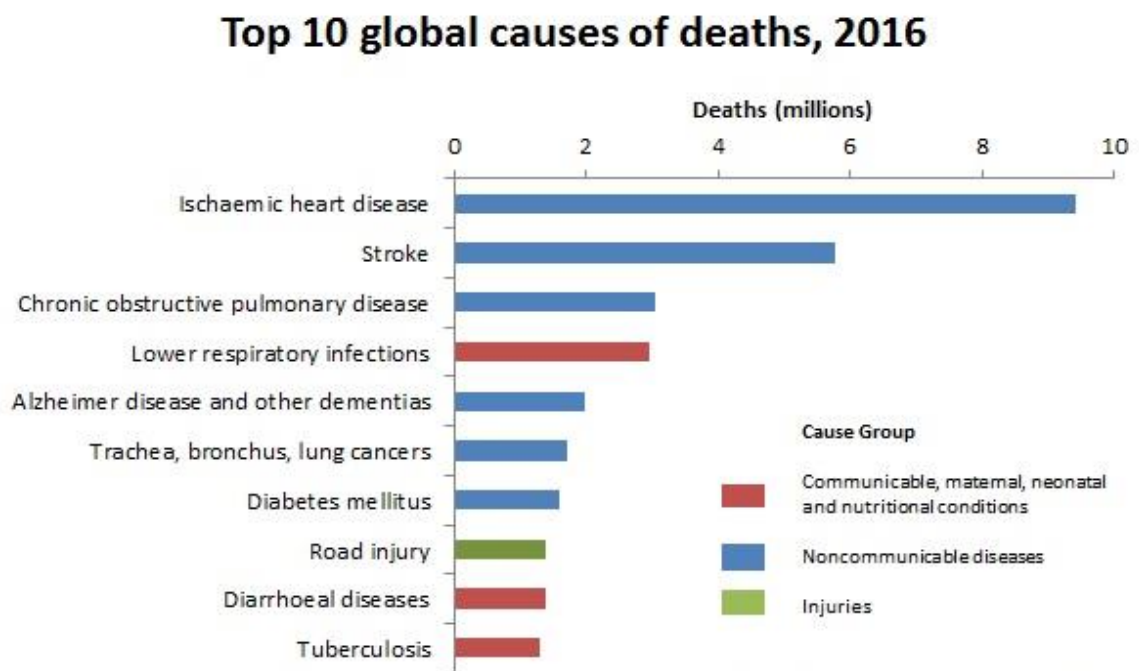
Source: Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva, World Health Organization; 2018.

Figure 3-**Top 10 Global Causes of Death 2000**

Top 10 global causes of deaths, 2000

Source: Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva, World Health Organization; 2018.
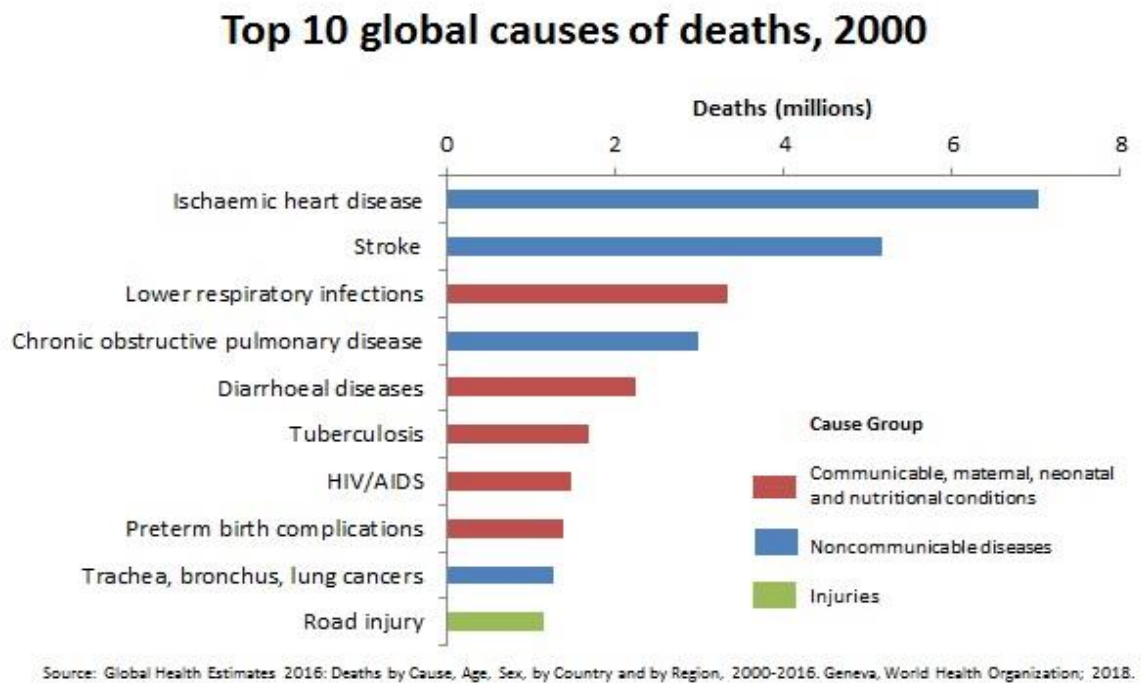
The impact of this increasing rate of heart disease comes with an increase in costs, beyond the human cost seen with the elevation in mortality. The costs to treat heart disease are, in many ways, directly proportional to the incidence of heart disease, with increased healthcare costs, for those treated for heart disease, at the highest in states with the highest diagnoses for heart disease (see Figures 4 and 5). [5, 6] Additionally, conditions related to heart disease account for 200 billion dollars in increased healthcare spending annually. [2] This number includes both the costs for treatment and reduced workforce capacity.

Figure 4-2015 Cost of Care Per Capita

Cost of Care per Capita for Medicare Beneficiaries*
Diagnosed with Heart Disease, 2015: Total Costs

Costs, $
- $3,153-$15,987
- $15,988-$17,843
- $17,844-$19,452
- $19,453-$21,565
- $21,566-$44,631
- Insufficient Data

National Mean = $18,917
National Median = $18,767

*Enrolled in fee–for–service Medicare.
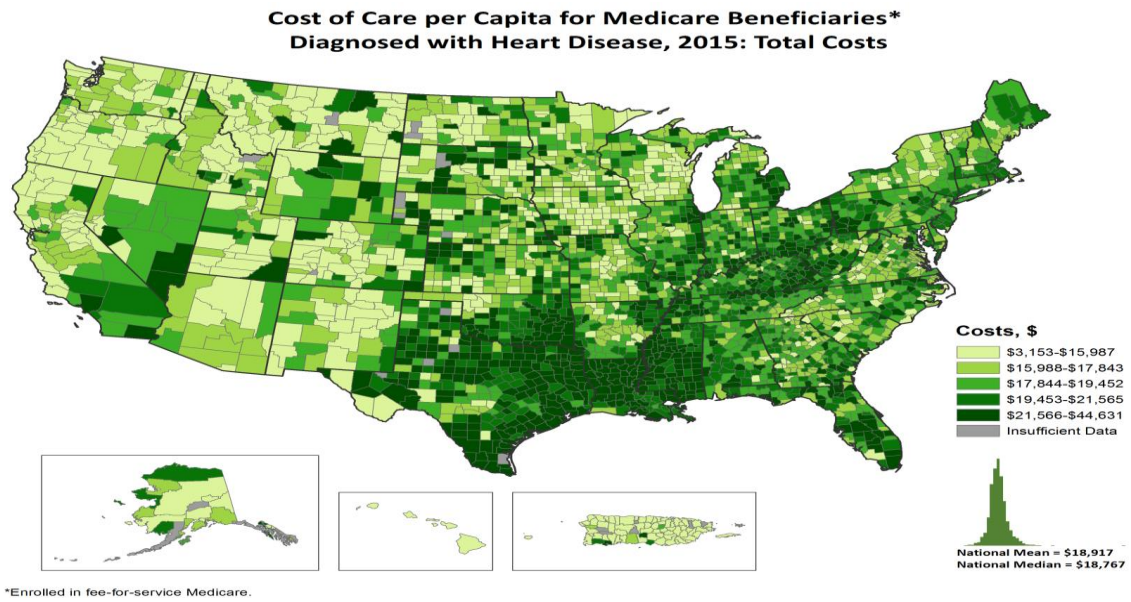
Source: CDC - National Center for Health Statistics

Figure 5-Heart Disease Death Rates 2014-2016

Heart Disease Death Rates, 2014-2016
Adults, Ages 65 +, by County

Age-Adjusted
Average Annual
Rates per 100,000
- 353.8 - 941.9
- 942.0 - 1055.6
- 1055.7 - 1172.0
- 1172.1 - 1328.0
- 1328.1 - 3665.2
- Insufficient Data

Rates are spatially smoothed to enhance
the stability of rates in counties with small
populations.

Data Source:
National Vital Statistics System
National Center for Health Statistics

www.cdc.gov/dhdsp/maps
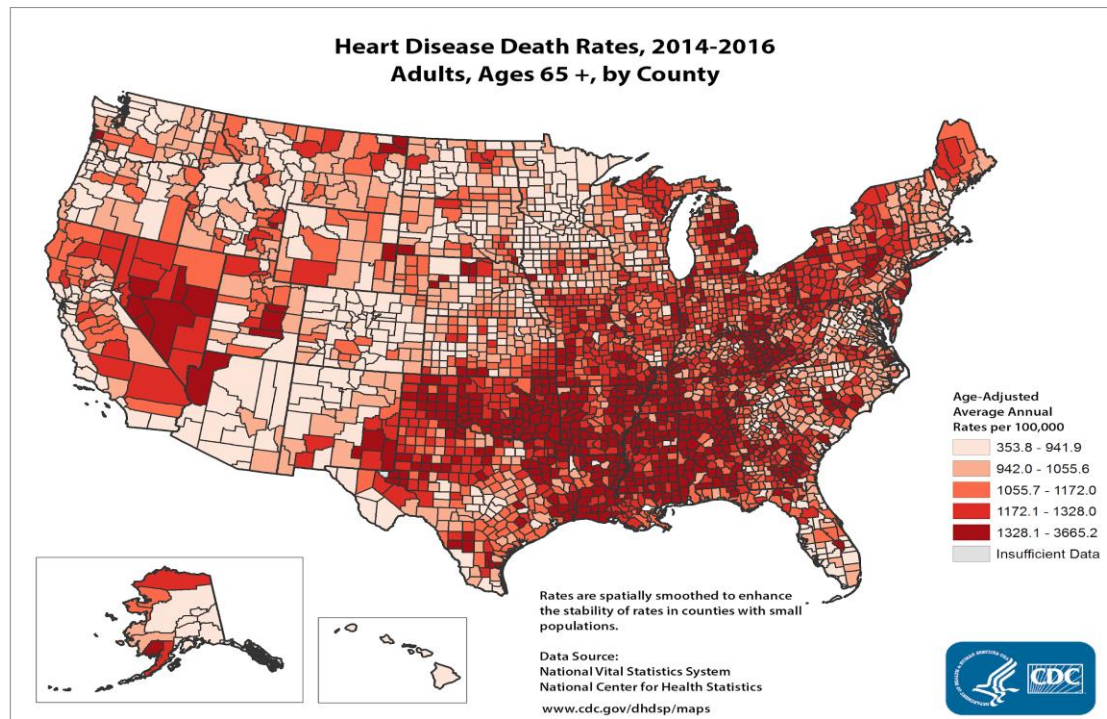
Source: CDC - National Center for Health Statistics

Given the health impact and financial concerns brought up by the increasing prevalence of cardiovascular disease, even a small reduction in the number of diagnoses or the potential effects of early intervention for cardiovascular disease may lead to significant impact, in both the number of annual deaths and costs associated with cardiovascular disease. [7] It is therefore imperative that further exploration into all avenues of preventative measures be explored. Although non-modifiable risk factors such as sex, age, or genetics may not be a viable target for prevention, the moderation of lifestyle factors such as diet, exercise, smoking, and stress account for some of the ways in which people can prevent cardiovascular disease and reduce cardiovascular events. Dietary changes would be a significant modifiable factor in the prevention of heart disease, as the impact of food on health has clearly been established and the alterations in food choices carry the potential to directly impact cardiovascular disease incidence and severity. It would therefore be an ideal remedy for the healthcare community to recommend dietary changes that would be most conducive to cardiovascular health. Unfortunately, in this regard, there has been little uniformity in the suggestions associated with the role that diet can play in the prevention of heart disease with changes in attitudes regarding the effects of specific nutrients and their effects on heart disease. There has been a school of thought, for example, over the past 60+ years that the overconsumption of dietary fats, particularly in their saturated form, play a large role in poor cardiovascular health, particularly in the accumulation of cholesterol buildup in the arteries. This diet heart hypothesis, as it came to be known, has its roots, in part, from the research in the ongoing Framingham study and the Seven Nation's study. In the Seven Nation's study Ancel Keys postulated that diets high in fats, particularly those high in the saturated form,

are associated with increased mortality from cardiovascular disease. Therefore, he

concluded, the reduction in dietary fats will reduce cardiovascular risk blood parameters.

[8] More recently, not only has the diet heart hypothesis come to be questioned, but in

some schools of thought, saturated fats have been exculpated and in fact have been

presented as potentially cardio protective. This thought is dependent on several factors

and new ways of looking at blood panels. [9] Additionally, the role of dietary

carbohydrates, both by themselves and in concert with dietary fat have come to be

postulated as a potential driver for increased cardiovascular risk. The theory here is that

the inflammation caused by the consumption of dietary carbohydrates, particularly higher

glycemic carbohydrates leads to a higher cardiovascular risk. Although, the importance

of dietary intervention in the prevention of heart disease remains imperative, the most

appropriate macronutrient ratios to support this goal remain in question.

Definitions

Dietary Macronutrients

Macronutrients

     Macronutrients are defined as the classification of nutrients that provide energy

for the body to carry on essential processes. The three main types of macronutrients are

carbohydrates, lipids (or fats), and proteins. [18] It is the blend of macronutrients that most

commonly define our energy yielding dietary makeup. Any discussion about the effects

of diet on cardiovascular disease needs to incorporate a discussion of the individual

macronutrients that make up the diet.

Carbohydrates

Carbohydrates are a classification of macronutrients identified as having an important role in cellular functioning, particularly through their role in energy production through glycolytic pathways and the TCA cycle. Although not the only nutrient that can be used for fuel, carbohydrates are the most common energy source used by the body. Carbohydrates are not essential in the diet, and accordingly, it is possible to generate energy for cellular use through alternative pathways using both protein and lipids as energy sources. Carbohydrate classification is made based on the number of saccharide groups in the molecule. One saccharide is called a monosaccharide, examples include glucose and fructose, two saccharides are called disaccharides and examples include sucrose (or table sugar), maltose, and lactose. Oligosaccharides are shorter in length and contain 3-10 monosaccharide groups. Polysaccharides are larger in length and contain more than 10 monosaccharide groups (up to thousands) and are considered to be a more complex type of carbohydrate due to the difficulty in breaking them down for absorption. Carbohydrates can also be classified as simple, which include mono-and disaccharides, and complex which include, oligo and polysaccharides. Carbohydrates are absorbed in their monosaccharide forms and the larger saccharides must be broken down for absorption and use. [18] Therefore, the longer the chain of saccharides the more work that must be performed in the body for absorption and therefore the higher the "complexity". Complex or longer chain carbohydrates have a lower impact on blood sugar. The impact of a carbohydrate on blood sugar is measured by the glycemic index.

Glycemic index

The glycemic index (GI) is a measure of the impact of carbohydrates on the elevation of blood sugar over time as compared to a reference monosaccharide. Glucose, in the case of the GI, is often used as the reference and has a GI score of 100, although some scales will use white bread as the reference food. Sucrose on the other hand, being a disaccharide, containing one glucose and one fructose molecule, has a lower GI score of 58 which means it does not elevate blood sugar as much as pure glucose. The lower the GI of a food, the lower the elevation in blood sugar over time (seen) following ingestion. Higher GI foods are associated with a higher health risk for conditions such as diabetes, heart disease, and obesity. [18]

Lipids

Dietary lipids (or fats) are a group of macronutrients made up of a glycerol molecule attached to three fatty acids. Fatty acids are comprised of a series of carbon atoms arranged in hydrocarbon chains with a carboxylic acids group on one end and a methyl group on the other. The length of the fatty acids range from 4 to 24 carbon atoms. Essential for function, fatty acids play an important role in the maintenance of hormone levels, cellular phospholipid layers, and proper nervous system function among other things. Dietary fats are composed of essential and non-essential fatty acids. Non-essential fatty acids can be produced endogenously and therefore are not required from the diet. Essential fatty acids, linoleic and alpha linolenic acid, must be obtained from the diet and cannot be produced endogenously. Calorically, fat consumption has been questioned for its impact on heart health due to both the association of dietary fat with serum cholesterol and due to the fact that fats contain 9 calories per gram, compared 4 calories per gram for

carbohydrates and protein. Indicating that on a gram by gram basis dietary fat consumption would contain more calories and may more significantly contribute to obesity, a strong risk factor for cardiovascular disease. [18] This opinion has been questioned, as dietary cholesterol is not absorbed directly as serum cholesterol and lower calorie macronutrients also do play a role in both serum cholesterol levels and obesity, as well as their subsequent associated health risks. In addition to categorizations based on essentiality, fatty acids can be subdivided based on their chemical structure. Specifically based on the number of double bonds per fatty acid into the subcategories of saturated, monounsaturated, and polyunsaturated fatty acids. [18]

Saturated Fatty Acids

Saturated-fatty acids (SFA), biochemically are distinguishable by the presence of zero double bonds being contributed to by the carbon atoms. This would mean that each carbon atom is participating in the maximum number of bonds and cannot further participate in double bonds, hence each carbon atom is fully saturated. Clinically, the consumption of excess dietary SFAs have been associated with an increased risk of morbidity and mortality from heart disease, although this relationship has been questioned in recent research. [18] Saturated fatty acids are highest in foods of animal origin (such as meats, butter, and milk) and coconut oils. [19]

Monounsaturated Fatty Acids

Monounsaturated Fatty Acids (MUFAs) biochemically are characterized by the presence of one double bond in the structure of the fatty-acid. Clinically, MUFAs have been linked to decreased overall correlation with cardiovascular risk blood parameters

and accordingly have tenuously been recommended, by some, as a suitable replacement for SFA in the diet. [18] Monounsaturated fatty acids are highest in oils from plant origin like olive oil, peanut oil, and canola oil. [19]

Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFAs) are characterized biochemically as having more than one double bond within their structure, many can have 6 double bonds. Clinically the correlation between PUFAs and decreased cardiovascular risk blood parameters have been more clearly delineated, leading to the recommendation from the American Heart Association that these may be the best substitution nutrient for SFAs. [7, 18] Polyunsaturated fatty acids are highest in oils also from plant origin like safflower oil, sunflower oil, corn oil, and soy oil. [19]

Blood Biomarkers

Total Cholesterol

Cholesterol is a lipid responsible for the production of steroid hormones, bile acids, and cellular membranes. Metabolized into its free form in the liver, cholesterol is released into the bloodstream bound to lipoproteins. Low Density Lipoproteins (LDLs) make up the majority of bound cholesterol, with High Density Lipoproteins (HDLs) binding a smaller, but significant amount of cholesterol. Clinically, elevation in the serum total cholesterol is associated with an increased risk for cardiovascular disease, atherosclerosis in particular. Cholesterol can also be used as a measure of liver function, as the liver is responsible for the production and metabolism of cholesterol. The relationship between serum total cholesterol and heart disease make the measurement of total cholesterol a measure of overall cardiovascular risk. Total cholesterol is measure of

the total cholesterol molecules floating in the bloodstream only and for years was used as a defacto measurement of cardiovascular risk. Numbers above 200 are correlated with a higher overall cardiovascular risk. It is important to note that, due the presence of cholesterol bound to lipoproteins, the measurement of total cholesterol, by itself, is an incomplete assessment of cardiovascular risk as it does not indicate the type and quantity of cholesterol in the blood. [12] As time went by, there came to be a greater understanding of the subdivisions of cholesterol and the fractions that make up total cholesterol. This increased understanding that total cholesterol is made up of several lipoprotein fractions, some of which may be beneficial, became an exemplar of the shortfalls of using total cholesterol as a measure of cardiovascular risk. A total cholesterol of 210, for example, that is made up of a larger portion of good HDL cholesterol would be less of a risk than a total cholesterol of 190 that is made up of a larger portion of bad LDL cholesterol. This would give rise to the need to look closer at not only the lipoprotein components but also the portion or ratio of the total cholesterol that they comprise.

High Density Lipoproteins

The amount of High Density Lipoproteins (HDL) or "good cholesterol" in the blood is often considered to be associated with cardiovascular health and lower risk of cardiovascular events. [12] Despite this, the impact of HDL on cardiovascular benefit is not universally accepted. The American Heart Association does not accept HDL as the sole positive indicator of cardiovascular health. [7] Epidemiological studies show that a higher level of HDL is associated with a lower risk of coronary artery disease. This association is not a linear relationship as lower HDL levels do not as strongly correlate to increased coronary artery disease. This would indicate that while HDL may be cardio

protective when high it may not be directly correlated with cardiovascular health. [13] The

HDL Mediated Cholesterol Efflux Capacity (CEC) is a measure of HDL cholesterol's

ability to remove cholesterol from circulation. The CEC is inversely associated with

cardiovascular risk and considered by some to be a better assessment of overall

cardiovascular risk. [13]

Low Density Lipoproteins

Low Density Lipoproteins (LDL) or "bad cholesterol" are positively associated

with cardiovascular risk. Individuals with higher levels of LDL cholesterol (particularly

in relation to lower levels of HDL cholesterol) are associated with increased risk of

cardiovascular disease and events. [12] There is some dissent with this thought,

however, particularly in relation to particle size. Givens points out that smaller particle

LDLs carry less overall cholesterol but carry a larger risk for cardiovascular disease. [22]

Pichler et al. [23] examined the effect of particle size on cardiovascular risk. The authors

noted that medium particle sized LDL cholesterol pose the largest risk followed by small

particles with larger particles having the least association with cardiovascular risk. [23]

This would indicate that although a patient may have a lower LDL count, that the

prevalence of medium or smaller particle LDL may pose more of a risk, questioning the

value of LDL itself as a biomarker for cardiovascular disease. [22] This leads to issues

with what is being tested. The traditional blood panel is measuring LDL-C, which is the

concentration of LDL cholesterol, instead of the percentage of LDL cholesterol which

may give a more complete picture of the size of the particle and associated risk. A

patient, for example, who had a large concentration of LDL but fewer particles could be

said to have a higher percentage of the more cardiovascular disease neutral large particle

LDL and accordingly lower risk. As the LDL-C is still positively correlated with cardiovascular risk of heart disease, it still remains a strong measurement but further evaluation into particle sub fractions is warranted.

Total Cholesterol to HDL Ratio

The serum total cholesterol to HDL ratio is a measure of the overall role that HDL, or good cholesterol, plays in the total cholesterol picture. The higher the HDL the lower the overall ratio and accordingly the lower the total cholesterol to HDL ratio the lower the overall risk. [12] The total cholesterol to HDL ratio has been cited as a superior laboratory measure of cardiovascular lipid risk. [12]

Triglycerides

Triglycerides (triglycerides) are a form of fat that is found in the bloodstream. They travel attached to both Very Low Density Lipoproteins (VLDLS) and LDLs. Triglycerides are produced in the liver using fatty acids and glycerol. Serum triglycerides are a test that is often performed with a lipid profile as part of an overall assessment of cardiovascular risk of heart disease. Elevated triglycerides are associated with an increased risk of cardiovascular disease. [12]

C - reactive protein

C - reactive protein (CRP) is a test for acute phase inflammatory conditions such as bacterial infections or other inflammatory conditions. Elevations in C - reactive protein are indicative of inflammation but do not provide insight as to the cause or location of the inflammation. [12] High-sensitivity C-reactive protein (hs-CRP) has been postulated as a potentially strong biomarker for increased cardiovascular risk of heart disease, particularly in women. [13]

Apolipoprotein B

Apolipoprotein B (Apo B) is the protein associated with the transport and metabolism of VLDL and LDL cholesterol. The measurement of Apo B is correlated strongly with LDL cholesterol particularly in that increases in Apo B are positively correlated with increased risk of cardiovascular heart disease risk. One area of differentiation with Apo B is that Apo B is a more direct measurement of the percentage of LDL particles and as such, is a better indicator of particle size for LDL cholesterol. [12] A larger Apo B with a high LDL-C would indicate a higher amount of large particle LDL and potentially lower cardiovascular risk of heart disease. [12]

Study Purpose, Goals and Objectives

This dissertation will examine the role of macronutrient ratios of dietary fats and carbohydrates on the biomarkers of cardiovascular health, particularly total cholesterol, High Density Lipoproteins (HDLs), Low Density Lipoproteins (LDLs), triglycerides, Apolipoprotein B, C-reactive protein, and total cholesterol to HDL ratios. The objective of this dissertation will be to ascertain the macronutrient profile, or profiles, most associated with cardiovascular health. A second objective of this dissertation will be to examine the impact of specific fatty acids on cardiovascular biomarkers.

Study Hypotheses

Hypothesis 1- The reduction in dietary saturated fat is associated with reduced cardiovascular risk blood parameters*.

Hypothesis 2- The replacement of saturated fat in the diet with polyunsaturated fat will reduce cardiovascular risk blood parameters*.

Hypothesis 3- The replacement of saturated fat in the diet with monounsaturated fat will reduce cardiovascular risk blood parameters*.

Hypothesis 4- The replacement of saturated fat in the diet with carbohydrates will reduce cardiovascular risk blood parameters*.

Hypothesis 5-A diet low in dietary fat is associated with reduced cardiovascular risk blood parameters*.

Hypothesis 6- A diet low in dietary carbohydrate is associated with decreased cardiovascular risk blood parameters*.

* Cardiovascular risk of heart disease blood parameters are defined as increased total cholesterol, LDL cholesterol, triglycerides, apolipoprotein B , C-reactive protein, total cholesterol to HDL ratio, and decreased HDL cholesterol

Study Significance

Heart disease is the leading cause of death in both the United States and worldwide leading to about 647,000 deaths per year in 2017 in the US. Among the causes of preventable risk factors for heart disease, diet plays a significant role. [2] Dietary choices that impact cardiovascular risk have the potential to alter the mortality as well as the costs associated with treating cardiovascular disease. The aim of this study is to examine the impact of different macronutrient ratios on the blood biomarkers, and by extension risk, for cardiovascular disease. Therefore the significance of this study is the potential to have a positive impact on the prevention of heart disease through dietary modifications. The potential outcome of a reduction on cardiovascular disease through dietary changes would be the reduction in the occurrence, mortality, and costs associated with cardiovascular disease.

Chapter II

LITERATURE REVIEW

History-Diet Heart Hypothesis

The origins of the diet heart hypothesis are credited in part with the research of Ancel Keys, starting in 1952, and continuing in 1958 with the Seven Nations study and further corroborated by the ongoing Framingham study, as well as, several other studies performed by several of Keys' contemporaries.[20] It was the findings of Dr. Keys, and others, that the consumption of dietary fat in general, and saturated fat in particular, was directly correlated with increases in the incidence of and mortality from cardiovascular disease. [8] The American Heart Association (AHA) followed in 1957 with a dietary guideline suggesting the reduction in dietary fat for lowering cardiovascular risk and in 1961 the AHA further recommended the reduction in saturated fat specifically. Although they noted, at the time, that this may have no impact on reduction in cardiovascular events. [8, 14] In 1977, the US Senate's select committee on Nutrition and Human Needs published the "Dietary Goals of the Unites States" which placed a national priority on the diet heart hypothesis and provided official governmental support for a low fat dietary approach. [14] Simply put, the diet heart hypothesis states that an increased consumption of dietary fat, particularly saturated fat, will lead to an increase in the deposition of cholesterol within the arterial walls and concomitantly lead to an overall increased risk for cardiovascular disease, which can manifest in a myriad of clinical outcomes, most

notably heart attack and stroke. In the diet heart hypothesis, the recommendation is to not only limit the overall intake of saturated fats to less than 10% of the diet but, depending on the origin of the recommendation, to limit the intake of total dietary fat. [20] Initial implications of this hypothesis were focused primarily on the limitation of saturated fatty acids as a whole group due to negative associations with cardiovascular risk factors, such as increases in LDL cholesterol and total cholesterol. Over time this approach has become more nuanced at it is understood that the removal of a nutrient like this does not happen in a vacuum and the nutrient is likely to be replaced by another nutrient in order to maintain energy balance. The focus therefore needed to shift from evaluating the effects of single nutrients into the role of the effect of replacing one nutrient with another. The Minnesota Coronary Experiment, which examined the effects of replacing saturated fats with dietary linoleic acid (a polyunsaturated essential fatty acid) on cholesterol, concluded that the removal of saturated fat and subsequent replacement with linoleic acid reduced both total cholesterol and LDL cholesterol. [9] Reexamination of the data from the Minnesota Coronary Experiment has shown, however, that although serum cholesterol was reduced in the study, the risk of cardiovascular mortality was not reduced. Suggesting that the clinical impact of the substitution has not been clearly established. [15] Part of the change from reducing saturated fat to the reduction in total fat can also be related to the caloric density of fat on a per gram basis, as well as the overall reduction in dietary cholesterol seen with a reduction in fat. As mentioned, in 1961, the recommendation was to replace the saturated fat in the diet with polyunsaturated fat, this recommendation was later changed to favor the reduction in total fat and replacement with lower glycemic carbohydrates following

the 1977 "Dietary Goals for the Unites States. [14] The Framingham report of 1983 cited obesity as a contributing risk factor for cardiovascular disease, further supporting the replacement of fat with carbohydrates. [14, 15] Another rationale, therefore, for limiting dietary fat is to control obesity, and by extension cardiovascular disease, through the reduction of caloric intake through decreased dietary fat. The assumption here is that since dietary fat contains 9 calories per gram, while dietary carbohydrates and protein contain 4 calories per gram, a gram for gram replacement of fat for carbohydrates or protein would theoretically reduce the overconsumption of calories further reducing cardiovascular risk. [14] Among the modifiable risk factors that increase the overall risk of cardiovascular disease obesity remains one of the top contributors. [2]

The recommendation to reduce total fat, and increase calories from carbohydrates led to the low fat, high carbohydrate diet craze of the 1980s and 1990s. [7] The effect of this substitution, however, did not yield the desired effect and rather than consuming complex carbohydrates from fruits and vegetables, dietary choices saw the increase in more simple carbohydrates. [14, 7] Consequently the national incidence rate of heart disease and obesity have risen during that time period. This increase is not reflective on consumer dietary habits as a whole however as the percentage of dietary calories derived from specific macronutrients has not seen statistically significant changes from 1999-2008 (See Figure 6). [16] Additionally, there has also been only a small change seen in dietary consumption of fatty acid subtype with a slight increase on polyunsaturated fat consumption from 1989-2014. (See Figure 7). [18]

Figure 6- Macronutrient Intake 1999-2008

Figure 2. Macronutrient intake (percent kcals) by sex in adults aged 20 and over, 1999–2008
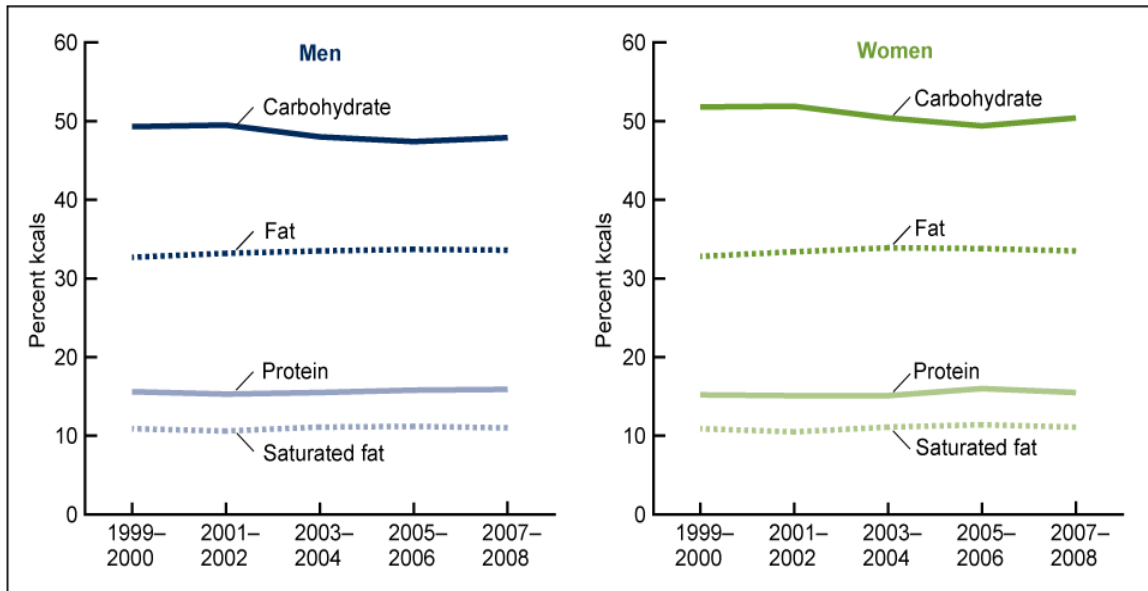
NOTE: Kcals are kilocalories.
SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey.

Figure 7- Fatty Acid Type 1994-2014
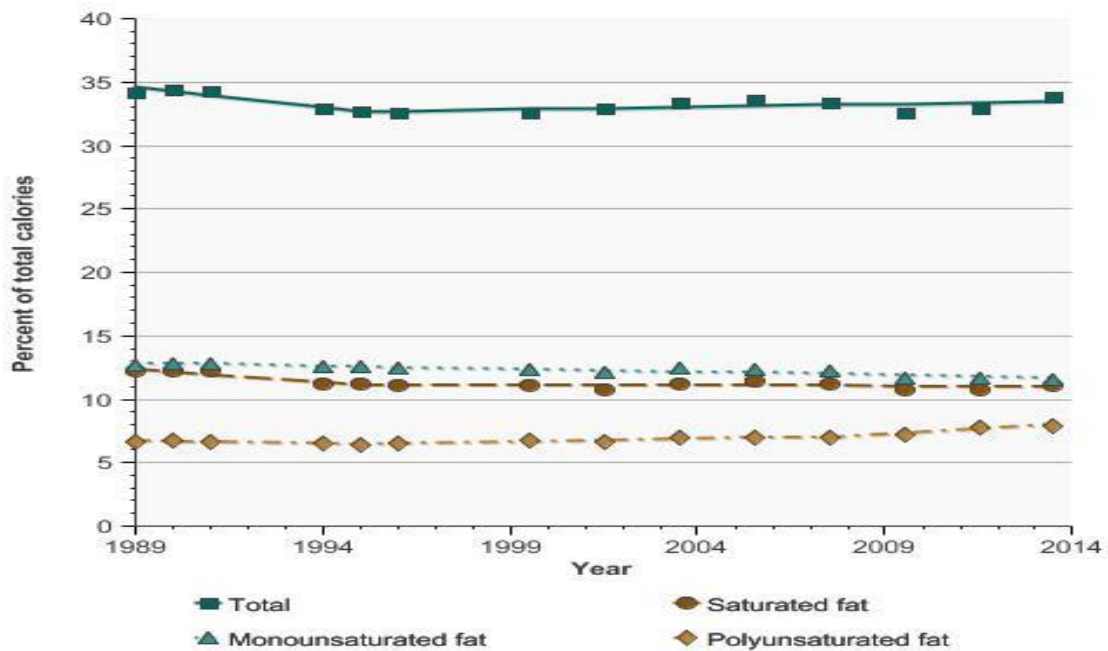
Source: NCI - National Cancer Institute

The recommendation to replace dietary fat, and saturated fat in particular, with carbohydrates did not appear to have had the desired effect. Currently it is the position of the American Heart Association that not only should saturated fat not be replaced with carbohydrates, but that the greatest benefit to cardiovascular health is achieved through the replacement of saturated fat with either poly- or monounsaturated fats, with a slight favor to polyunsaturated fats. [7] The debate over the role of macronutrients and their impact on cardiovascular disease has not stopped and to this day we have some who still support the low fat diet approach while supporters of a low carbohydrate, high fat diet feel that their approach is ideal to positively impact cardiovascular health. The following literature review will examine the current state of the research regarding the role of nutrients in cardiovascular health with a particular emphasis on the effects of replacement of saturated fats with other nutrients.

Literature search strategy

This literature review was based off of research articles, and other peer reviewed sources, attained through the Web of Science Database. The Web of Science Database includes nine indexes of research data, including the MEDLINE research index, and covers over 12,000 high impact research journals. [24] The expanse of research journals covered in this database makes it a suitable search tool for conducting a review. The search terms selected for the review were chosen so that a broad enough result is achieved while still limiting the articles to those with the most relevance to the review. A search was performed using the terms commonly associated with macronutrients (carbohydrates; high fat; low carbohydrate; fatty acids; saturated fatty acids; monounsaturated fatty acids; polyunsaturated fatty acids) and "cardiovascular risk of

heart disease". The search yielded significant results but still needed to be further

modified to include English articles only and with a date range from the past 25 years

(1993 to present). The results were then narrowed to peer review articles, randomized

control trials, meta-analyses, and review type articles as these would have the highest

degree of quality and correlation to this review. [25] Following this process, each article

was then reviewed individually and the appropriateness for inclusion was examined. In

addition to the database search articles, research articles that were referenced in the

search as being seminal to the field were reviewed for inclusion.

Results

Saturated Fat and Biomarkers

The role that saturated fat may play on clinical laboratory indicators of

cardiovascular health is a vast topic and one that can be influenced by the context in

which the labs are being viewed. If, for example, one is primarily concerned with the

effect on overall LDL cholesterol, as the American Heart Association is, then the

collective research shows that LDL cholesterol is raised overall by SFA in the diet. [7]

The conclusion, therefore would be, that dietary SFAs are antithetical to cardiovascular

health, particularly if we are only looking at total LDL and not particle sub fraction

effects, which would be the case with LDL-C. If we were to look at the dietary effects of

SFA on HDL cholesterol, we see research that shows that SFA will increase the amount

of HDL cholesterol in the blood. [26] This would be more associated with a decreased

cardiovascular risk. [21] Accordingly, since there is an increase in both LDL cholesterol

and HDL cholesterol, the ratio of total cholesterol to HDL may not change dramatically

in diets high in saturated fats, though this finding is not universal. [26] Additionally,

Woodside points out that the elevation in HDL cholesterol seen with SFA is higher than

seen with unsaturated fatty acid classifications. [27] This may indicate a cardiovascular

protective element to SFAs. A diet higher in SFAs has been shown to reduce the level of

triglycerides in the blood. Lower triglyceride levels are associated with improved

cardiovascular health. If we look at more of the ratio based biomarkers, the ratio of total

cholesterol to HDL will be reduced with a diet high in SFA, as well as the triglycerides to

total cholesterol ratio. [26] Clinically, if we look beyond the laboratory values into patient

presentations we get a larger picture. Zong et al. [28] examined the effects of a diet high in

SFA in their longitudinal cohort study. In this study the diets of 73,147 women and

42,635 were evaluated. It was their conclusion that for those with diets high in SFA, there

was a positive relationship with increased rates of cardiovascular disease. [28] In their

observational study, Virtanen et al. [29] noted, however, that despite the increased

association between diets high in SFAs and LDL cholesterol, there was no increased risk

for fatal coronary heart disease. The LDL cholesterol raised with dietary saturated fatty

acids, according to the author, is the larger particle variety which are not as associated

with cardiovascular risk when compared to the smaller LDL particles. [29] Saturated fat

has also been associated with enhanced apo B production, associated with increased large

particle LDL and potentially decreased cardiovascular risk (depending on the relationship

with LDL). [29] Additionally, the impact of elevations in LDL may be attenuated by the

reduction in small particle LDL which would be seen by a reduction in dietary

carbohydrates. [26] Virtanen et al. point out that evaluating the effects of reducing SFA in

the diet must be done within the context of the replacement nutrient. [29] Collectively the

research shows that while many elements of a diet high in SFA have been associated with positive cardiovascular laboratory values for cardiovascular health, the effects on LDL cholesterol could justifiably lead one to recommend the reduction in the SFA content.

It is also apparent that the type and source of the SFA are an equally important part of the equation. Praagman et al. [30] note that a diet high in SFAs are associated with higher LDL levels. The effect of chain length is important in relation to increased heart disease risk, with longer chain fatty acids showing an increase in correlation. Short and medium chain fatty acids, on the other hand had no association with increased risks. [30] Zong et al. agreed with this assertion, noting that the replacement of longer chain saturated fatty acids was associated with decreased cardiovascular risk while replacement of medium and short chain fatty acids with other macronutrients was not beneficial. [28] Lichtenstein [31] also noted that saturated fat chain length has a role in the effects on lipid biomarkers. Short chain fatty acids with 6-10 carbons and longer chain stearic acid at 18 carbons have minimal effects on lipid biomarker changes. While lauric (12), myristic (14), and palmitic (16) acids were associated with larger increases in LDL cholesterol, as well as higher increases in HDL cholesterol, indicating that the emphasis may not need to be the reduction in all SFAs equally if looking to improve cardiovascular biomarkers. [31] Stearic acid is the only exception noted to the correlation between increased chain length and increased cardiovascular risk of heart disease. Some of this impact on cardiovascular risk may be the effect on overall cholesterol ratios. Since short chain fatty acids are associated with a proportionally larger increase in HDL vs LDL cholesterol, there is a reduction in the overall total cholesterol to HDL ratio, which would be more associated with decreased cardiovascular risk. Unlike longer length saturated fats that have similar

effects on both the LDL and HDL levels and accordingly minimal to no effect on the ratio. [26] Longer chain acids within the group that have been associated with cardiovascular risk (i.e. 12, 14, and 16 carbons) is a factor as well, meaning that a 16 carbon fatty acid would be more associated with risk when compared to a 12 carbon fatty acid. Palm oil, for example, with 40% of its fat coming from 16 carbon chains shows an increased risk when compared to palm kernel oil or coconut oil which contains 45% 12 carbon chains. [28] Woodside et al. [27] points out, as well, that the effect of saturated fats on the lipid profile is not uniform to the classification and that even some long chain fatty acids have different effects. Stearic acid (with 18 carbons), for example, has little effect on serum cholesterol, likely due to its rapid conversion to oleic acid. [27] Fernandez and West [32] confirm the neutrality of stearic acid in relation to cardiac lipid biomarkers. They also point out that lauric (12), myristic (14), and palmitic (16) saturated fatty acids decrease LDL receptor activity, protein, and mRNA abundance, which in turn would be associated with increased LDL cholesterol in the blood. [32] Lauric acid (12 carbons) can be found in foods like palm kernel oil and coconut oil. Palm kernel oil, coconut oil, and butter are rich in myristic oil (14 carbons). Palmitic acid (16 carbons) can be found in foods such as beef and dairy, particularly of the grain fed variety. [28, 33] Although these foods have significant portions of the above fatty acids, it is important to note that these foods also contain other fatty acids and thus, despite the fact that the fatty acids are associated with LDL elevation, the foods may not necessarily be in the ratio to most negatively impact cardiovascular disease. If you look, for instance, at the above examples, the fatty acid content of coconut oil is not uniform and may contain multiple fatty acid types, even within the 12-16 carbon group, which would impact associated risk.

Coulston [34] looked at the effects of specific saturated fats noting that 14 carbon myristic acids lead to the largest increases in both LDL and HDL cholesterol followed by palmitic (16 carbon) and lauric acid (12 carbon). They also report that 18 carbon Stearic acid is not cardio-neutral, like others have noted, but in fact lowers serum LDL cholesterol indicating some cardiovascular benefit. [34]

Evaluation of the literature shows that there is an overall consensus that, as a whole, saturated fatty acids will increase the serum HDL levels, lower triglyceride levels, and improve many ratio based metrics. The effects of saturated fatty acids on LDL cholesterol is somewhat controversial. While most research indicates that saturated fats will elevate the LDL levels (and by extension the total cholesterol), the impact of the elevation of LDL cholesterol is not uniform, based on both fatty acid chain length and the source of the saturated fatty acid. It therefore can be assumed that a diet can contain some types of saturated fatty acids without impacting the LDL cholesterol negatively. Possibly indicating that foods that are dominant in 12, 14, and 16 carbon chain length are more important to potentially avoid or reduce. There is also some discussion, according to the research, as to the need to look deeper at the types of LDL cholesterol elevated by saturated fatty acids. Larger particle LDL cholesterol, the type that is less associated with cardiovascular heart disease risk, is more associated with SFA consumption rather than the more dangerous small particle LDL. This would indicate that the type of LDL may help to illuminate the risks associated with dietary saturated fat and may be where the debate should be focused on. The effect of chain length on LDL sub fraction type has not been fully established and firm conclusions may be premature. That being said, the association between total elevated LDL cholesterol and cardiovascular risk would

indicate that the replacement of saturated fat with an appropriate nutrient in the diet may provide some degree of cardiovascular protection.

Polyunsaturated Fats and Biomarkers

Polyunsaturated fatty acids or PUFAs are obtained, in large part, from plant based sources and can be further subdivided into omega 3 and omega 6 fatty acids. The lack of saturation in their structure leads to their ideal candidacy as a potential replacement for SFA in the diet. In reviewing the literature, a comparison between SFAs and PUFAs does show some similarities and differences to their effects on biomarkers. A diet high in PUFAs will raise HDL cholesterol, but does not raise the HDLs to the same degree as SFA (and subsequently may not lower the Total Cholesterol to HDL ratio to the same degree) and therefore may have less of a cardio protective benefit. Triglycerides are lowered in diets higher in PUFA but not to the same degree as diets higher in SFA as well. The main benefit achieved through diets high in PUFAs is the overall reduction in LDL cholesterol. It is this reduction in overall LDL cholesterol that when viewed in the context of the total lipid profile (high HDL, low LDL, low triglycerides, low total cholesterol to HDL) where a diet high in PUFAs appears to have cardiovascular benefit. In epidemiological studies, the risk of coronary heart disease is less for those who consume diets higher in unsaturated fatty acids. [38] Lichtenstein [31] makes the further assertion that of the specific types of PUFA, the omega 3 fatty acid carries the largest benefit on the reduction in cardiovascular risk. [31]

Overall the collective research seems to agree that among the fatty acid types, PUFAs are the most cardio-beneficial. This is due in large part to the fact that they tend to be associated with higher HDL cholesterol, lower triglycerides, improved ratio based

biomarkers and lower LDL cholesterol. Despite the fact that the impact of unsaturated fatty acids on HDLs are not as high as with SFAs, the general consensus of the literature is that the effects of PUFAs on cardiovascular risk blood parameters are beneficial.

Monounsaturated Fats and Biomarkers

The effects of diets high in MUFAs tend to be similar to SFAs with respect to HDLs, triglycerides, and ratio based metrics in that they are largely cardio protective for these measures. The extent of this similarity however is not to the same and does not extend to LDL cholesterol. MUFAs, unlike SFAs, are associated with decreased LDL cholesterol making them, theoretically, more cardio protective when compared to SFAs. [40] Another noted effect of dietary MUFAs, are the reduction in LDLs without reduction in HDLs, reducing the LDL to HDL ratio. [26] This effect on LDL cholesterol is disputed in the literature, as it has been inconsistent. [7] One reason for this inconsistency could be the source of the MUFAs. Studies looking at MUFAs are often based on diets with differing food sources. Western diet MUFAs tend to come from animal sources while European diet sources tend to be vegetable based sources like olive oil. [41] This could account for the differing impacts on LDL cholesterol. Additionally, Virtanen et al. [29] noted that there was a slight, yet not statistically significant, increase in coronary heart disease risk for diets that are higher in MUFAs. [29] One possible explanation for this would be the timeframe from which these studies were evaluating and the prevalence of hydrogenated MUFAs leading to the overconsumption of dangerous trans-fats. Another benefit of MUFAs is that a diet higher in SFAs would be associated with decreased insulin sensitivity and increased inflammation both presenting cardiovascular and other health risks. A diet rich in MUFAs would not have this association. [26]

27

The literature regarding the effects of MUFA on lipid biomarkers examined has been mixed and limited. The impact of the addition of MUFAs to the diet are lower LDL cholesterol, lower triglyceride levels, and improved ratio based metrics. The HDL cholesterol is improved with MUFAs as well although, as noted earlier, the impact of unsaturated fatty acids on HDL cholesterol is not as significant as with SFAs. Overall this would indicate that there is a cardio protective benefit to MUFAs and they may be more correlated with cardiovascular health. One issue with MUFAs, and all unsaturated fatty acids, is the impact of processing and heat, particularly with the potential for MUFAs to be consumed as trans-fats which would be much worse for cardiovascular health than unprocessed MUFAs would normally be.

Carbohydrates and Biomarkers

Collectively, diets higher in carbohydrates are associated with poor cardiovascular biomarkers, i.e. decreased HDL cholesterol, increased triglycerides, etc. Although the impact on biomarkers is largely dependent on the quality and source of the carbohydrate. The removal of refined carbohydrates and replacement with an appropriate fatty acid can actually reduce the overall risk of heart failure. [38] In an evaluation of the dietary effects on cardiovascular disease for Asian Indians, Misra et al. [42] noted that for those who consumed higher levels of carbohydrates, particularly with low fiber intake, there was an increase in the lipid triglycerides. [42] Brunner et al. [43] also points out that diets higher in carbohydrates are associated with high lipid triglycerides increasing cardiovascular risk. [43] Clinically, the overall risk of coronary heart disease was increased for those consuming higher glycemic or refined carbohydrates. This risk is not as high when the source of carbohydrates are more complex and lower glycemic. Higher glycemic foods

28

contain more dietary fiber. As fiber has been shown to have a beneficial effect on lipoproteins, a diet that is higher in whole food sources and contains a larger concentration of fiber may be more cardio protective. [31]

The impact, according to the literature, of carbohydrates on cardiovascular health, is less than ideal. Although it is true that carbohydrates are associated with lower overall LDL cholesterol, they are also lower in the more cardio protective HDL cholesterol, and by extension, more neutral to negative on the total cholesterol to HDL ratio. Unfortunately, diets high in carbohydrates are also associated with higher triglyceride levels and therefore the overall impact is that of a worsened cardiovascular risk. The type of carbohydrates do need to be taken into account as whole food unrefined carbohydrates are less detrimental when compared to more simple or processed varieties.

The Importance of Nutrient Substitution

The diet heart hypothesis was predicated on the idea that total dietary fat and saturated fat in particular, are directly responsible for the deposition of cholesterol within the arteries and the corresponding increases in the morbidity and mortality associated with cardiovascular disease. This has led to the targeting of saturated fatty acids for reduction in the American diet. [22] It is therefore understandable that the reduction of dietary fats, particularly SFAs, should be accompanied by a replacement nutrient with a less negative effect on cardiovascular risk factors. It is with this in mind, that the low fat high carbohydrate trend grew for reasons previously mentioned. Evaluation of the literature shows not only that the reduction of dietary fat, particularly SFA, does not positively affect cardiovascular lab values, but there is potentially a slight increase in cardiovascular risk when we replace fat with carbohydrates. Zong et al [28] attributes this

inconsistency in the literature to a failure to take into account the nutrient used for substitution citing that, when unspecified, carbohydrates were more likely to be the nutrient used for substitution. They went on to point out that when the study had the replacement of SFA with PUFAs there was a reduction in the cardiovascular risk but when the substitution macronutrient was carbohydrate, then the risk increased. Additionally, Zong et al. [28] noted that the replacement of longer chain saturated fatty acids was associated with decreased cardiovascular risk while replacement of medium and short chain fatty acids with other macronutrients was not beneficial. [28] Indicating again that chain length may be an important consideration. Coming from a different approach, Coulston [34], examined the effects of dietary fat, primarily from plant source, on lipid biomarkers. The author noted that even a small replacement of dietary carbohydrates with fats, of any type, is associated with an increase in HDL cholesterol and lower triglycerides, but if the replacement is either PUFA or MUFA, then the effect also will include a lowering of LDL cholesterol and total cholesterol. [34] Baum et al. [44] agreed that the impact of replacing saturated fat was dependent on the type of nutrient used as replacement. [44]

The focus on single nutrient reductions and their effects on preventing or treating heart disease have brought mixed benefits. Although research, for example, has linked the consumption of SFA with cardiovascular disease, the reduction of SFA as a plan of action has not been an effective means of combating cardiovascular disease. Brassard et al. [35] point out that while there is a correlation between diets higher in SFA and some adverse, biomarkers there has been inconsistency in the literature pointing out that Meta-analyses show that a diet high in SFAs does not raise the risk of death from

cardiovascular disease despite impacting the biomarkers, particularly LDL cholesterol. [35] The reduction of SFAs by definition have the requirement that the nutrient be replaced with another in order to maintain a net iso-caloric environment. The effect of replacing SFA in the diet, therefore, must be taken within the context of the nutrient being used for the replacement, with Li et al [45] citing it as "critically important". [45, 46] According to the American Heart Association, recent meta-analyses that show reductions in SFA have no relation to reduced cardiovascular risk, fail to take into account the replacement macronutrient. In many cases the replacement macronutrient in the standard American diet has been simple refined carbohydrates. [7] Additionally, Jakobsen and Overvad [47] point out that examining ischemic heart disease risk from the myopic view of single nutrient removal fails to take into account the impact of the substitution nutrient and is problematic, as often the replacement nutrient is a higher glycemic carbohydrate which is more associated with cardiovascular risk. The authors indicate the imperative need to examine the issue from the perspective of the replacement nutrient to be considered. [47]

It is therefore essential to evaluate the effect of nutrient substitution from the perspective of the replacement nutrient. The replacement nutrient selected, as it turns out, plays an important role in determining the efficacy of the dietary changes in cardiovascular disease. As stated, in an iso-caloric environment, the removal of SFAs will necessitate their replacement by another nutrient. As it turns out, if left unspecified, the dietary nutrient that is selected to replace SFA is often a carbohydrate and even more commonly higher glycemic carbohydrate in the standard American diet. It is therefore imperative, given the impact of specific nutrients on biomarkers, that the impact of

reducing dietary SFA must be evaluated from the context of the nutrient used as a replacement.

SFA substitution for PUFA

A primary focus for the reduction of cardiovascular risk has been the reduction of LDL cholesterol. In fact, the American Heart Association takes the viewpoint that the most important biomarker to consider when trying to combat heart disease is LDL cholesterol, making it a prominent part of their position statement on diet and cardiovascular risk. [7] The consumption of SFAs in the diet has been associated with several cardio beneficial effects, as previously mentioned, but has also been associated with an increase in LDL cholesterol, which is the reason for concern. PUFAs on the other hand carry many of the cardiovascular blood biomarker benefits of SFAs but do not raise LDL cholesterol. In fact, the replacement of SFAs with PUFAs has been associated with reduced LDL cholesterol. [7, 48] It would then stand to reason that the overall reduction in SFAs and subsequent replacement with PUFAs will reduce the overall cardiovascular risk, due in large part to the reduction in LDL cholesterol. The American Heart Association recommends limiting dietary SFAs in the diet and replacing them with PUFAs preferably. According to the American Heart Association, the replacement of SFAs in the diet with PUFAs, has been shown to reduce cardiovascular disease by 30% in randomized control trials. [7] The AHA's first recommendation to replace SFAs with PUFAs dates back to 1961, predating recommendations to replace SFAs with carbohydrates. [7] Schwab et al. [49] noted convincing evidence that the replacement of SFA with PUFA will reduce serum LDL cholesterol. [49] Hu and Willet [50] evaluated the dietary effects of different macronutrients and their effects on the reduction of

cardiovascular risk factors. They concluded that the reduction of saturated fatty acids and their subsequent replacement with non-hydrogenated unsaturated fatty acids can provide a useful means of reducing cardiovascular risk due to the reduction in LDL cholesterol. [50] Wang and Hu [51] evaluated the effects of replacing SFAs in the diet with different forms of unsaturated fatty acids, noting that replacement of SFAs with unspecified unsaturated fatty acids led to a reduction in risk. They support the replacement of SFAs with PUFAs as the most effective means of reducing cardiovascular risk. [51] Brunner et al. [43] looked at the effect of macronutrient concentrations on cardiovascular biomarkers. Based on their study, a diet that is high in PUFAs is associated with a more ideal LDL and HDL profile with lower triglycerides. This would indicate that a diet higher in PUFAs would be associated with a more favorable cardiovascular risk when compared to a diet higher in saturated fats. [43] Mente et al. [39], however, does not support looking only at the effects of LDL cholesterol, citing that the evaluation of cardiovascular risk based on LDL cholesterol alone is a myopic viewpoint and fails to take into account the total risk. They point to the total cholesterol to HDL ratio citing it as a global marker "likely to provide the best overall indication of the effect of an intervention such as diet on CVD risk" [39]. The authors then go on to point out that while the replacement of SFAs with unsaturated fatty acids, such as, PUFAs will lower the total cholesterol, blood pressure, and the LDL cholesterol, there will not be the concomitant reduction in triglycerides or increase in HDL cholesterol to the same degree. [39] The replacement of SFAs in the diet with PUFAs and MUFAs has been associated with lower HDL cholesterol levels, but would see higher HDL levels when compared to the replacement of SFAs with protein

and carbohydrates, indicating that the use of unsaturated fats as a replacement nutrient can be associated with a decreased overall cardiovascular risk. [27]

Looking at disease risk and clinical impact, a reduction of 10% of saturated fatty acids is associated with a 27% reduction in heart disease risk and a 5% reduction in saturated fat carries a 10% reduction in risk. [22] Li et al. [45] noted a 25% risk reduction in cases where saturated fat was replaced with PUFAs. [45] In a large-scale longitudinal cohort study, it was noted that for each 1% reduction in SFAs and replacement with PUFAs, there is a reduction in risk by 34.4 cases per 100,000. Virtanen et al. [29] reported that the effect of replacement of SFAs with PUFAs correlate to a slight decrease in coronary heart disease risk, pointing to the effect of PUFAs on positively affecting cardiovascular laboratory values. [29] Additionally, the impact of replacing SFAs with PUFAs may not be limited to the effects on cholesterol biomarkers as PUFAs can also have benefits of decreased inflammatory markers and increased insulin sensitivity. [26] Jakobsen et al. [52] examined the effects of replacing dietary SFAs with PUFAs, as well as other various replacement nutrients, they looked at the impact of substitution on coronary events and coronary deaths. They concluded that the replacement of 5% SFAs in the diet with PUFAs was inversely associated with both coronary events as well as coronary deaths. [52] Jakobsen and Overvad [47] supported the conclusion that the replacement of dietary saturated fatty acids with PUFAs was inversely associated with Ischemic Heart Disease. [47] Schwab et al. [49] echoed this point that a partial replacement of SFAs with PUFAs can reduce the likelihood of cerebrovascular disease. [49] Alternatively, looking at the substitution of SFAs with Omega 6 PUFAs, it was determined that this substitution was not likely to reduce cardiovascular events and mortality, indicating that the type and

source of replacement nutrient is an important consideration. [53] Virtanen [54] does not feel, however, that the evidence supports recommending reduction in consumption of omega 6 PUFAs. It was still his position that the replacement of SFAs with Omega 6 PUFAs provided a beneficial impact on cardiovascular risk. [54]

According to the literature reviewed, the replacement of SFAs with PUFAs allows for the benefits of SFAs to be realized without the negative effects associated with high LDLs. Dietary replacement of SFAs with PUFAs shows a lipid profile that is higher in HDL cholesterol, when compared to protein and carbohydrates as the substitution nutrient, and lower in LDL cholesterol, lower in triglycerides and improved ratio-based lipid parameters. Additionally the impact of the reduction of SFAs and replacement with PUFAs has a positive impact on not only lipid biomarkers but also inflammatory markers, insulin sensitivity, and a reduction in cardiovascular events, indicating a tangible benefit to replacing SFAs with PUFAs.

SFA Substitution for MUFA

MUFAs share the effect, with PUFAs, of lowering LDL cholesterol and therefore, have also been reported as a potential replacement for SFAs in the diet. Like PUFAs, MUFAs will increase HDL cholesterol, lower triglycerides and improve ratio-based metrics, but their effect on these, as well as LDL cholesterol, is not as significant in intensity. According to the research, the replacement of SFAs with MUFAs has not shown to be consistent, with some studies touting the benefits while others make the benefits less conclusive, some of this could be due to the aforementioned impact of MUFA source (i.e. Trans-fats). Despite inconclusive research regarding the effect of MUFAs as a replacement for SFAs, an inverse relationship has been shown, indicating a

possible benefit and reduction in cardiovascular risk. [22] A diet high in MUFAs would reduce abdominal adiposity, Triglycerides, and BP when compared to a diet higher in PUFAs as a replacement for SFAs. [22] Givens [22] notes that there is mounting evidence that replacing SFAs with MUFAs has beneficial effects on cardiovascular risk factors. [22] Schwab et al. [49] noted as well, that the replacement of SFAs with MUFAs will reduce LDL cholesterol. [49] The American Heart Association notes that the research showing beneficial effects of replacing SFAs with MUFAs is scarce and the AHA falls short of recommending it to the degree that they recommend PUFAs as the replacement nutrient for saturated fats. [7] In an examination of the data from the nurses' health study, Tamasecu [55] noted that the replacement of saturated fats with monounsaturated fats was consistent with decreased cardiovascular risk for patients with type 2 diabetes. [55] Kris-Etherton [56] examined the effects of replacement of carbohydrates with MUFAs on cardiovascular lipid risk factors. The author noted that the LDL cholesterol and triglycerides were lower in the MUFA diet. [56] Jakobson et al. [52] found that the impact of dietary replacement of SFAs with MUFAs showed a decrease in coronary events but not a decrease in coronary deaths. [52] Baum et al. [44] noted that the data regarding the replacement of SFA with MUFAs remains inconsistent, suggesting it may still be a suitable replacement. [44]

The research regarding the replacement of SFAs with MUFAs is in many ways similar to the impact of replacing SFAs with PUFAs in that we see a reduction in LDL cholesterol, lower triglycerides and ratio-based metrics. The evidence of this is not as strong as there is not enough research. Additionally, the reduction in SFAs and increase in MUFAs is associated with a decrease in cardiovascular events but not a decrease in

mortality. Indicating that there is a possibility that the effect of using MUFAs as a replacement for SFAs may not be as clinically beneficial as other nutrients like PUFAs.

SFA substitution for Carbohydrates

The replacement of both total fat and SFAs with carbohydrates has been postulated as a recourse to treat cardiovascular disease for decades. It is this theory that has promulgated the low fat, high carbohydrate diet craze in the latter part of the 1900s. The rationale for this was two-fold. First, since fat contains 9 calories per gram, whereas carbohydrates contain 4 calories per gram, the replacement of total fats, and specifically saturated fats, has the effect of reducing caloric consumption and this would potentially impact obesity, and by extension reduce a significant cardiovascular risk factor. Additionally, since a diet higher in fat would also be higher in cholesterol overall, then the replacement of fat with carbohydrates would be a particularly beneficial one as it would lead to a reduction in the overall consumption of dietary cholesterol. According to the research, the reality may not be the same as the theoretical benefit. Several recent studies and meta-analyses show that the replacement of SFAs with carbohydrates (both with and without taking into account the type of carbohydrate) do not, in fact, have the effect of reducing the cardiovascular risk blood parameters. Givens [22] points out that the reduction of SFAs and replacement with carbohydrates may be associated with no decrease or even an increase in risk. [22] Shrapnel [12] also similarly noted this, reporting that the replacement of SFAs with carbohydrates was at best cardiovascular risk neutral. [12] Harris et al. [46] reported that the replacement of SFAs with carbohydrates may reduce LDL cholesterol, but will also reduce HDL cholesterol, possibly mitigating any beneficial effects. Substitution analysis shows that replacing SFAs with carbohydrates is associated

with decreased LDL, increased triglycerides, and increased total cholesterol to HDL ratio in adolescent females, a finding consistent with the adult population. [46] According to Siri-Tarino [26], the biggest impact of replacing SFAs with carbohydrates is seen in the increase in triglycerides. [26] The American Heart Association noted in their position paper that there is no reduction in cardiovascular risk when SFAs are replaced with refined carbohydrates. [7] HDL cholesterol is either not increased or possibly even lowered. LDL cholesterol also remains the same or even will increase. There is also an elevation in triglycerides from a higher carbohydrate diet. It would also stand to reason then that the negative cardiovascular risk factors will also negatively impact the ratio based risk factors. [7] Mente et al. [39] noted that the replacement of SFAs with carbohydrates will have an adverse effect on lipid-based risk factors. The authors stated that diets higher in carbohydrates, as would be seen with SFA replacement, have the effect of raising LDL cholesterol, triglycerides, total cholesterol while reducing HDL cholesterol all the while elevating systolic blood pressure. [39] This has led the AHA to take the viewpoint that the reduction in SFAs and replacement with carbohydrates, regardless of type (simple or complex), is not an effective means of reducing the cardiovascular risk. [7] Additionally, the replacement of total fat with unspecified carbohydrates is not associated with reductions in cardiovascular disease. [7] Shrapnel [21] took a different, and slightly reverse, view on the topic in his examination of the effects of replacing macronutrients with carbohydrates. In his study he examined the effect of the removal of polyunsaturated fatty acids, monounsaturated fatty acids, protein, and saturated fat and replaced them with carbohydrates. He concluded that the replacement of saturated fat with carbohydrates posed no change in the risk of cardiovascular disease,

but the replacement of polyunsaturated fatty acids, monounsaturated acids, and protein with carbohydrates actually increased cardiovascular risk. An additional finding of this study was that increasing carbohydrates was positively correlated with an increase in dietary sugar and an overall decrease in carbohydrate quality. It was therefore, the author's conclusion that dietary recommendations regarding the amount of carbohydrates should be reconsidered and potentially lowered. [12] Lichtenstein [31] notes that dietary increases in carbohydrates, as a result of reducing SFAs, present with an increase in the VLDL cholesterol, low HDL cholesterol, a high total cholesterol to HDL ratio, and increases in small particle LDL cholesterol, the extent of the impact is dependent on the type and quality of the carbohydrate used as a replacement nutrient. [31] Brunner et al. [48] corroborated the effect of a diet high in carbohydrates as a replacement for saturated fats as lowering HDL cholesterol, but also showed that there is a lowering in LDL cholesterol and triglycerides. This is in contrast to other studies that cite increases in both LDLs and triglycerides. [48] The authors did not specify the type of carbohydrates consumed, however which may have impacted results. [43] Jakobson et al. [52] looked at the impact of replacing SFAs in the diet with carbohydrates. It was their conclusion that the replacement of SFAs with carbohydrates was actually associated with an increased risk of coronary events but not in overall coronary deaths. The authors did point out that the amount of fiber, extent of processing, and glycemic index may play a role on the impact of carbohydrates as a replacement nutrient. [52] Tanasescu et al. [55] points out that the replacement of saturated fats with carbohydrates not only did not decrease cardiovascular risk for type 2 diabetics but it increased their risk. [55]

Effect of Glycemic index

Part of the reason that there is some discrepancy as to the clinical benefit of replacing SFAs with carbohydrates has to do with the source of the carbohydrates used for replacement. Etherton and Fleming [40] supported this case asserting that when considering the replacement of SFAs with carbohydrates, we must consider the source of the carbohydrates. Carbohydrates from refined sources are more positively correlated with increased LDL cholesterol and increased triglycerides. Fibrous carbohydrates, on the other hand, do not carry the same increases in, and may even decrease, cardiovascular biomarker effect as more processed or refined varieties. [40] Several more studies have also called the impact of the type of carbohydrate into question. Studies have shown that the effect of higher glycemic carbohydrates, as seen with refined grains or sugars, on cardiovascular lab values have shown itself to be more detrimental overall. This is not the case when the dietary carbohydrates are of a lower glycemic index, such as whole grains and vegetables, which show more beneficial impact on some measures of cardiovascular health, particularly the LDL levels. [27, 51] As far as the HDL cholesterol levels are concerned, the American Heart Association shows no difference between low and high glycemic carbohydrates, as they both lower HDL compared to SFAs. The same can be said for triglycerides, as replacement for SFAs with either high or low glycemic carbohydrates increasing triglycerides. [7] Although this increase in triglycerides is not the same for different carbohydrate types. Replacing higher glycemic carbohydrates with lower glycemic carbohydrates has been associated with a 15-25% reduction in triglycerides. There is also evidence that diets higher in sugar and fructose are associated with increased atherogenic dyslipidemia. [26] Interestingly, replacing carbohydrates with

the specific saturated fatty acids lauric, myristic, and palmitic has been shown to increase LDLs, but will also increase HDLs and lower triglycerides. [7] This makes sense when we look at the effects of these particular SFAs on the lipid markers. As stated previously, this could potentially indicate a cardio protective element to SFAs that does not exist with carbohydrates, depending on the biomarkers evaluated. Hu and Willet [50] concluded that the replacement of dietary saturated fat with carbohydrates was not effective as a means of reducing cardiovascular risk due to the neutral impact on the ratio-based risk factors and elevations in the triglyceride levels. The authors do leave room for the replacement of saturated fats with whole grain carbohydrates as an acceptable means of reducing cardiovascular risk due to the higher levels of fiber and lower glycemic index. The authors also point to the reduction in cardiovascular risk seen with higher whole grain consumption. [50] Clarke et al. [57] noted the replacement of dietary saturated fatty acids with complex carbohydrates resulted in a reduction in both total cholesterol and LDL cholesterol. They did, however, note that the subsequent replacement of the complex carbohydrates with PUFA led to a further reduction in LDL cholesterol, replacement with MUFAs did not have the same effect. [57] This may indicate that PUFAs are a better replacement for SFAs than even low glycemic carbohydrates. Wang and Hu [51] noted that, while the replacement of SFAs with carbohydrates does increase cardiovascular risk, the risk is reduced if the carbohydrates used to replace SFAs are whole grains. [51] Woodside [27] further supports the view that the replacement of dietary saturated fat with refined carbohydrates would have a negative effect on cardiovascular risk due to the elevation seen in the lipid triglycerides. The authors postulated that a carbohydrate higher in fiber, such as from whole grains, is more likely to be a cardio beneficial replacement

for saturated fats. [27] Despite the fact that carbohydrates do not show increased cardiovascular risk, compared to SFAs, when taken on aggregate there is an elevation in risk seen when the carbohydrates are from higher glycemic index sources as compared to lower glycemic index sources. Replacing SFAs with low glycemic carbohydrates did show a decreased risk of myocardial infarction. The authors noted that studies that fail to account for the type of carbohydrates used for substitution tend to show null effects from the substitution. [45] Astrup et al. [58] supported the assertion that lower glycemic carbohydrates have more beneficial effects on LDL and HDL cholesterol than higher glycemic carbohydrates. They go so far as to recommend that the carbohydrate content of a diet should be comprised primarily of fiber rich, lower glycemic foods. [58] According to Virtanen et al. [29], replacing SFAs with carbohydrates has been shown to be beneficial in reducing coronary heart disease when the carbohydrates are of a lower glycemic index, noting that higher glycemic carbohydrates increased the risk in previous studies, but the authors were unable to substantiate these correlations in their own research. [29]

In reviewing the research regarding the impact of replacing dietary SFAs with carbohydrates, we get a certain degree of consensus, with some small areas of caveat. Overall, the impact of replacing SFAs with carbohydrates can be associated with either increased cardiovascular risk or no change in risk as seen by lipid profile risk factors with elevations in lipid LDLs and triglycerides, reductions in HDLs, and elevated total cholesterol to HDL ratio. The source of dietary carbohydrates is a consideration as there is some mitigation of any increased risk if the carbohydrates are from lower glycemic, higher fiber sources. Indicating that carbohydrates used as a potential replacement for SFAs should be of a lower glycemic and higher fiber source, rather than more refined

42

simple carbohydrates. PUFAs fared better, when compared to carbohydrates, on effecting risk factors which may indicate that they are still the better option.

SFA for Protein

Although not the focus of this literature review, the effect of replacing dietary SFAs with protein was examined. Zong et al. [28] noted a reduction in risk of 31.5 cases per 100,000 when SFAs were replaced with plant-based proteins. [28] It would be important to differentiate the use of plant based protein as they would not contain the saturated fat of an animal based protein. Lichtenstein [31] examined the impact of dietary proteins on the plasma lipoprotein content. The conclusion was that there is a dearth in the research regarding the impact of total protein on LDL cholesterol levels. Additionally, despite earlier studies that indicated the positive impact of plant-based protein, particularly soy, on LDL cholesterol, the overall impact may be more modest than originally thought. [31] Parker et al. [59] studied the impact of a diet high in protein, replacing dietary carbohydrate, on lipid biomarkers. They reported that the higher protein diet was positively correlated with lower triglycerides, lower LDL cholesterol, and had no effect on HDL cholesterol. [59] Indicating that a diet higher in protein may represent a good replacement option when compared to carbohydrates.

Despite the lack of research regarding the impact of replacing saturated fat with plant based protein, it remains a promising area of future research as a possible replacement option.

Dietary Intervention Effects and Lipid Biomarkers

The replacement of SFAs with carbohydrates has been suggested for decades but according to the research may not be beneficial to reducing cardiovascular disease. If the carbohydrate type is left unspecified, the replacement of SFAs with carbohydrate is associated with minimally lower (and potentially higher) LDL cholesterol, but unfortunately also lower HDL cholesterol and higher triglycerides. The impact of this is neutral to negative as to the effect on cardiovascular health. If we consider the replacement of SFA with lower glycemic carbohydrates, we do see an improvement in cardiovascular risk factors particularly lower triglycerides and LDL cholesterol. Additionally, if we differentiate LDL cholesterol by particle subtype, we see an increase in smaller denser LDL cholesterol with diets higher in carbohydrates, which are more detrimental to cardiovascular health. The effects of two dietary interventions, each at the opposite end of the spectrum, in terms of SFA replacement, should be evaluated for their impact.

Low Fat Diets

The replacement of fat, saturated fat in particular, with carbohydrates has been a particular focus of the diet industry for, among other reasons, the fact that SFAs have the effect of raising LDL cholesterol. The low fat, high carbohydrate diet is often seen with macronutrient distributions of approximately 60-65% carbohydrates, 15-20% proteins and 15-20% fats, with minimal fat (>10%) coming from saturated sources. [60] These ratios may fluctuate depending on the source of the recommendation, but the overall concept remains the same. One reason that low fat diets have been recommended for cardiovascular health would be due to the fact that they are effective for weight loss and

for reductions in type 2 diabetes, both of which are predisposing factors to cardiovascular disease. [58] Vafeiadou et al. [61] noted that a low-fat diet, when compared with a diet high in SFAs, resulted in improved vascular reactivity, which would be associated with decreased cardiovascular risk. [61] These diets are purported to be beneficial for not only weight loss, but also for cardiovascular health due in large part to the low level of fat and cholesterol in the diet. [12] In particular, studies have shown that diets that are lower in both total fat and that contain no more than ~ 9.3% of their calories from SFAs have been correlated with both lower total and LDL cholesterol in the long term when compared to diets higher in SFAs. Due to the prevalence of total cholesterol and LDL cholesterol as risk factors for heart disease, this would indicate a cardio protective benefit to a low fat, higher carbohydrate diet. In contrast, however diets lower in fat have also been associated with higher triglycerides, and lower HDL which would negate some of the aforementioned benefits. The interplay between lower LDL and TC levels with lower HDL and higher triglycerides levels calls into question the value of a lower fat diet. Interestingly, as the fat levels rise in the diet there is a corresponding elevation in HDL cholesterol as well as LDL cholesterol. A strong argument for lower fat diets remains that they have the effect of lowering LDL cholesterol, while other non-dietary lifestyle factors, such as physical exercise, may increase HDL cholesterol. The authors also noted that carbohydrate rich diets tend to increase the smaller, more dense LDL particles which have been shown to be more detrimental to cardiovascular health. [62] Astrup [58] noted that the clinical effect of low-fat diets have on lipid biomarkers are associated with reduced LDL and HDL cholesterol, and elevated triglycerides. [58] According to Schwingshackl [62], in Meta analyses low fat diets have been associated with lower levels

of LDL cholesterol, due in large part to reductions in saturated fat. This is mitigated by the decrease in HDL cholesterol and increases in triglycerides calling the impact of a low-fat diet into question. [62] Coulston [34], interestingly, noted that de novo synthesis of fatty acids tended to favor the production of palmitic (16 carbon) fatty acid more when dietary fat was kept low. This would indicate that a low fat diet may worsen cardiovascular risk due to endogenous production of 16 carbon saturated fat in excess of that found in consumption. [34]

After reviewing the literature on the impact of low fat diets, it is clear that despite the clinical impact on obesity, etc. there remains some concerns as to the potential to increase cardiovascular risk due to the ability to decrease HDL cholesterol and increase both small particle LDL cholesterol and triglycerides. This is particularly true if the diet is higher in refined or higher glycemic carbohydrates, as lower glycemic carbohydrates do not carry the same impact on lipid biomarkers.

Carbohydrate Restricted Diets

Noting the potentially negative effect of carbohydrates as the substitution nutrient for SFAs, leads one to question the effect of reducing carbohydrate intake on cardiovascular risk. A low carbohydrate or ketogenic diet is one in which the dietary carbohydrates are reduced, usually below 50g / day. Although different varieties exist, most low carbohydrate diets make up for the reduction in carbohydrates through increases in dietary fat (often 70-75%). Ketogenic diets are purported to have a variety of effects, from reduction in obesity, control of childhood seizures, and decreased cardiovascular risk. [63] Nakamura et al. [65] looked at the effect of three different low carbohydrate diets on the risk of cardio metabolic risk that each diet had on differing

46

levels of carbohydrate restriction. As a measure of cardiovascular risk, they looked at

LDL, HDL, CRP, total cholesterol, A1C, and uric acid. All three diets increased HDLs

indicating some degree of cardiovascular benefit. The plant based, low carbohydrate diet

lowered CRP, low carb diets with higher PUFAs, MUFAs, and plant-based proteins and

lower SFAs reduced all cardio metabolic risk factors. All three nutrients, SFAs, PUFAs,

and MUFAs, will increase HDLs, but only plant based, low carbohydrate diets lowered

the CRP. There was no significant difference between a high or low MUFA diet and

CRP. Plant based low carbohydrate diets lowered CRP, while animal-based diets did not

relate to CRP. Indicating no added ill effect of an animal-based diet, but rather no

increased benefit. [41] Wang and Hu [51] noted that the replacement of carbohydrates in the

diet with PUFAs showed a reduction in the cardiovascular risk. [51] The LDL elevations

seen in higher SFA diets are associated with larger particle LDL types which are less

detrimental to cardiovascular health. [62] This would indicate that despite the elevation of

LDL cholesterol, we may not see an increase in cardiovascular risk with lower

carbohydrate diets. Hu et al. [64] looked at the effects of low carbohydrates on

cardiovascular risk factors in their analysis of epidemiological studies. It was their

conclusion that a diet low in carbohydrates will increase HDL cholesterol, decrease LDL

and total cholesterol, and decrease triglycerides to a greater extent when compared to an

iso-caloric low-fat diet. The authors did not take the viewpoint that a low carbohydrate

diet is superior to a low-fat diet but that it is a viable alternative that may not have

previously been considered and that dietary guidelines should be reevaluated keeping this

in mind. [64] Bazzano et al. [65] looked at the impact of a low carbohydrate diet on

cardiovascular risk factors in a randomized control trial. Their results suggested that the

total cholesterol to HDL ratio did improve, but they surprisingly, also noticed a reduction in both the HDL and LDL cholesterol levels of subjects. Additionally, there were reductions in triglycerides and CRP. The authors concluded that a low carbohydrate diet was more suited to reduction in cardiovascular risk than a low fat diet. [65] Schwingshackl and Hoffmann's [62] meta-analysis took a look at the effects of long-term low fat and low carbohydrate diets on lipid profiles, and therefore cardiovascular risk, for overweight and obese individuals. They felt that there was no way to make any particular recommendation but noted that diets higher in saturated fat did tend to increase LDL cholesterol, whereas diets higher in carbohydrates increase triglycerides. They did point out that when the dietary saturated fatty acids were reduced and replaced with PUFAS there was a reduction in LDL cholesterol. [62] Lichtenstein [31] noted that the replacement of dietary carbohydrates with fat, undifferentiated, was associated with favorable cardiovascular biomarkers, in terms of reduced triglycerides, increased HDL cholesterol, and lower total cholesterol to HDL ratios. Interestingly few studies have examined the impact of moderate reduction in carbohydrates, but Lichtenstein [31] points out that moderate restrictions in carbohydrates have similar impact on cardiovascular biomarkers but with no increased benefit. [31] Shrapnel [21] noted that the effect of higher dietary carbohydrates on cardiovascular risk needs to be evaluated from within the context of the macronutrients the carbohydrates are replacing. Carbohydrates replacing SFA are associated with a neutral effect on cardiovascular risk. Replacing unsaturated fatty acids with carbohydrates will elevate the cardiovascular risk as will the replacement of protein with carbohydrates indicating that diets that are higher in carbohydrates increase the overall cardiovascular risk when compared with all nutrients, except saturated fat. [21]

There still remains some controversy regarding the healthful impact of low carbohydrate diets on cardiovascular risk. The literature points to the use of a low carbohydrate diet, particularly a ketogenic diet, as a potentially effective means of approaching a diet with decreased cardiovascular risk. The replacement of carbohydrates in the diet have been associated with decreased cardiovascular risk biomarkers, more so when the dietary fat is made up of a larger portion of PUFA's. The maximum percentage of carbohydrates in the diet that can still achieve this effect has yet to be established, indicating that there may be room to evaluate the appropriate macronutrient ratios which can still achieve the benefits of carbohydrate restriction.

Conclusions

After reviewing the literature there appears to be some consensus when it comes to the overall recommendations regarding the effects of different macronutrients on cardiovascular blood biomarkers, and by extension overall risk. Saturated fat has shown itself to increase the lipid levels of LDL cholesterol for almost all groups, while simultaneously raising HDL cholesterol, lowering triglycerides and generally improving ratio-based biomarkers. Some research points out that, despite the effect of raising LDL cholesterol, the particle size that is elevated is the more cardiovascular neutral large particle LDL, which would give an overall picture that the effect of saturated fat on cardiovascular biomarkers may not be all bad. That being said, due to the impact on LDL and total cholesterol, the replacement of saturated fats with another macronutrient may show itself to be beneficial for reducing cardiovascular risk. Interestingly, there is some data to suggest that the type and source of the saturated fat may also come into play with some saturated fat chain lengths having less effect than others. Stearic acid, for example,

which is found in some foods like grass fed beef, have been demonstrated to have less effect of serum LDL levels than palmitic acid which, for example, is more associated with grain fed beef. This may be a good driver of future research which looks into the individual foods rather than macronutrient makeup of diets. Carbohydrates not only were associated with an elevation in large particle LDL cholesterol risk, particularly with high glycemic carbohydrates, but also a reduction in HDL cholesterol and increased triglycerides. The research is clear that a diet high in refined carbohydrates is going to increase cardiovascular risk, while a diet higher in fiber and lower glycemic carbohydrates may be better for cardiovascular health, although not necessarily ideal. Polyunsaturated fatty acids and monounsaturated fatty acids were both associated with decreased LDL cholesterol, increased HDL cholesterol, decreased triglycerides, and improved cardiovascular ratio-based assessments. This would indicate these to be the most favorable of the nutrients evaluated, with polyunsaturated fats being the most favorable. Collectively, there are strengths and weaknesses associated with differing macronutrients as each has a different impact on cardiovascular risk when looked at through the lens of removal. This necessitates the evaluation of the effect of macronutrient replacement within the context of the replacement nutrient. The replacement of saturated fat with carbohydrates, for example, showed no benefit in the reduction of cardiovascular risk factors, although there is some reduction in risk when lower glycemic carbohydrates are used to replace saturated fat. The issue here is that in most instances for the standard American diet, the carbohydrates used to replace saturated fat have been largely higher glycemic refined carbohydrate. This finding is in contrast to years of dietary recommendations that both total fat and saturated fat be

reduced in favor of carbohydrates. The replacement of saturated fat with polyunsaturated fats and monounsaturated fats have been shown to have the effects of lowering LDL cholesterol and triglycerides, while having a high HDL cholesterol and improving ratio-based measures. As the research has been less conclusive regarding the impact of monounsaturated fats, the replacement of saturated fats with polyunsaturated fats has shown itself to be a strong potential mediator of cardiovascular risk. Interestingly, the impact of replacing low glycemic carbohydrates with polyunsaturated fatty acids reduced cardiovascular risk, indicating that polyunsaturated fats were more beneficial to the heart when compared to low glycemic carbohydrates. This of course does not take into account the potential impacts of individual foods on the overall cardiovascular risk. Some studies have suggested that different foods containing specific fatty acids will have differing cardiovascular impacts, this may be due to the prevailing chain lengths of the fatty acids, however.

Overall, after reviewing the literature it is clear that future directions looking to examine macronutrient ratios and their effects on cardiovascular risk should focus on a diet that is higher in fat than current recommendations suggest, with a higher percentage of those fats coming from both mono and, preferentially, polyunsaturated fats. Carbohydrate reductions from the current recommendations should be considered with an increased focus placed on the more cardiovascular beneficial lower glycemic and fiber rich carbohydrates, rather than the more detrimental higher glycemic carbohydrates.

Research Gaps

The review of the literature has shown a few gaps that need to be addressed by future research. There is a strong amount of data that indicates the effects of either higher

51

or lower levels of fatty acids and carbohydrates and their subsequent effects on cardiovascular risk blood parameters. Despite this, the macronutrient ratios where we start to see changes in cardiovascular disease blood based risk factors have not been established. The research has been, in large part, focused on the effect of substitutions of differing percentages, but no research examined determined the exact ideal percentages or ratios. If, for example, we can assume that replacing 10% of dietary consumption of SFAs with PUFAs will reduce LDL cholesterol and cardiovascular risk, then what would be the effect of a 50% reduction? We do see some information indicating a cardiovascular benefit to a high fat, low carbohydrate diet, but at what level of carbohydrate do we see increases in cardiovascular risk blood parameters? The same can be said for the percentages of SFAs vs PUFAs that are associated with cardiovascular risk blood parameters. Additionally, the impact of having a larger percentage of carbohydrates coming from fibrous foods should be evaluated in the context of their impact on cardiovascular risk. Effectively, which macronutrient ratios are the most associated with a beneficial cardiovascular lipid profile? Attempts have been made to establish the effects of macronutrients on these biomarkers, but more emphasis needs to be made to quantify the ratios of each nutrients. Additionally, the effects of differing fatty acids need to be evaluated. Research has shown some correlation between chain length and type of fatty acids and cardiovascular risk. A strong correlation has been shown, for example, that foods rich in the fatty acids lauric, myristic, and palmitic acids are more associated with negative lipid profiles and an increased risk for cardiovascular disease. Shorter and longer chain saturated fats do not share this same effect and Stearic acid, for example, is considered to be cardiovascular neutral. This would indicate that, although at

a holistic level saturated fats may be positively correlated with increased cardiovascular risk blood parameters, research should look at the impact of differing chain length and their impact on cardiovascular biomarkers. The same is true for individual unsaturated fatty acids. The effect of this type of research may point us to individual foods, rather than nutrient categories, that need to be avoided or limited. To date, there has been little in terms of research into this topic, but the impact of the effects of specific fatty acids on future directions of diet could potentially lead us to a more cardiovascular beneficial diet. Therefore, it is perhaps possible to design a diet that does not limit macronutrient groups, but rather centers around foods that are known to be beneficial or not detrimental to cardiovascular health.

Chapter III

Research Methods

Research Overview

The association between diet and cardiovascular biomarkers for disease has been established in the literature. There has been a developed understanding as to the impact that certain nutrients can and should have on these biomarkers. Unfortunately, the research to date has largely either looked at the impact of these nutrients without regard to other nutrient interactions or as replacement nutrients holistically. There have been studies, for example, that show the positive impact of replacing simple carbohydrates with complex carbohydrates, studies showing biomarker improvement with the replacement of saturated fats with polyunsaturated fats, and diets that have shown some positive effects when looking at diets that restrict the use of an entire micronutrient from the diet. There has been little in the research however, that has examined the impact of differing macronutrient ratios or attempts to find the most cardiovascular beneficial ratios of nutrients. Additionally, there has been little attempt to quantify the impact of restriction of specific fatty acids and their effect on cardiovascular biomarkers. After reviewing the literature, it is apparent that it is theoretically possible to evaluate the diet from the perspective of finding the optimal cardiovascular beneficial ratio of

macronutrients and possibly to determine the most appropriate specific nutrients to avoid, or limit, in the diet.

Research Design

In this retrospective, cross-sectional, correlational, observational study, we will be examining the relationship between variables, specifically in this case the average dietary habits of a pre-selected population and the impact of those dietary habits on that population's cardiovascular lipid biomarkers. The aim is to correlate the patients' objective biomarker data to specific dietary patterns and to use this information to determine the impact of diet on cardiovascular health.

Retrospective data will be used in this study due to its ready availability to be found in a pre-existing database. This allows for a large-scale investigation into the impact of dietary patterns and their relationship to specific biomarkers. The main strength of this study will be the availability of the information as well as the ability to use any conclusions obtained in future prospective studies. As the data has been collected already, there is no ability to conduct any intervention, and no ability to have a control group, therefore this study is going to be based on observations. A cross sectional design indicates that the sample population comes from a predetermined subset of the population as a specific point in time. This is a type of observational study that can be used to evaluate the physiological condition of a subset of the target population at the time the data was collected. A limitation to this research design is that it can only establish correlation between nutrients and biomarkers and fails to establish any causation. Correlational research, by design, is intended to show relationships between variables but

cannot attribute this relationship through any specific mechanism of action that would establish that variable A leads to outcome B. [66, 67]

Research Question

The clear relationship between dietary macronutrient ratios and cardiovascular risk blood parameters demonstrates the importance of further examining the impact of macronutrient manipulation to optimize cardiovascular impact, specifically in relation to dietary fats and carbohydrates. Therefore, the purpose of this study is to answer the following research question: What are the macronutrient ratios associated with the most favorable cardiovascular serum biomarker profile?

Hypotheses

Hypothesis 1- The reduction in dietary saturated fat is associated with reduced cardiovascular risk blood parameters*.

Null Hypothesis 1- The reduction in dietary saturated fat is not associated with reduced cardiovascular risk blood parameters*.

Hypothesis 2- The replacement of saturated fat in the diet with polyunsaturated fat will reduce cardiovascular risk blood parameters*.

Null Hypothesis 2- The replacement of saturated fat in the diet with polyunsaturated fat will not reduce cardiovascular risk blood parameters*.

Hypothesis 3- The replacement of saturated fat in the diet with monounsaturated fat will reduce cardiovascular risk blood parameters*.

Null Hypothesis 3- The replacement of saturated fat in the diet with monounsaturated fat will not reduce cardiovascular risk blood parameters*.

Hypothesis 4- The replacement of saturated fat in the diet with carbohydrates will reduce cardiovascular risk blood parameters*.

Null Hypothesis 4- The replacement of saturated fat in the diet with carbohydrates will not reduce cardiovascular risk blood parameters*.

Hypothesis 5-A diet low in dietary fat is associated with reduced cardiovascular risk blood parameters*.

Null Hypothesis 5-A diet low in dietary fat is not associated with reduced cardiovascular risk blood parameters*.

Hypothesis 6- A diet low in dietary carbohydrate is associated with decreased cardiovascular risk blood parameters*.

Null Hypothesis 6- A diet low in dietary carbohydrate is not associated with decreased cardiovascular risk blood parameters*.

* Cardiovascular risk blood parameters are defined as increased total cholesterol, LDL cholesterol, triglycerides, apolipoprotein B, C-reactive protein, total cholesterol to HDL ratio, and decreased HDL cholesterol.

Figure 8-*Theoretical Model*
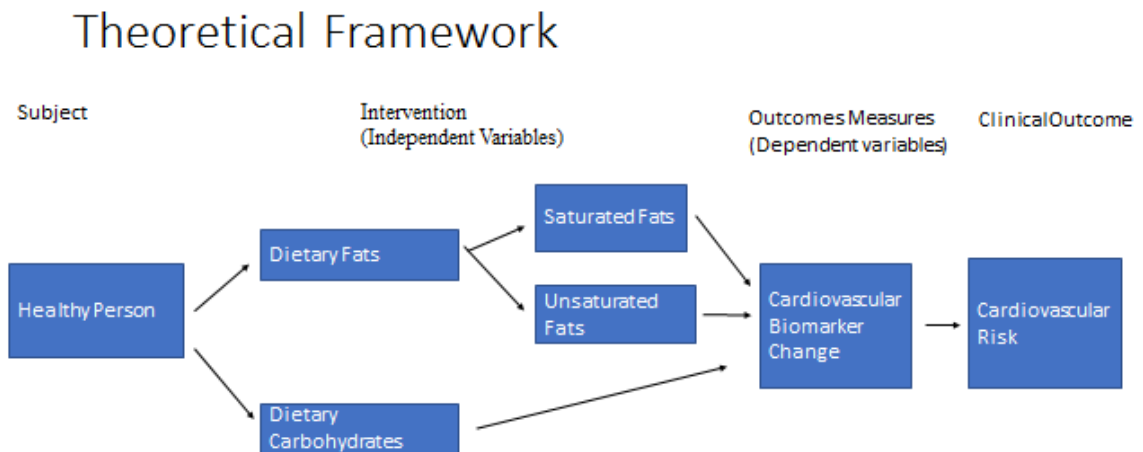
Data Sources

     The data source for this study will come from the National Health and Nutrition Examination Survey (NHANES) from the years 2005-2016. Started in the 1960s the NHANES data comes from a series of studies that combine interviews and other survey type data with patient examinations and laboratory values. The data set is comprised of 5000 participants annually from counties around the United States. The data is collected continuously to allow for greater correlations to be made from the larger data sets. To aid this ease of use, all datasets use the same or similar identification tags. The data of most pertinent use for this study will be the food diary data, demographic data, and the laboratory work that was conducted during this timeframe. The intended use of this data is for epidemiological studies to determine the prevalence of specific traits and physical

presentations. The NHANES data is part of the National Center for Health Statistics (NCHS) under the Centers for Disease Control (CDC). [68]

Study Period Inclusion/exclusion

The data used in this study will come from the annual data sets of NHANES collected from 2005-2016. These years were included because they contain the appropriate combination of the variables needed for analysis. Specifically, these years all included an examination of dietary habits of study participants, as well as the appropriate cardiovascular laboratory values, specifically total cholesterol, high density lipoproteins, low density lipoproteins, triglycerides, C - reactive protein (all years except 2011-2014, Apolipoprotein B, and the total cholesterol to HDL ratio, to be calculated from the above data. The availability of this data will allow for us to examine the impact of diet on these cardiovascular biomarkers addressing the 6 study hypotheses. Inclusion criteria will be adults between the ages of 18-65 with no known preexisting conditions, which may impact current dietary practices. For example, a participant who has a current diagnosis of diabetes may be eating a diet to positively impact their diabetes but may also have cardiovascular disease which is a known comorbidity for diabetes. Therefore, it would be difficult to associate dietary patterns with blood lipid biomarkers with any degree of accuracy. [68]

Instruments

The instruments used for this study will be a combination of surveys and examinations (physical and laboratory). It is this combination that leads to the conclusion that this is a mixed study design combining both quantitative and qualitative methods.

The qualitative data in this study will come in the form of surveys of the participants. Specifically, the evaluation of diet using 48-hour food diaries. The data will be quantified through examination and extrapolation, but the original data will be from surveys which are quantitative in nature.

The quantitative portion of the data will come from laboratory examination of the participant's blood. These samples were collected through yearly evaluation of the survey participants.

*Figure 9-***Study Variables**

Variable Category	Variable Tag in NHANES	Variable Name	Range
Nutritional Variables	Energy	DR1TKCAL, DR2TKCAL	kcal
	Carbohydrate	DR1TCARB, DR2TCARB	gm
	Total Sugars	DR1TSUGR, DR2TSUGR	gm
	Dietary Fiber	DR1TFIBE, DR2TFIBE	gm
	Total Fat	DR1TTFAT, DR2TTFAT	gm

	Total Saturated Fatty Acids	DR1TSFAST, DR2TSFAST	gm
	Total Monounsaturated Fatty Acids	DR1TMFAT, DR2TMFAT	gm
	Total Polyunsaturated Fatty Acids	DR1TPFAT, DR2TPFAT	gm
	Dodecanoic acid (Lauric acid)	DR1TS120, DR2TS120	gm
	Tetradecanoic acid (Myristic acid)	DR1TS140, DR2TS140	gm
	Hexadecanoic acid (Palmitic acid)	DR1TM161, DR2TM161	gm
	Octadecanoic acid (Stearic acid)	DR1TM181, DR2TM181	gm
	Polyunsaturated to Saturated Fat Ratio	PUFAsfratio	
	Monounsaturated to Saturated Fat Ratio	MUFAsfratio	
	Carbohydrate to Saturated Fat Ratio	Carbsfratio	

	Carbohydrate to Total Fat Ratio	Carbtffratio	
	Sugar to Saturated Fat Ratio	Sugsfratio	
	Fiber to Saturated Fat Ratio	Fibersfratio	
	Sugar to Total Fat Ratio	SugTfratio	
	Fiber to Total Fat Ratio	FiberTfratio	
Laboratory Variables	Direct HDL-Cholesterol	LBDHDD	mg/dl
	Direct HDL-Cholesterol	LBDHDDSI	mmol/l
	Triglyceride	LBXTR	mg/dl
	Triglyceride	LBDTRSI	mmol/l
	LDL-Cholesterol	LBDLDL	mg/dl
	LDL-Cholesterol	LBDLDLSI	mmol/l
	Total Cholesterol	LBXTC	mg/dl
	Total Cholesterol	LBDTCSI	mmol/l
	C-Reactive Protein	LBXCRP	mg/dl
	Apolipoprotein B	LBXAPB	mg/dl

	Apolipoprotein B	LBDAPBSI	g/dl
	Total Cholesterol to HDL ratio	TCratio	

Data analysis method

As this data has already been collected, we will be employing a secondary data analysis to look at the data and to determine the correlation and predictive abilities of the data. The use of secondary data has the positive attribute of being readily accessible and does not require many of the issues associated with Institutional Review Board, expediting the ability to evaluate the research quickly. The negative side of this examination is that the data collected in the NHAINES study, while useful in this case, was not specifically collected with this study's hypotheses in mind. This could lead to issues with reliability, as this dietary data may not have been collected with the current studies intended use. [66, 67]

Pearson r correlation - The Pearson correlation is used to determine the degree of correlation, measured in an r score or coefficient of correlation, between two linearly related variables. One factor to be considered would be the normality of the data, which would need to be present for the Pearson correlation to be useful. Given the central limit theorem, it can be assumed that due to the size of the dataset, normality can be assumed. Despite this, the data will be examined to assess normality and to ensure that there is no skew or kurtosis to the data. To that end, the identification and elimination of outlier data will assist with the skew and kurtosis of the data. [66, 67]

Measures of significance- In this study the level of significance will be evaluated using a p value. This p value allows us to determine the likelihood that the results are correct or merely by chance, i.e. statistical significance. The significance level in this study will be a p value of $<.05$. [66, 67]

Linear regression-Linear regression, both simple and multivariate, is a statistical tool that will help to define a linear relationship between variables. This is different than a correlational examination that determines if a relationship exists between two variables, a linear regression evaluation will allow us to attempt to quantify the relationship between two variables. This will allow for the analysis of the data and help to establish a possible predictive algorithm to the data. [66, 67]

Analysis of Variance- The analysis of variance allows for the evaluator to test a hypothesis by determining if the means of a variable differ between categories. In other words, the difference that outcome variable A will be for different classification categories. In this case, we are looking to see if the means of outcome variables are different for groups of differing macronutrient distributions. Post-hoc testing using Tukey's test will help to determine which specific groups differed for statistically significant tests. This will help to test the hypothesis that for differing macronutrient distributions, there will be a difference in biomarker outcomes, and to determine which ratio categories are associated with these differences. [66, 67]

*Figure 10- **Relationship between variables and Statistical evaluation***

Hypothesis	Intervention Measures (Independent Variables)	Outcomes Measures (Dependent variables)	Statistical tools

1-The reduction in dietary saturated fat is associated with reduced cardiovascular risk blood parameters.	Dietary Saturated Fat Consumption	Cardiovascular Risk Blood Biomarkers	Pearson correlation between Intervention vs Outcomes measures Linear Regressions for Intervention vs. Outcomes measures ANOVA hypothesis testing
2-The replacement of saturated fat in the diet with polyunsaturated fat will reduce cardiovascular risk blood parameters.	Dietary Saturated Fat Consumption Dietary Polyunsaturated Fat Consumption	Cardiovascular Risk Blood Biomarkers	Pearson correlation between Intervention vs Outcomes measures Linear Regressions for Intervention vs. Outcomes measures ANOVA hypothesis testing
3-The replacement of saturated fat in the diet with monounsaturated fat will reduce cardiovascular risk blood parameters.	Dietary Saturated Fat Consumption Dietary Monounsaturated Fat Consumption	Cardiovascular Risk Blood Biomarkers	Pearson correlation between Intervention vs Outcomes measures Linear Regressions for Intervention vs. Outcomes measures ANOVA hypothesis testing

4-The replacement of saturated fat in the diet with carbohydrates will reduce cardiovascular risk blood parameters.	Dietary Saturated Fat Consumption Dietary Carbohydrates Fat Consumption	Cardiovascular Risk Blood Biomarkers	Pearson correlation between Intervention vs Outcomes measures Linear Regressions for Intervention vs. Outcomes measures ANOVA hypothesis testing
5-A diet low in dietary fat is associated with reduced cardiovascular risk blood parameters.	Dietary Fat Consumption	Cardiovascular Risk Blood Biomarkers	Pearson correlation between Intervention vs Outcomes measures Linear Regressions for Intervention vs. Outcomes measures ANOVA hypothesis testing
6-A diet low in dietary carbohydrate is associated with decreased cardiovascular risk blood parameters.	Dietary Carbohydrate Consumption	Cardiovascular Risk Blood Biomarkers	Pearson correlation between Intervention vs Outcomes measures Linear Regressions for Intervention vs. Outcomes measures ANOVA hypothesis testing

Data analysis plan

The data for this study will be analyzed using SAS 9.4 statistical software

package. The SAS statistical software allows for the examination of multivariate data

quickly using more than 90 different prewritten procedures. The software package allows

for the analysis of large-scale datasets using up to date statistical methods and allows for its presentation in a variety of tabular and graphical formats. Additionally, the data obtained from the NHANES data set comes in a format that is readily usable by the SAS software package allowing for ease of transition to analysis. [69]

Data Organization and Cleaning

Datasets containing relevant information were downloaded from the 2005-2016 NHANES website. This data came from the demographics, laboratory, dietary, and examination datasets. This data was then merged by the assigned unique participant identifier. All datasets used identical tags for the variable data so no further processing needed to be performed at this point. These merged datasets yielded 20,007 results. The combined dataset was then filtered to remove data that may be incomplete or incorrect. The following filters were used:

- Those who did not complete both days of the food diary
- Those who responded that the food diary was not representative of their usual dietary intake
- Those who did not complete laboratory examination
- Those who indicated that they had altered their diet or were following a special diet- This data may still be used in evaluating specific dietary practices and their impact of cardiovascular health
- Those eating fewer than 1500 calories
- Those under the age of 18 or over the age of 65

Outliers were then removed by determining the interquartile range, multiplying this number by 1.5, and then adding or subtracting this number from the third and first

quartiles. After filtering, the dataset now contained 3089 food diaries with corresponding demographic information, physical characteristics, and laboratory values. Calculations were then performed on the datasets to develop averages for all dietary data (to average the food diary information by category and percent of daily caloric intake), as well as some other ratio based calculations. All statistical analyses were performed on these cleaned and processed datasets.

Chapter IV

Results

Introduction

This chapter presents the results of the analysis of data obtained from the 2005-2016 NHANES database. This data has been cleaned and processed, as discussed in Chapter 3, to remove any participants whose information may be either incomplete or contain issues that could impact the results in a way that interferes with testing these 6 hypotheses. After collection and processing, the data was analyzed to look first at the general descriptive statistics, to get a frame of reference for the current dietary trends of the sample population. Following this, the data was then analyzed within the context of each of the hypotheses and presented individually.

Descriptive Statistics

Statistical analysis of the data involved using 48-hour food diaries obtained thorough the 2005-2016 NHAINES databases, as well as laboratory and demographic data. In looking at the descriptive statistics for caloric consumption, in the sample, it is seen that the average caloric consumption for the sample population is 2462.24 calories (2602.12cal male, 2257.28cal female). The macronutrient breakdown of these calories would be 95.87g/day or 383.48cal protein (103.38g male, 84.87g female) , 300.88g/day or 1203.52cal carbohydrates (313.71g male, 282.09g female), and 93.15g/day or

838.35cal in total fat (97.44g male, 86.88g female). This would come to a dietary makeup of 15.6% protein, 48.9% carbohydrates, and 34.05% fat. This macronutrient ratio is in line with recommendations set forth in the Acceptable Macronutrient Distribution Range (AMDR). [70] The saturated fat consumption of 30.02 grams or 11% is almost double the American Heart Association's recommendation of 5-6% and slightly above the Dietary Guideline recommendation of 10%. [70, 71] The sugar consumption of 131.08 g/day or 21.1% of daily calories is significantly above dietary guidelines of <10% of daily calories. [70]Additionally, fiber consumption of 19.67g is below the guideline recommendations for adults which range from 22.4g/day to 33.6g/day (depending on age and sex). [70] (See Figures 11 and 12)

Figure 11 - **Descriptive Statistics Nutritional Variables**

Nutritional Variables						
Variable	Mean	Minimum	Maximum	Percent	AMDR 18+ [70]	Dietary Guidelines [70]
Average Calories	2462.24	1523	4235.5			
Males	2602.12	1523	4235.5			
Females	2257.28	1523	3919			
Average Protein	95.87	29.46	221.2	15.6%	10-35%	
Males	103.38	29.46	221.2			
Females	84.87	23	35.16			
Average Carbohydrates	300.88	115.73	674.32	48.9%	45-65%	
Males	313.71	133	674.32			
Females	282.09	72.19	115.73			
Average Fiber	19.67	1	80.25			
Males	20.3	1	80.25			
Females	18.75	3.35	61.45			
Average Sugar	131.08	10.3	443.77	21.1%		<10%
Males	134.51	12.23	443.77	20.7%		
Females	126.07	10.29	386.425	22.3%		
Average Total Fat	93.15	33.67	214.66	34.05%	20-35%	
Males	97.44	33.67	214.66			
Females	86.88	40.55	195.9			
Average Saturated Fat	30.02	8.29	52.84	11%		<10%
Males	31.4	8.29	52.84	10.9%		
Females	28.01	9.32	52.3	11.2%		
Average Polyunsaturated Fat	21.15	4.38	73.74			
Males	21.77	4.38	73.75			
Females	20.23	6.03	62.23			

	33.67	10.87	107.09			
Average Monounsaturated Fat						
Males	35.47	10.87	107.09			
Females	31.04	13.378	90.35			

Figure 12- **Macronutrient Intake by Percent Graph**
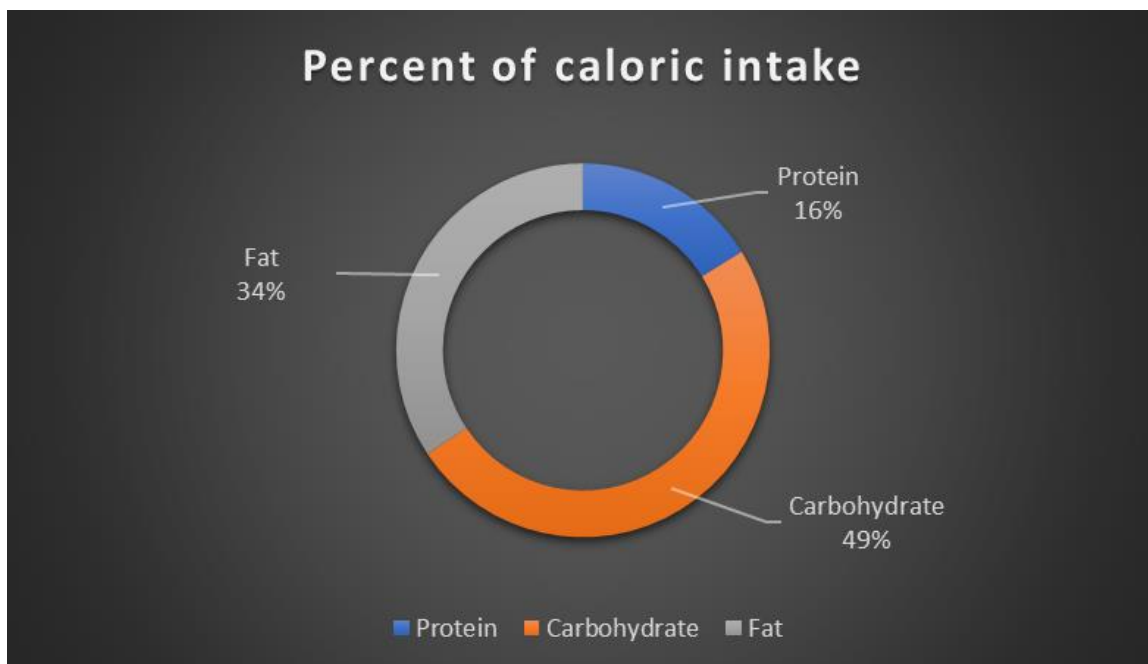
Evaluation of the cardiovascular biomarkers shows a population that is within the acceptable ranges for each biomarker. The average person, as a whole or differentiated by sex, in this study does not fall outside of the normal ranges for any of the clinical cardiovascular biomarkers. [12](See Figure 13)

Figure 13- *Descriptive Statistics Biomarker Variables*

Biomarker Variables	Mean	Minimum	Maximum	Reference Range [12]
Total Cholesterol	193.07	69	463	<200mg/dl
HDL Cholesterol	53.12	17	164	>45mg/dl male >55mg/dl female
Males	48.52	17	164	
Females	59.85	21	127	
LDL Cholesterol	114.45	23	375	<130 mg/dl
Triglycerides	130.66	12	1761	

Males	141.9	22	1761	<160mg/dl male <135mg/dl female
Females	114.33	12	1377	
Apolipoprotein-B	92.74	20	228	<125md/dl male <120mg/dl female
Males	94.72	32	211	
Females	89.86	20	228	
C-Reactive Protein	0.45	0.01	17.5	<1mg/dl
Total Cholesterol to HDL Ratio	3.91	1.36	14.39	<5 males <4.4 female
Males	4.22	1.37	14.39	
Females	3.46	1.64	10.53	

Hypothesis 1

Hypothesis number one is: "The reduction in dietary saturated fat is associated with reduced cardiovascular risk blood parameters." Evaluation of this hypothesis placed the percent of saturated fat and individual saturated fatty acids as the independent variables and the individual blood biomarkers as the dependent variables.

Pearson Correlation

In looking at the Pearson correlations for total saturated fat consumption as a percent of caloric intake, no statistically significant associations were noted between the percent of saturated fat consumed by the sample population and cardiovascular biomarkers. The data, when broken down by sex, shows statistically significant correlations when looking at male participants where a statistically significant weak negative correlation was noted between HDL cholesterol and saturated fat consumption ($r = -.054$, $p = .020$, $n = 1850$) and a statistically significant negative correlation was seen between low carbohydrate consumption in males and total cholesterol ($r = -.073$, $p = .0393$, $n = 8$).

Looking at Pearson correlations between the individual fatty acids allows us a deeper look into the impact of the percent of individual saturated fatty acids consumed and health. The saturated fat butyric acid shows a statistically significant weak positive correlation with HDL cholesterol. (r = .038, p = .05, n = 2630). Saturated fat caprylic acid shows a statistically significant weak positive correlation with HDL cholesterol. (r = .042, p = .0324, n = 2630). Saturated fat lauric acid shows a statistically significant weak positive correlation with HDL cholesterol and a weak negative correlation with the total cholesterol to HDL ratio (r = .039, p = .046, n = 2630 and r=-.043, p = .03, n=2630). Saturated fat stearic acid shows a statistically significant weak negative correlation with HDL cholesterol and a weak negative correlation with the total cholesterol to HDL ratio (r = .053, p = .006, n = 2630 and r=-.054, p = .006, n=2630). (See Figure 14, 15)

Figure 14- **Pearson Correlations**

Pearson Correlations				
Independent Variable	Dependent Variable	Probability	Coefficient	Number
Male Percent SF				
	HDL	0.02	-0.0541	1850
Male Low SF				
	TC	0.03938	-0.73129	8
Butyric Acid (SF 040)	HDL	0.0006	0.06253	2986
	TC	0.0038	0.05296	2986
Caproic (SF 060)	HDL	0.0039	0.05275	2986
	TC	0.018	0.05701	2986
	LDL	0.0365	0.03892	2986
Caprylic Acid (SF 080)	HDL	0.001	0.06032	2986
Capric Acid (SF 100)	HDL	0.0002	0.06888	2986
	TC/HDL	0.0287	-0.04266	2630
Lauric Acid				

(SF 120)	TRIG	0.0187	-0.04324	2959
	TC/HDL	0.0229	-0.04164	2986
Stearic Acid				
(SF 180)	TC/HDL	0.0090	0.04778	2986

Figure 15- **Correlation of individual saturated fatty acids with HDL cholesterol**
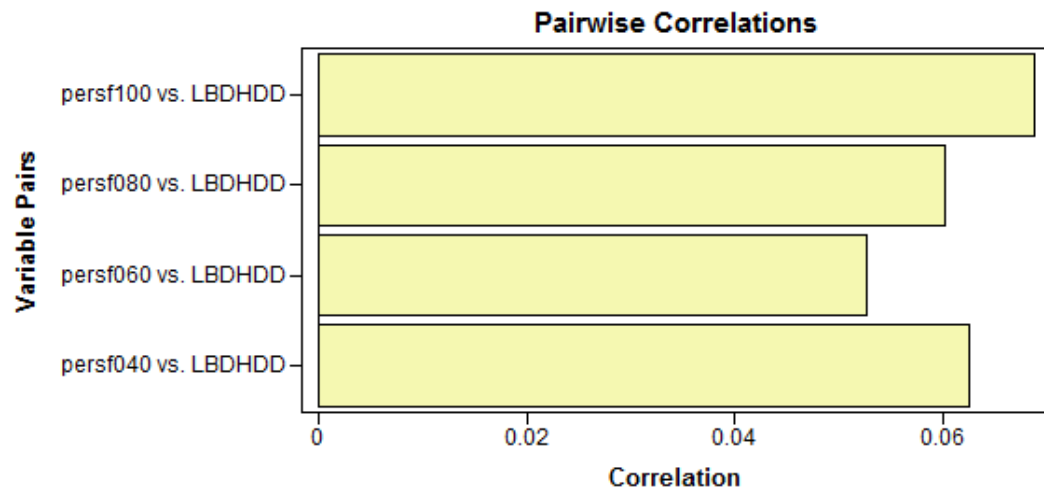
Simple Linear Regression

Simple linear regression was performed to examine the impact of saturated fat consumption in males on serum HDL cholesterol levels. Simple linear regression showed a significant relationship with the model:

HDL Cholesterol = Percent of Saturated Fat *-27.95 + 51.6 $(p=.0200, r^2=.0029)$

Although this regression shows a statistically significant model, the low R-Squared involved demonstrates that the impact of the model (percent saturated fat in males on serum HDL) is not strong by itself and other variables are relevant factors in predicting HDL.

Analysis of Variance

An analysis of variance was performed to look at the effect that saturated fat consumption of differing percentage groups (low (<5%), medium (5-10%), or high

(>10%)) may have on differing cardiovascular biomarkers. The hypothesis under consideration is: The reduction in dietary saturated fat is associated with reduced cardiovascular risk blood parameters. The results of the analysis of variance indicate there were no statistically significant differences seen between the groups of differing saturated fat percentages on any cardiovascular biomarkers at the p<.05 level for the three categories.

Multivariate Regression Analysis

A multivariate regression analysis was performed to look at the role that individual saturated fatty acids making up the dietary intake may have. The independent variables were the individual fatty acids and the dependent variables were the blood biomarkers. In evaluating the role that the individual fatty acids, in combination, play on HDL cholesterol levels a statistically significant model was noted as:

HDL Cholesterol = (Percent of Butyric Acid* 7218.78) + (Percent of Caprylic Acid*12106) + (Percent of Lauric Acid*-798.7) + (Percent of Stearic Acid*-1670.3) + 55.4 (p<.0001, Adj. R^2=.0085)

It is worth noting that although the model shows statistical significance as a whole, Caprylic and Lauric acid had no statistically significant associations individually (p=.197 and p=.581) indicating that although the model is significant, these individual are likely not contributing strongly to the HDL cholesterol results. In evaluating the role that the individual fatty acids in combination play on total cholesterol to HDL ratio, a statistically significant model was noted as:

Total Cholesterol to HDL ratio = (Percent of Lauric Acid* -178.91) + (Percent of Stearic Acid*100.24) + 3.67 (p=.0008, Adj. R^2=.0041)

The low adjusted R^2 values indicate more variables than just the consumption of these saturated fatty acids were contributing to the serum biomarker.

Hypothesis 2

Hypothesis number two is: "The replacement of saturated fat in the diet with polyunsaturated fat will reduce cardiovascular risk blood parameters". Evaluation of this hypothesis placed the percent of saturated fat, the percent of polyunsaturated fat, the polyunsaturated to saturated fat ratios, and individual polyunsaturated fatty acids as the independent variables and the individual blood biomarkers as the dependent variables.
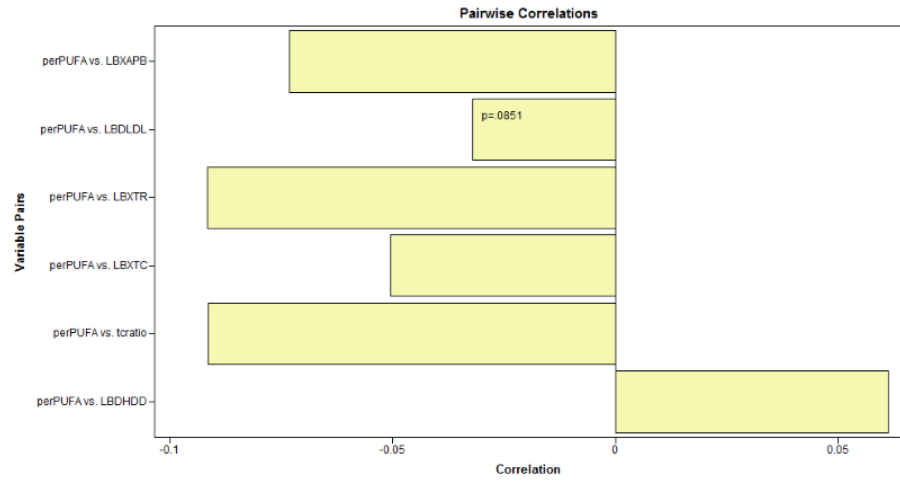
Pearson Correlation

In looking at the Pearson correlations, statistically significant correlations were seen with a few variables. There is a statistically significant weak positive correlation between HDL cholesterol and polyunsaturated fat consumption. ($r = .061$, $p = .0008$, $n = 2986$). There is a statistically significant weak negative correlation between triglycerides and polyunsaturated fat consumption. ($r = -.092$, $p < .0001$, $n = 2959$). There is a statistically significant weak negative correlation between apolipoprotein B and polyunsaturated fat consumption. ($r = -.073$, $p < .0001$, $n = 2958$). There is a statistically significant weak negative correlation between total cholesterol to HDL ratio and polyunsaturated fat consumption. ($r = -.091$, $p < .0001$, $n = 2986$). There is a statistically significant weak negative correlation between total cholesterol and polyunsaturated/saturated fat ratio. ($r = -.055$, $p = .0027$, $n = 2986$). There is a statistically significant weak negative correlation between LDL cholesterol and polyunsaturated/saturated fat ratio. ($r = -.0381$, $p = .0405$, $n = 2888$). There is a statistically significant weak positive correlation between HDL cholesterol and polyunsaturated/saturated fat ratio. ($r = .043$, $p = .018$, $n = 2986$). There is a statistically significant weak negative correlation between triglycerides and polyunsaturated/saturated fat ratio. ($r = -.079$, $p < .0001$, $n = 2959$). There is a statistically significant weak negative

correlation between apolipoprotein-B and polyunsaturated/saturated fat ratio. (r = -.065, p = .0004, n = 2958). There is a statistically significant weak negative correlation between total cholesterol to HDL cholesterol ratio and polyunsaturated/saturated fat ratio. (r = -.081, p <.0001, n = 2986). (See Figure 16, 17, 18)

Looking at Pearson correlations between the individual fatty acids, polyunsaturated fat linoleic acid shows a statistically significant weak negative correlation with total cholesterol (r = -.053, p = .0035, n = 2986), weak positive correlation with HDL cholesterol (r = .058, p = .0015, n= 2986), weak negative correlation with triglycerides (r = -.095, p<.0001, n = 2959), weak negative correlation with apolipoprotein-B (r = -.072, p < .0001, n = 2958), and a weak negative correlation with the total cholesterol to HDL ratio (r = -.09256, p <.0001, n = 2986). Polyunsaturated fat linolenic acid shows a statistically significant weak positive correlation with HDL cholesterol (r = .066, p = .0003, n= 2986), weak negative correlation with triglycerides (r = -.045, p =.0153, n = 2959), weak negative correlation with apolipoprotein-B (r = -.066, p = .0003, n = 2958), and a weak negative correlation with the total cholesterol to HDL ratio (r = -.063, p = .0006, n = 2986). Polyunsaturated fat Stearidonic acid shows a statistically significant weak negative correlation with apolipoprotein-B (r = -.041, p = .0246, n = 2958). Polyunsaturated fat Arachidonic acid shows a statistically significant weak positive correlation with apolipoprotein-B (r = .04514, p = .0141, n = 2958), and a weak positive correlation with the total cholesterol to HDL ratio (r = .05230, p = .0043, n = 2986). Polyunsaturated fat Cervonic acid shows a statistically significant weak positive correlation with total cholesterol (r = .033, p = .0326, n = 2986). (See Figure 18)

*Figure 16-***Graph of Pearson Correlations between the percent of polyunsaturated fatty acids and biomarkers (p<.05 except where noted)**

Figure 17- **Graph of Pearson Correlations between the polyunsaturated to saturated fatty acid ratios and biomarkers (p<.05)**
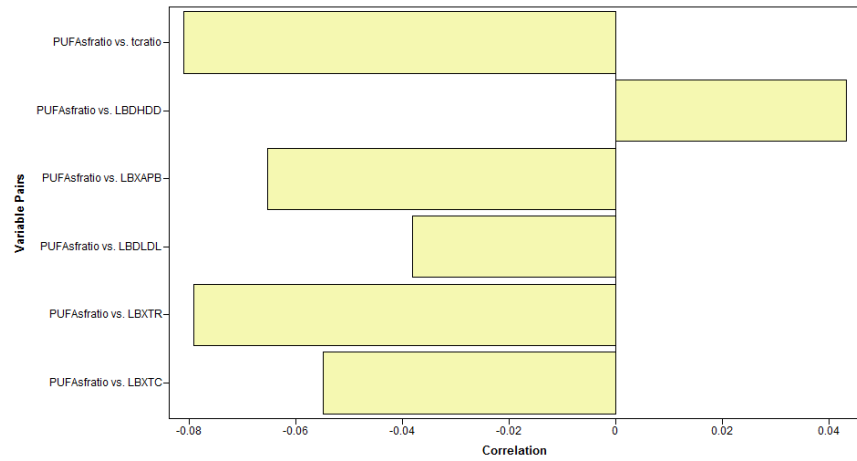
Figure 18- **Pearson Correlations**

Pearson Correlations				
Independent Variable	Dependent Variable	Probability	Coefficient	Number
Percent PUFA				
	HDL	0.0008	0.06121	2986

	TRIG	<.0001	-0.09166	2959
	APO-B	<.0001	-0.07324	2958
	TC/HDL	<.0001	-0.09147	2986
PUFA/SF Ratio				
	TC	0.0027	-0.0549	2986
	LDL	0.0405	-.03812	2888
	HDL	0.0182	0.04320	2986
	TRIG	<.0001	-0.07916	2959
	APO-B	0.0004	-0.06533	2958
	TC/HDL	<.0001	-0.08101	2986
Linoleic				
	TC	0.0035	-0.05347	2986
	HDL	0.0015	0.05805	2986
	TRIG	<.0001	-0.09485	2959
	APO-B	<.0001	-0.0715	2958
	TC/HDL	<.0001	-0.09256	2986
Linolenic				
	HDL	0.0003	0.06568	2986
	TRIG	0.0153	-0.04457	2959
	APO-B	0.0003	-0.0661	2958
	TC/HDL	0.0006	-0.06291	2986
Stearidonic				
	APO-B	0.0246	-0.04134	2958
Arachidonic				
	APO-B	0.0141	0.04514	2958
	TC/HDL	0.0043	0.0523	2986
Cervonic				
	TC	0.0326	0.03911	2986

Simple Linear Regression

Simple linear regression was performed to examine the impact of the

polyunsaturated fat to saturated fat ratio on serum triglyceride levels, simple linear

regression showed a significant relationship with the model:

Triglycerides= Polyunsaturated Fat to Saturated Fat ratio *-28.81 + 152.18 ($p<.0001$, $r^2=.0063$)

Simple linear regression showed a statistically significant relationship between

total cholesterol and the polyunsaturated to saturated fat ratio with the model:

Total Cholesterol = Polyunsaturated Fat to Saturated Fat ratio *-7.31 + 198.53 ($p=.0027$, $r^2=.0030$)

Simple linear regression showed a statistically significant relationship between

LDL cholesterol and the polyunsaturated to saturated fat ratio with the model:

LDL Cholesterol = Polyunsaturated Fat to Saturated Fat ratio *-4.27 + 117.65 ($p=.0405$, $r^2=.0015$)

79

Simple linear regression showed a statistically significant relationship between HDL cholesterol and the polyunsaturated to saturated fat ratio with the model:

HDL Cholesterol = Polyunsaturated Fat to Saturated Fat ratio *2.21+ 51.47 (p=.0182, r^2=.0019)

Simple linear regression showed a statistically significant relationship between apolipoprotein-B and the polyunsaturated to saturated fat ratio with the model:

Apolipoprotein B = Polyunsaturated Fat to Saturated Fat ratio *-5.46 + 96.81 (p=.0004, r^2=.0043)

Simple linear regression showed a statistically significant relationship between the total cholesterol to HDL ratio and the polyunsaturated to saturated fat ratio with the model:

Total Cholesterol to HDL Ratio= Polyunsaturated Fat to Saturated Fat ratio *-0.35 + 4.17 (p<.0001, r^2=.0066)

Although this regression shows a statistically significant model the low R-squared value demonstrates that other variables than the percent of polyunsaturated fat to saturated fat ratio alone are relevant factors in predicting cardiovascular biomarkers.

Analysis of Variance

An analysis of variance was performed to look at the effect that polyunsaturated fat to saturated fat ratio groups of differing ranges (0-.5, .5-1, and 1+) may have on differing cardiovascular biomarkers. The hypothesis under consideration is: The reduction in dietary saturated fat and replacement with polyunsaturated fats is associated with reduced cardiovascular risk blood parameters. The results of the ANOVA showed a statistically significant difference between the three categories when total cholesterol was the dependent variable [$F_{(2, 2983)}$ = 4.89, p=.0076]. Further analysis using Tukey's test showed that the primary difference is between the first (m= 197.19mg/dl) group and the second (m= 192.64 mg/dl) and third (m=189.69 mg/dl) groups. It is clear from these

results that there is a significant difference in the means of these groups. The results of the ANOVA showed a statistically significant difference between the three categories when total cholesterol to HDL ratio was the dependent variable [F (2, 2983) = 7.56, p=.0005). Further analysis using Tukey's test showed that the primary difference was between the first (m= 4.06) group and the second (m= 3.9) and third (m= 3.75) groups. It is clear from these results that there is a significant difference in the means of these groups. The results of the ANOVA showed a statistically significant difference between the three categories when triglycerides were the dependent variable [F (2, 2956) =8.72, p=.0002]. Further analysis using Tukey's test showed that the primary difference was between the first group (m= 145.57 mg/dl) and the second (m= 129.19 mg/dl) and third (m= 118.13 mg/dl) groups. It is clear from these results that there is a significant difference in the means of these groups. The results of the ANOVA showed a statistically significant difference between the three categories when apolipoprotein-B was the dependent variable [F (2, 2955) = 5.49, p=.0042]. Further analysis using Tukey's test showed that the primary difference was between the first (m= 95.32 mg/dl) group and third (m= 90.24 mg/dl) group. It is clear from these results that there is a significant difference in the means of these groups.

Multivariate Regression Analysis

A multivariate regression analysis was performed to look at the impact of the percentages of saturated fat, polyunsaturated fat, and individual polyunsaturated fatty acids on cardiovascular biomarkers. The independent variables were the percentage saturated fat, the percentage of polyunsaturated fat, and the individual fatty acids. The

dependent variables were the blood biomarkers. Looking at the impact of poly and saturated fat on total cholesterol, a statistically significant model was noted of:

Total Cholesterol = (Percent polyunsaturated fat*-84.89) + (Percent saturated fat*35.40) + 195.75

(p=.0024, Adj. R^2=.0024)

Although the model shows statistical significance, the percent of saturated fats had no statistically significant association individually (p=.228), indicating that they do not contribute significantly to the model. Looking at the impact of poly and saturated fat on triglycerides, a statistically significant model was noted of:

Triglycerides = (Percent polyunsaturated fat*-419.32) + (Percent saturated fat*42.11) + 158.43

(p<.0001, Adj. R^2=.0078)

Although the model shows statistical significance, the percent of saturated fats had no statistically significant association individually (p=.5995), indicating that they do not contribute significantly to the model. Looking at the impact of poly and saturated fat on apolipoprotein-B a statistically significant model was noted of:

Apolipoprotein B= (Percent polyunsaturated fat*-76.93) + (Percent saturated fat*10.39) + 97.55

(p=.0003, Adj. R^2=.0048)

Although the model shows statistical significance, the percent of saturated fats had no statistically significant association individually (p=.5732), indicating that they do not contribute significantly to the model. Looking at the impact of poly and saturated fat on HDL cholesterol, a statistically significant model was noted of:

HDL Cholesterol = (Percent polyunsaturated fat*39.35) + (Percent saturated fat*1.86) + 49.87

(p=.0037, Adj. R^2=.0031)

Although the model shows statistical significance, the percent of saturated fats had no statistically significant association individually (p=.869), indicating that they do

not contribute significantly to the model. Looking at the impact of poly and saturated fat on total cholesterol to HDL ratio, a statistically significant model was noted of:

Total Cholesterol to HDL ratio = (Percent polyunsaturated fat*-4.99) + (Percent saturated fat*.89) + 4.2 (p<.0001, Adj. R^2=.008)

Although the model shows statistical significance, the percent of saturated fats had no statistically significant association individually (p=.348), indicating that they do not contribute significantly to the model.

In evaluating the role that the individual fatty acids play on apolipoprotein-B a statistically significant model was noted as:

Apolipoprotein B= (Percent of Linoleic Acid*-65.22) + (Percent of Linolenic Acid*-275.72) + (Percent of Stearidonic Acid*-10079) + (Percent of Arachidonic Acid*3912.9) + 97.2 (p<.0001, Adj. R^2=.0092).

Although the model shows statistical significance, polyunsaturated fatty acid Linolenic acid had no statistically significant association individually (p=.186), indicating that it does not contribute significantly to the model. In evaluating the role that the individual fatty acids play on the total cholesterol to HDL ratio, a statistically significant model was noted as:

Total Cholesterol to HDL ratio = (Percent of Linoleic Acid*-5.61) + (Percent of Linolenic Acid*-2.86) + (Percent of Arachidonic Acid*223.44) + 4.17 (p<.0001, Adj. R^2=.0113).

Although the model shows statistical significance, polyunsaturated fatty acid Linolenic acid had no statistically significant association individually (p=.7905), indicating that it does not contribute significantly to the model. In evaluating the role that the individual fatty acids play on triglycerides, a statistically significant model was noted as:

Triglycerides = (Percent of Linoleic Acid*-579.97) + (Percent of Linolenic Acid*1165.06) + 162.25 (p<.0001, Adj. R^2=.0089)

Although the model shows statistical significance, polyunsaturated fatty acid Linolenic acid had no statistically significant association individually (p=.198), indicating that it does not contribute significantly to the model. In evaluating the role that the individual fatty acids play on HDL cholesterol, a statistically significant model was noted as

HDL Cholesterol = (Percent of Linoleic Acid*-18.6) + (Percent of Linolenic Acid*255.28) + 50.08 (p=.0009, Adj. R^2=.004)

Although the model shows statistical significance, polyunsaturated fatty acid Linolenic acid had no statistically significant association individually (p=.277), indicating that it does not contribute significantly to the model. In evaluating the role that the individual fatty acids play on total cholesterol, a statistically significant model was noted as:

Total Cholesterol = (Percent of Linoleic Acid*-101.60) + (Percent of Cervonic Acid*2931.33) + 199.13 (p=.0011, Adj. R^2=.0039)

The low adjusted R^2 values indicated much more than just the consumption of these specific polyunsaturated fatty acids alone were contributing to the serum biomarker.

Hypothesis 3

Hypothesis number three is: "The replacement of saturated fat in the diet with monounsaturated fat will reduce cardiovascular risk blood parameters". Evaluation of this hypothesis placed the percentage of monounsaturated fat, monounsaturated to saturated fat ratios, and individual monounsaturated fatty acids as the independent variables and the individual blood biomarkers as the dependent variables.

Pearson Correlation

In looking at the Pearson correlations, statistically significant correlations were seen with a few variables. Evaluation of the percent of monounsaturated fat consumption, as they relate to cardiovascular biomarkers, revealed no statistically significant correlations. There is a statistically significant weak positive correlation between triglycerides and monounsaturated to saturated fat ratio. ($r = .039$, $p = .033$, $n = 2959$). (See Figure 19)

Looking at Pearson correlations between the individual fatty acids, monounsaturated fat Palmitoleic acid shows a statistically significant weak negative correlation with HDL cholesterol ($r = -.055$, $p = .003$, $n = 2986$), weak positive correlation with triglycerides ($r = .050$, $p = .007$, $n = 2959$), weak positive correlation with apolipoprotein-B ($r = .049$, $p = .008$, $n = 2958$), and a weak positive correlation with the total cholesterol to HDL ratio ($r = .073$, $p < .0001$, $n = 2986$). (See Figure 19)

*Figure 19-***Pearson Correlations**

Pearson Correlations				
Independent Variable	Dependent Variable	Probability	Coefficient	Number
Mufa/SF Ratio				
	TRIG	0.0327	0.03925	2959
Palmitoleic Acid				
	HDL	0.0029	-0.0545	2986
	TRIG	0.0071	0.04952	2959
	APO-B	0.008	0.04878	2958
	TC/HDL	<.0001	0.07346	2986

Simple Linear Regression

Further evaluation of the relationship between the consumption of monounsaturated fatty acids consumed and cardiovascular biomarkers led to the performance of a simple linear regression with the percent of the monounsaturated fat

Palmitoleic acid as the independent variable and the cardiovascular biomarkers as the dependent variable. Simple linear regression between the percent of Palmitoleic acid consumed and total cholesterol to HDL ratio, showed a significant relationship with the model:

Total Cholesterol to HDL ratio = Percent of Palmitoleic Acid*53.18 + 3.65 (p<.0001, r^2=.0054)

Simple linear regression between the percent of Palmitoleic acid consumed and triglycerides, showed a significant relationship with the model:

Triglycerides= Percent of Palmitoleic Acid*2999.23 + 115.89 (p=.0071, r^2=.0025)

Simple linear regression between the percent of Palmitoleic acid consumed and apolipoprotein-B, showed a significant relationship with the model:

Apolipoprotein-B= Percent of Palmitoleic Acid*679.1 + 89.39 (p=.008, r^2=.0024)

Simple linear regression between the percent of Palmitoleic consumed and HDL cholesterol, showed a significant relationship with the model:

HDL Cholesterol= Percent of Palmitoleic Acid*-466.52 + 55.42 (p=.0029, r^2=.0030)

Although this regression shows a statistically significant model, the low R-squared involved demonstrates that other variables are relevant factors in predicting cardiovascular biomarkers.

Analysis of Variance

An analysis of variance was performed to look at the effect that monounsaturated fat to saturated fat ratios of differing percentage groups (0-1, 1-1.5, 1.5-2, and 2+) may have on differing cardiovascular biomarkers. The hypothesis under consideration is: The reduction in dietary saturated fat and replacement with monounsaturated fats is associated with reduced cardiovascular risk blood parameters. The results of the ANOVA showed a statistically significant difference between the four categories when triglycerides were the

dependent variable [F (3, 2955) = 5.15, p=.0015). Further analysis using Tukey's test

showed that the primary difference was between the fourth (m= 203.34 mg/dl) group and

the third (m= 134.00 mg/dl), second (m= 128.14 mg/dl), and first (m= 125.54 mg/dl)

groups with the mean of the fourth group being significantly higher than the other three.

It is clear from these results that there is a significant difference in the means of these

groups.

Multivariate Regression Analysis

A multivariate regression analysis was performed to look at the impact of all

individual monounsaturated fatty acids on cardiovascular biomarkers. The independent

variables were the percent of the individual fatty acids and the dependent variables were

the blood biomarkers. In evaluating the role that the individual fatty acids play on the

HDL cholesterol, a statistically significant model was noted as:

HDL Cholesterol = (Percent of Palmitoleic Acid*-614.06) + (Percent of Vaccenic Acid*32.1)

+(Percent of Paullinic Acid*-839.13) + (Percent of Erucic Acid*1595.35) + 53.31 (p=.0022, Adj. R^2=.0042)

Although the model shows statistical significance as a whole, Paullinic acid and

Erucic acid had no statistically significant association individually (p=.103 and p=.165)

indicating that they do not contribute significantly to the model. In evaluating the role

that the individual fatty acids play on the total cholesterol to HDL ratio, a statistically

significant model was noted as:

Total Cholesterol to HDL ratio   = (Percent of Palmitoleic Acid*65.32) + (Percent of Vaccenic

Acid*-2.21) + (Percent of Paullinic Acid*11) +(Percent of Erucic Acid*-83.01) + 3.84 (p=.0003, Adj. R^2=.0058)

Although the model shows statistical significance as a whole, Paullinic acid and

Erucic acid had no statistically significant association individually (p=.800 and p=.393),

indicating that they do not contribute significantly to the model. In evaluating the role

that the individual fatty acids play on triglycerides, a statistically significant model was noted as:

Triglycerides = (Percent of Palmitoleic Acid*4132.84) + (Percent of Vaccenic Acid*-152.61) + (Percent of Paullinic Acid*-5526.13) + (Percent of Erucic Acid*-4793.70) + 134.98 (p=.0021, Adj. R^2=.0043)

Although the model shows statistical significance, Oleic, Paullinic acid and Erucic acid had no statistically significant association individually (p=.093, p=.133, and p=.563), indicating that they do not contribute significantly to the model. In evaluating the role that the individual fatty acids on apolipoprotein-B, a statistically significant model was noted as:

Apolipoprotein-B= (Percent of Palmitoleic Acid*687.51) + (Percent of Vaccenic Acid*17.86) +(Percent of Paullinic Acid*-1696.87) +(Percent of Erucic Acid*-1245.65) + 89.52 (p=.0012, Adj. R^2=.003)

Although the model shows statistical significance as a whole, Paullinic acid and Erucic acid had no statistically significant association individually (p=.103 and p=.117), indicating that they do not contribute significantly to the model. The low adjusted R^2 values indicated much more than just the consumption of these monounsaturated fatty acids were contributing to the serum biomarkers.

Hypothesis 4

Hypothesis number four is: "The replacement of saturated fat in the diet with carbohydrates will reduce cardiovascular risk blood parameters". Evaluation of this hypothesis placed the percent of total carbohydrate and the ratio of carbohydrate to saturated fat consumption as the independent variables and the individual blood biomarkers as the dependent variables.
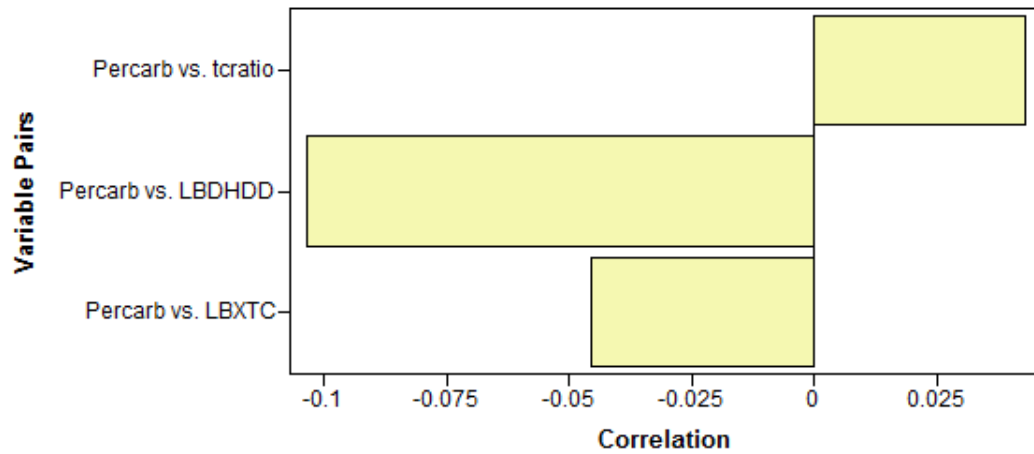
Pearson Correlation

In looking at the Pearson correlations, statistically significant correlations were seen with a few variables. A weak negative correlation was noted between the percent of carbohydrates consumed and total cholesterol (r = -.045, p = .013, n = 2986). There is a statistically significant weak negative correlation between percent carbohydrates and HDL cholesterol (r = -.103 p<.0001 n = 2986). There is a statistically significant weak positive correlation between percent carbohydrates and the total cholesterol to HDL ratio (r = .043, p = .018, n = 2986). There is a statistically significant weak positive correlation between percent fiber and HDL cholesterol (r = .061 p = .0008 n = 2986). There is a statistically significant weak negative correlation between percent fiber and the total cholesterol to HDL ratio (r = -.059, p = .0013, n = 2986). There is a statistically significant negative correlation between percent sugar and HDL cholesterol (r = -.11 p <.0001 n = 2986). There is a statistically significant weak positive correlation between percent carbohydrates and the total cholesterol to HDL ratio. (r = .074, p <.0001, n = 2986). (See Figure 20, 21).

Looking at the ratios between carbohydrates, fiber, sugar, and saturated fat, we noted a few correlations. There is a statistically significant weak negative correlation between carbohydrate to saturated fat ratio and HDL cholesterol (r =-.044, p =.016, n = 2986). There is a statistically significant weak positive correlation between fiber to saturated fat ratio and HDL cholesterol (r =.037, p =.041, n = 2986). There is a statistically significant weak negative correlation between fiber to saturated fat ratio and the total cholesterol to HDL ratio (r =-0.052, p =.004, n = 2986). There is a statistically significant weak negative correlation between sugar to saturated fat ratio and HDL cholesterol (r =-.084, p <.0001, n = 2986). There is a statistically significant weak

89

positive correlation between carbohydrate to saturated fat ratio and the total cholesterol to

HDL ratio (r =.049, p =.007, n = 2986). (See Figure 21)

Figure 20- **Graph of Pearson Correlations between the percentage of carbohydrate consumption and biomarkers (p<.05)**

Figure 21- **Pearson Correlations**

Pearson Correlations				
Independent Variable	Dependent Variable	Probability	Coefficient	Number
Percent Carb				
	TC	0.0132	-0.04537	2986
	HDL	<.0001	-0.10345	2986
	TC/HDL	0.0184	0.04313	2986
Percent Fiber				
	HDL	0.0008	0.06117	2986
	TC/HDL	0.0013	-0.05873	2986
Percent Sugar				
	HDL	<.0001	-0.10970	2986
	TC/HDL	<.0001	0.07420	2986
Carb/SF Ratio				
	HDL	0.0155	-0.04431	2986
Fiber/SF Ratio				
	HDL	0.0407	0.03746	2986
	TC/HDL	0.0043	-0.05226	2986
Sugar/SF Ratio				
	HDL	<.0001	-0.08442	2986
	TC/HDL	0.0069	0.04945	2986

Simple Linear Regression

Further evaluation of the relationship between the carbohydrate, fiber, sugar to saturated fat ratios, and cardiovascular biomarkers with simple linear regression was performed with the carbohydrate, fiber, and sugar to saturated fat ratios as the independent variable and the cardiovascular biomarkers as the dependent variable. Simple linear regression between the percent of carbohydrates consumed and total cholesterol to HDL ratio showed a significant relationship with the model:

Total Cholesterol to HDL ratio = Percent Carbohydrates*.74 + 3.55 (p=.018, r^2=.0019)

Simple linear regression between the carbohydrate to saturated fat ratio and HDL cholesterol showed a significant relationship with the model:

HDL Cholesterol= Carbohydrate to Saturated fat ratio *9.19 + 50.97 (p=.011, r^2=.0022)

Simple linear regression between the fiber to saturated fat ratio consumed and HDL cholesterol showed a significant relationship with the model:

HDL Cholesterol = Fiber to Saturated fat ratio *2.92 + 52.18 (p=.04, r^2=.0014)

Simple linear regression between the fiber to saturated fat ratio consumed and total cholesterol to HDL ratio showed a significant relationship with the model:

Total Cholesterol to HDL ratio = Fiber to Saturated fat ratio *-.35 + 4.02 (p=.004, r^2=.0027)

Simple linear regression between the sugar to saturated fat ratio consumed and total cholesterol to HDL ratio showed a significant relationship with the model:

Total Cholesterol to HDL ratio = Sugar to Saturated fat ratio *.07 + 3.77 (p=.007, r^2=.0024)

Simple linear regression between the sugar to saturated fat ratio consumed and HDL cholesterol showed a significant relationship with the model:

HDL Cholesterol= Sugar to Saturated fat ratio*-1.33 + 55.89 (p<.0001, r^2=.0071)

Although these regressions show a statistically significant model, the low R-squared involved demonstrates that the impact of the model alone is not strong and other variables are relevant factors in predicting cardiovascular health.

Analysis of Variance

An analysis of variance was performed to look at the effect that the carbohydrate to saturated fat ratio of differing percentage groups (low (0-5), medium (5-10), or high (>10)) may have on differing cardiovascular biomarkers. The hypothesis under consideration is: The replacement of saturated fat in the diet with carbohydrates will reduce cardiovascular risk blood parameters. The results of the analysis of variance indicate that there were no statistically significant differences seen between the groups of differing carbohydrate to saturated fat ratios on any cardiovascular biomarkers at the $p<.05$ level for the three categories indicating no differences in the means of the three groups.

Multivariate Regression Analysis

A multivariate regression analysis was performed to look at the impact of the percent of saturated fat and carbohydrates on cardiovascular biomarkers. The independent variables were the percent of total carbohydrates and percent saturated fat, and the dependent variables were the blood biomarkers. In evaluating the role that the dietary makeup plays on the total cholesterol to HDL ratio, a statistically significant model was noted as:

Total Cholesterol to HDL ratio = (Percent Carbohydrates*1.02) + (Percent saturated fat*2.11) + 3.18 $(p=.0082, \text{Adj. } R^2=.0025)$

In evaluating the role that the dietary makeup plays on the HDL cholesterol, a statistically significant model was noted as:

HDL Cholesterol = (Percent Carbohydrates*-24.75) + (Percent saturated fat*-28.41) + 68.35

(p<.0001, Adj. R^2=.012)

In evaluating the role that the dietary makeup plays on the total cholesterol, a statistically significant model was noted as:

Total Cholesterol = (Percent Carbohydrates*-23.13) + (Percent saturated fat*5.81) + 203.75

(p=.046, Adj. R^2=.0014).

The low adjusted R^2 values indicate much more than just the consumption of these nutrients alone were contributing to the serum biomarkers.

Hypothesis 5

Hypothesis number five is: "A diet low in dietary fat is associated with reduced cardiovascular risk blood parameters". Evaluation of this hypothesis placed the percentage of total fat, percentage of carbohydrates, and the carbohydrate to total fat ratio as the independent variables and the individual blood biomarkers as the dependent variables.

Pearson Correlation

In looking at the Pearson correlations, statistically significant correlations were seen with a few variables. A weak negative correlation was noted between the carbohydrate to total fat ratio (Cho/Fat) and HDL cholesterol (r = -.070, p <.0001, n = 2986). There is a statistically significant weak positive correlation between the Cho/Fat ratio and triglycerides (r = .041, p = .0248, n = 2959). There is a statistically significant weak positive correlation between the Cho/Fat ratio and the total cholesterol to HDL ratio (r = .045, p =.0140, n = 2986). (See Figure 22).

Subdividing the carbohydrate to total fat ratio into four categories (0-1, 1-1.5, 1.5-2, and 2+) produced the following significant correlations. There is a statistically significant weak positive correlation between the Cho/Fat ratio (1-1.5) and triglycerides

(r = .083, p = .0032, n =1255). There is a statistically significant weak negative correlation between the Cho/Fat ratio (2+) and HDL cholesterol (r = -.109, p =.0194, n =464). There is a statistically significant weak positive correlation between the Cho/Fat ratio (2+) and triglycerides (r = .098, p =.036, n =462). There is a statistically significant weak positive correlation between the Cho/Fat ratio (2+) and the total cholesterol to HDL ratio (r = .124, p =.008, n =464). (See Figure 22)

In looking at the correlations associated with both a low carbohydrate (<45%), high fat (>35%) diet and a high carbohydrate (>50%), low fat (<30%) diet the following correlations were noted. No correlations were noted between low carbohydrate, high fat diets and cardiovascular biomarkers. There is a statistically significant weak negative correlation between percentage of carbohydrate consumption (in a high carbohydrate, low fat diet) and HDL cholesterol (r = -.122, p = .001, n =713). There is a statistically significant positive correlation between percentage of carbohydrate consumption (in a high carbohydrate, low fat diet) and C-reactive protein (r = .211, p =.022, n =117). There is a statistically significant weak positive correlation between percentage of total fat consumption (in a high carbohydrate, low fat diet) and HDL cholesterol (r = .083, p =.027, n =713). There is a statistically significant weak negative correlation between percentage of total fat consumption (in a high carbohydrate, low fat diet) and triglycerides (r = -.087, p =.021, n =709). There is a statistically significant weak negative correlation between percentage of total fat consumption (in a high carbohydrate, low fat diet) and total cholesterol to HDL ratio (r = -.089, p =.018, n =713). (See Figure 22).

Figure 22-**Pearson Correlations**

Pearson Correlations				
Independent Variable	Dependent Variable	Probability	Coefficient	Number
Carb/TF ratio				
	HDL	<.0001	-0.0702	2986
	TRIG	0.0248	0.04125	2959
	TC/HDL	0.014	0.04496	2986
Carb/TF ratio 1-1.5				
	TRIG	0.0032	0.08309	1255
Carb/TF ratio 2+				
	HDL	0.0194	-0.10853	464
	TRIG	0.0357	0.09777	462
	TC/HDL	0.0075	0.12393	464
Percent Carb (High carb diet)				
	HDL	0.0011	-0.12204	713
	CRP	0.0223	0.2111	117
Percent Fat (High carb diet)				
	HDL	0.0268	0.08294	713
	TRIG	0.0208	-0.08678	709
	TC/HDL	0.0178	-0.08874	713

Simple Linear Regression

Further evaluation of the relationship between the ratio of carbohydrates to total

fat consumed and cardiovascular biomarkers led to the performance of a simple linear

regression with the carbohydrate to total fat ratio as the independent variable and the

cardiovascular biomarkers as the dependent variable. Simple linear regression between

the carbohydrate to fat ratio consumed and HDL cholesterol showed a significant

relationship with the model:

HDL Cholesterol = Carbohydrate to Fat ratio*-2.47 + 56.88 $(p<.0001, r^2=.0052)$

Simple linear regression between the carbohydrate to fat ratio consumed and

triglycerides showed a significant relationship with the model:

Triglycerides = Carbohydrate to Fat ratio *10.08 + 115.43 $(p=.025, r^2=.0017)$

Simple linear regression between the carbohydrate to fat ratio consumed and total cholesterol to HDL ratio showed a significant relationship with the model:

Total Cholesterol to HDL ratio = Carbohydrate to Fat ratio *.135 + 3.7 (p=.0115, r²=.0022).

Although this regression shows a statistically significant model, the low R-squared involved demonstrates that other variables are relevant additional factors in predicting biomarkers.

Analysis of Variance

An analysis of variance was performed to look at the effect that carbohydrate to total fat ratios of differing percentage groups (0-1, 1-1.5, 1.5-2, and 2+) may have on differing cardiovascular biomarkers. The hypothesis under consideration is: The reduction in dietary total fat and replacement with carbohydrates are associated with reduced cardiovascular risk blood parameters. The results of the ANOVA showed a statistically significant difference between the four categories when HDL cholesterol was the dependent variable [F (3, 2982) = 4.54, p=.0035). Further analysis using Tukey's test showed that the primary difference is between the first (m=55.38 mg/dl) and third (m=52.59 mg/dl) group and the first (m= 55.38 mg/dl) and fourth group (m=51. mg/dl). It is clear from these results that there is a significant difference between the groups.

Multivariate Regression Analysis

A multivariate regression analysis was performed to look at the impact of both carbohydrates and total fat on cardiovascular biomarkers. The independent variables were the percent of total fat and carbohydrates and the dependent variables were the blood biomarkers. In evaluating the role that the carbohydrate and fat concentrations play on the HDL cholesterol, a statistically significant model was noted as:

HDL Cholesterol = (Percent of Total Fat*41.68) + (Percent Carbohydrates*-32.84) + 59.23

(p=.0014, Adj. R^2=.016)

In evaluating the role that the carbohydrate and fat concentrations play on the

total cholesterol to HDL ratio, a statistically significant model was noted as:

Total Cholesterol to HDL ratio = (Percent of Total Fat*-4.56) + (Percent Carbohydrates*.94) +

4.69 (p=.0441, Adj. R^2=.006)

The low adjusted R^2 values indicate much more than just the consumption of total

fat and carbohydrates were contributing to the serum biomarker outcomes.

Hypothesis 6

Hypothesis number six is: "A diet low in dietary carbohydrate is associated with

decreased cardiovascular risk blood parameters". Evaluation of this hypothesis placed the

percent of total carbohydrate, percentage of sugar, and percentage of fiber as the

independent variables and the individual blood biomarkers as the dependent variables.

Pearson Correlation

In looking at the Pearson correlations, statistically significant correlations were

seen with a few variables. A weak negative correlation was noted between the percent of

carbohydrates consumed and total cholesterol (r = -.045, p = .013, n = 2986). There is a

statistically significant weak negative correlation between percent carbohydrates and

HDL cholesterol (r = -.103 p <.0001 n = 2986). There is a statistically significant weak

positive correlation between percent carbohydrates and the total cholesterol to HDL ratio

(r = .043, p = .018, n = 2986). There is a statistically significant weak positive correlation

between percent fiber and HDL cholesterol (r = .061 p = .0008 n = 2986). There is a

statistically significant weak negative correlation between percent fiber and the total

cholesterol to HDL ratio (r = -.059, p = .0013, n = 2986). There is a statistically

97

significant negative correlation between percent sugar and HDL cholesterol (r = -.11 p

<.0001 n = 2986). There is a statistically significant weak positive correlation between

percent carbohydrates and the total cholesterol to HDL ratio (r = .074, p <.0001, n =

2986). (See Figure 23).

In looking at the correlations associated with both a low carbohydrate (<45%) and

a high carbohydrate (>65%) dietary consumption, no statistically significant correlations

were noted. In looking at diets that contained 45-65% carbohydrate consumption group,

in line with current AMDR recommendations, the following correlations were noted.

There is a statistically significant weak negative correlation between percentage of

carbohydrate consumption and HDL cholesterol (r = -.061, p =.005, n =2127). There is a

statistically significant weak positive correlation between percentage of carbohydrate

consumption and triglycerides (r = .061, p =.005, n =2109). There is a statistically

significant weak positive correlation between percentage of fiber consumption and HDL

cholesterol (r = .091, p <.0001, n =2127). There is a statistically significant weak

negative correlation between percentage of fiber consumption and total cholesterol to

HDL ratio (r = -.078, p =.0003, n =2127). There is a statistically significant weak

negative correlation between percentage of sugar consumption and HDL cholesterol (r = -

.087, p <.0001, n =2127). There is a statistically significant weak positive correlation

between percentage of sugar consumption and triglycerides (r = .072, p =.001, n =2109).

There is a statistically significant weak positive correlation between percentage of sugar

consumption and total cholesterol to HDL ratio (r = .078, p =.0003, n =2127). (See

Figure 23).

Figure 23- **Pearson Correlations**

Pearson Correlations				
Independent Variable	Dependent Variable	Probability	Coefficient	Number
Percent Carb				
	TC	0.0132	-0.04537	2986
	HDL	<.0001	-0.10345	2986
	TC/HDL	0.0183	0.04313	2986
Percent Fiber				
	HDL	0.0008	0.06117	2986
	TC/HDL	0.0013	-0.05873	2986
Percent Sugar				
	HDL	<.0001	-0.1097	2986
	TC/HDL	<.0001	0.0742	2986
Percent Carb (45-65%) carbohydrate diet				
	HDL	0.0052	-0.06058	2127
	TRIG	0.0053	0.06065	2109
Percent Fiber (45-65%) carbohydrate diet				
	HDL	<.0001	0.09053	2127
	TC/HDL	0.0003	-0.07786	2127
Percent Sugar (45-65%) carbohydrate diet				0
	HDL	<.0001	-0.08735	2127
	TRIG	0.001	0.07168	2109
	TC/HDL	0.0003	0.07763	2127

Simple Linear Regression

Further evaluation of the relationship between the percentage of carbohydrates consumed and cardiovascular biomarkers led to the performance of a simple linear regression with the percent of carbohydrates as the independent variable and the cardiovascular biomarkers as the dependent variable. Simple linear regression between the percent of carbohydrates consumed and total cholesterol to HDL ratio showed a significant relationship with the model:

Total Cholesterol to HDL ratio = Percent Carbohydrates*.74 + 3.55 $(p=.018, r^2=.0019)$

Simple linear regression between the percent of carbohydrates consumed and HDL showed a significant relationship with the model:

HDL Cholesterol = Percent Carbohydrates*-20.95 + 63.38 (p<.0001, r^2=.011)

$\qquad$ **S**imple linear regression between the percent of carbohydrates consumed and total cholesterol showed a significant relationship with the model:

Total Cholesterol = Percent Carbohydrates*-23.9 + 204.77 (p=.013, r^2=.0021)

$\qquad$ Although this regression shows a statistically significant model, the low R-squared involved demonstrates that more than this variable is relevant in predicting cardiovascular biomarkers.

Analysis of Variance

$\qquad$ An analysis of variance was performed to look at the effect that carbohydrate consumption of differing percentage groups (low (<45%), medium (45-65%), or high (>65%)) may have on differing cardiovascular biomarkers. The hypothesis under consideration is: The reduction in dietary carbohydrates are associated with reduced cardiovascular risk blood parameters. The results of the ANOVA showed a statistically significant difference between the three categories when HDL cholesterol was the dependent variable [F (2, 2983) = 10.64, p<.0001]. Further analysis using Tukey's test showed that the primary difference is between the low (m=55.06 mg/dl) and medium (m= 52.34 mg/dl) carbohydrate consumption group. It is clear from these results that there is a significant difference in the groups, particularly between the low and medium carbohydrate groups.

Multivariate Regression Analysis

$\qquad$ A multivariate regression analysis was performed to look at the impact of multiple types of carbohydrates on cardiovascular biomarkers. The independent variables were the percent of total carbohydrates, percent sugar, and percent fiber and the dependent variables were the blood biomarkers. In evaluating the role that the

carbohydrate concentration plays on the total cholesterol to HDL ratio, a statistically significant model was noted as:

Total Cholesterol to HDL ratio = (Percent of Fiber*-5.19) + (Percent of Sugar*1.24) + 3.81 (p<.0001, Adj. R^2=.0088)

The low adjusted R^2 value indicates much more than just the consumption of dietary fiber and sugar were contributing to the serum biomarker.

Chapter V

Discussion

Introduction

This study involved the performance of a retrospective correlational research

assessment from a sample population obtained from the 2005-2016 NHAINES database.

The data, once collected, cleaned, and processed, was evaluated first from the perspective

of the descriptive statistics. This was important for contextual purposes, defining where

our sample population is currently, as well as to be evaluated for clinical correlation with

the results of hypothesis testing. Each hypothesis was then examined to determine their

validity. Following that with the results tabulated and discussed, a final summary and

potential clinical implications will be possible. It will also be possible, from that point, to

note any flaws in the study and point to future directions of research.

Discussion

Hypothesis 1

In evaluating this hypothesis, it was important to first look at the review of the

literature as it is our best indication of what we expect to see. According to the literature,

we expect to see that as the study participant increased the percentage of calories in their

diet from saturated fat, there should be an elevation in the total cholesterol, the LDL

cholesterol, the HDL cholesterol, as well as a possible elevation in the total cholesterol to

HDL ratio (depending on how elevated the LDL is in comparison to the HDL cholesterol). Additionally, based off the review, we should expect to see a reduction in serum triglycerides. These biomarkers do have some fluctuation dependent on the source of the data. Some authors, for example, noted no elevation in LDL cholesterol, while others noted that the impact on HDL cholesterol was different depending on the specific saturated fatty acid source.

Examining the impact of saturated fat consumption, as it relates to this hypothesis, it is important to look at the descriptive statistics as they can help to shine a light on what we would expect to see. The descriptive statistic show that the average overall saturated fat consumption averaged 11% (10.9% in males and 11.2% in females), which is in excess of the 10% or less recommendation of the Dietary Guidelines and nearly double the 5-6% recommendation from the American Heart Association. [70, 71] Despite this elevation, the population's laboratory biomarkers are all within normal limits.

Deeper examination looking at the correlation between the percent of saturated fat consumed daily and the clinical biomarkers tested showed no statistically significant associations. There were neither positive nor negative associations with the consumption of saturated fat and cardiovascular lipid biomarkers. Indicating that the changes in the percent of saturated fat is not associated with these outcomes. This contrasts with what would have been expected based on the literature. A deeper look at the impact of dietary saturated fat on the male population does show that there is a slight elevation in HDL, but this would be a low correlation and may not impact the total cardiovascular risk. In males with low saturated fat consumption, we do see a statistically significant reduction in total cholesterol, which has been used as an indicator of risk, but the effect does not

stretch to other biomarkers, so there is no indication if the impact is positive (reduction in LDL cholesterol) or negative (reduction in HDL cholesterol). Evaluation of the individual fatty acids did show that there are some positive effects seen with the consumption of short and medium chain fatty acids Butyric, Caproic, Caprylic, and Capric, as would be expected. Indicating some beneficial health impact from these fatty acids, specifically in the minor elevation in HDL cholesterol and total cholesterol to HDL ratio. Lauric acid shows the beneficial impact of both reducing the triglycerides and reducing the total cholesterol to HDL ratio, indicating a potential benefit. Stearic acid shows a small increase in the total cholesterol to HDL ratio, indicating that if anything, it could increase risk, but with no other biomarker change it is speculative.

The analysis of variance showed that for groups of differing percentages of saturated fat consumption, there were no differences noted in their impact on cardiovascular lipid biomarkers. This would indicate that accepting the null hypothesis "the reduction of saturated fat in the diet is not associated with reduced cardiovascular risk blood parameters" would be the appropriate assessment in this case.

Looking at the multivariate regression analysis did allow us to develop algorithms to demonstrate the relationship with and predict the HDL cholesterol and the total cholesterol to HDL ratio from the dietary consumption of specific individual fatty acids. This may be beneficial as part of an overall strategy to target specific dietary fats to impact these biomarkers.

In totality, the data suggested that there is no strong evidence that the reduction of saturated fat in the diet would impact biomarkers enough to reduce the incidence of cardiovascular disease and the null hypothesis would have to be accepted. There is some

evidence that the increased consumption of saturated fats, Butyric, Caproic, Caprylic, Capric, and Lauric acid may provide some benefit, but the effect would not be large and may not have a clinical impact; further study would be appropriate.

Hypothesis 2

Hypothesis number two "The replacement of saturated fat in the diet with polyunsaturated fat will reduce cardiovascular risk blood parameters" involved the examination of both the effects of polyunsaturated fat consumption, as well as the impact of the ratio of polyunsaturated to saturated fat in the diet and the impact that alterations here will have on lipid biomarkers. The review of the literature indicated that the polyunsaturated fatty acids in the diet specifically should improve lipid biomarkers through elevations in HDL cholesterol and reductions in LDL cholesterol, total cholesterol, triglycerides, apolipoprotein-B, and the total cholesterol to HDL ratios.

The descriptive statistics currently show that the average participant eats approximately 21.15g daily of polyunsaturated fat (21.77g for males and 20.23g for females) and an average polyunsaturated fat to saturated fat ratio of .70 (.69 for males and .72 for females).

Evaluation of the correlational data on pure percent of polyunsaturated fat in the diet, we can see that there are statistically significant, but weak, associations with elevation of HDL cholesterol and reductions in triglycerides, apolipoprotein B, total cholesterol, and the total cholesterol to HDL ratio. Further evaluation however, when looking at the ratio between polyunsaturated to saturated fat consumptions, reveals the same correlations, but now there is a small reduction in LDL cholesterol noted. Indicating

105

that, while the total percent of polyunsaturated fat showed cardio-protective benefit, the addition of a ratio-based metric by which we examined the role of polyunsaturated fat as it relates to saturated fat consumption to have an increased cardiovascular benefit in decreasing LDL cholesterol.

Looking at individual polyunsaturated fatty acids shows similar cardio protective benefits with linoleic and linolenic acids, both associated with increases in HDL cholesterol and reductions in triglycerides, apolipoprotein-B, and the total cholesterol to HDL ratio. Arachidonic acid on the other hand, is associated only with negative cardiovascular impact, specifically the increase in apolipoprotein-B and the total cholesterol to HDL ratio. It is interesting to note, that linoleic and linolenic are essential fatty acids and must be obtained through dietary sources, a poor diet may be deficient in these, and arachidonic acid is most associated with the inflammatory reaction. [10]

Simple linear regression allowed us to develop a serviceable model to predict the triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, apolipoprotein-B, and the total cholesterol to HDL ratio from the polyunsaturated to saturated fat consumption ratio. This may be useful in guiding clinical recommendations. As these biomarkers are contributed to by so many modifiable factors it is important to note that the model, while significant, did have a low R^2 value indicating that it is accurate in only a few cases.

The participants of the study were then subdivided into three groups based on their polyunsaturated to saturated fat ratios. The groups consisted of those who ate a .5 or less ratio, a group that at a .5-1.0 ratio, and a group that ate over a 1.0 ratio. These groups were then compared to see if there were any significant differences in the means as they relate to the clinical biomarkers. This is done through an ANOVA test. Significant

differences in the means would indicate that there is a clinical effect that is prevalent in one or more groups that differs from the others. Following the ANOVA test, a Tukey's test was then performed to isolate the groups that showed effect. The results of the ANOVA testing showed that there was significant difference in the groups for total cholesterol, total cholesterol to HDL ratio, triglycerides, and apolipoprotein B. The Tukey's test for the total cholesterol and total cholesterol to HDL ratio showed that the .5 group was significantly different than the .5-1.0 and the >1.0 groups, but that the .5-1 group and the >1.0 groups did not significantly differ from each other. This would indicate that there is a significant difference in the reduction in total cholesterol and the total cholesterol to HDL ratio as the polyunsaturated fat ratio increased over .5, but was not significantly different over 1.0. The Tukey's test showed that, in terms of the triglyceride outcome, the .5 group was significantly different than the .5-1.0 and the >1.0 groups, but that the .5-1 group and the >1.0 groups did not significantly differ from each other. Apolipoprotein B saw a significant change when going from the low (<.5) group to the high (>.1) group. Looking at these results together gives the impression that to see cardiovascular benefit, it is important that the ratio of polyunsaturated to saturated fat be higher than .5 and that 1.0 and higher would have the greatest benefit, as it would improve all 4 biomarkers significantly. It is important to note from this context that, as previously mentioned, the sample population average of .70 is less than the more ideal 1.0 ratio, which we see gives the most favorable result. Additionally, since no biomarker favored the <.5 group, there may be concern that the average ratio for the sample population may be too close to this ratio for ideal risk mitigation.

Multivariate regression analysis looked at the percent of polyunsaturated fat and saturated fat and their impact on biomarker outcomes allowed us to develop statistically significant models to predict total cholesterol, triglycerides, apolipoprotein B, HDL cholesterol, and the total cholesterol to HDL ratio. This model may be useful to allow for the prediction of biomarker outcomes based on the individual variables and accordingly, may allow us to more customize the diet accordingly. We can also further subdivide the percent polyunsaturated fat in the individual polyunsaturated fats to develop a working model between Linoleic, Linolenic, Stearidonic, Arachidonic acids, and Cervonic acids and apolipoprotein-B, total cholesterol to HDL ratio, triglycerides, HDL cholesterol, and total cholesterol. Although statistically valid, the models all had low adjusted R^2 values. This is due in part to the multifactorial nature of modifiable risk factors on the outcomes.

Overall, the data, in relation to this hypothesis, shows that the ratio between polyunsaturated to saturated fat consumption demonstrates that a higher ratio is more directly associated with cardiovascular biomarker improvement. First, the correlational results demonstrating statistically significant relationships between the ratio of polyunsaturated to saturated fatty acid and decreased risk blood parameters. Second, the linear regressions showing a statistically significant linear relationship between an increase in the polyunsaturated fatty acid ratio and decreased triglycerides, total cholesterol, LDL cholesterol, apolipoprotein B, the total cholesterol to HDL ratio and increased HDL cholesterol. Third, the results of the ANOVA analysis demonstrate that for groups who consume larger polyunsaturated to saturated fat ratios, there is a statistically significant difference with reduced mean total cholesterol, triglycerides, apolipoprotein B, and the total cholesterol to HDL ratio. It is demonstrated through these

results that, although a diet high in polyunsaturated fat does have a positive effect, this effect is increased when we view it from the lens of the ratio between polyunsaturated to saturated fat. This would lead to a rejection of the null hypothesis and acceptance of the hypothesis that "The replacement of saturated fat in the diet with polyunsaturated fat will reduce cardiovascular risk blood parameters".

Hypothesis 3

Hypothesis number three "The replacement of saturated fat in the diet with monounsaturated fat will reduce cardiovascular risk blood parameters" involved the examination of both the effects of monounsaturated fat consumption, as well as the impact of the ratio of monounsaturated to saturated fat in the diet and the resultant effects of dietary manipulation will have on cardiovascular lipid biomarkers. The review of literature was less clear as to the expected impact of replacing saturated fat with monounsaturated fat. Overall, like polyunsaturated fat, there should be a reduction in LDL cholesterol, minimal change in HDL cholesterol, reduction in triglycerides, reduction in total cholesterol, and reduction in the total cholesterol to HDL ratio. These results would indicate an overall positive cardiovascular profile that would be associated with the replacement of saturated fat with monounsaturated fat.

The descriptive statistics for the monounsaturated fat consumption of the sample population shows that the average participant consumed 12.3% of their dietary calories from monounsaturated fat (11.6% for males and 12.4% for females). Looking at the monounsaturated to saturated fat ratio in the sample population, shows the average participant has a 1.12 ratio. This ratio is 1.13 for males and 1.11 for females.

A review of the Pearson correlations between the percent of monounsaturated fat consumption and cardiovascular biomarkers reveals no correlations of any kind, indicating that dietary consumption of monounsaturated fats is not associated with cardiovascular biomarkers. Looking at the monounsaturated to saturated fat ratio, a statistically significant correlation was noted with an increase in triglycerides from an increase in the ratio. Although just one biomarker, this is a slightly negative outcome for cardiovascular health. Looking deeper into the correlation and evaluating the individual monounsaturated fatty acids, shows correlations between palmitoleic acid and decreased HDL cholesterol, increased triglycerides, increased apolipoprotein-B, and increased total cholesterol to HDL ratio.

Simple linear regression with palmitoleic acids shows statistically significant models between palmitoleic acids and increased total cholesterol to HDL ratio, increased triglycerides, increased apolipoprotein-B, and lowered HDL cholesterol. These regression analyses would allow for the potential to model the impact of palmitoleic acid on cardiovascular risk.

For comparative purposes, the participants were separated into 4 categories by their differing monounsaturated to saturated fat ratios (0-1, 1-1.5, 1.5-2, and >2). Analysis of variance was performed to see if the 4 groups differed in their respective means of cardiovascular biomarkers. A statistical significance was noted between the 4 groups and serum triglycerides. Analysis using Tukey's test showed that the fourth group revealed a more statistically significant deviation than the other three groups. This data would suggest that at a ratio of >2 monounsaturated fat to saturated fat consumption,

there is a statistically significant increase in serum triglycerides, which would increase risk. The average monounsaturated to saturated fat ratio was below this >2 threshold.

Multivariate regression analysis looked at the impact of the individual monounsaturated fatty acids and cardiovascular outcomes that allowed for the development of statistically significant models for the prediction of HDL cholesterol, the total cholesterol to HDL ratio, triglycerides, and apolipoprotein-B. Although the low adjusted R^2 of the model, due to the multifactorial nature of these biomarkers, reduced the effect of the model.

In looking at the hypothesis, the data failed to show that there is any significant positive benefit in terms of the replacement of saturated fat with monounsaturated fat on cardiovascular biomarkers. One monounsaturated fatty acid, palmitoleic acid, is associated with an increase in cardiovascular risk blood parameters. Based on the data presented, we must reject the hypothesis "The replacement of saturated fat in the diet with monounsaturated fat will reduce cardiovascular risk blood parameters" and accept the null hypothesis.

Hypothesis 4

Hypothesis number four "The replacement of saturated fat in the diet with carbohydrates will reduce cardiovascular risk blood parameters" involved the examination of both the effects of total carbohydrate consumption, as well as the impact of the ratio of carbohydrate to saturated fat consumption in the diet and their effects on cardiovascular lipid biomarkers. The review of literature was consistent in many regards when looking at the results of replacing dietary saturated fat with carbohydrates.

According to the literature, there should be a lowering of the HDL cholesterol, an increase, decrease, or no change (depending on carbohydrate source) in LDL cholesterol, an increase in triglycerides, and an increase in the total cholesterol to HDL ratio. These biomarker findings would be consistent with a negative impact from the replacement of saturated fat with cholesterol in the diet. One caveat would be that if a higher percentage of those carbohydrates were in the form of fiber, we may be able to expect a larger benefit when compared to either undefined carbohydrates or even sugar.

Examination of the descriptive statistics for the sample population shows that the average participant consumed 48.9% of their daily calories (48.2% males and 50% females). This is within, if not on the low end, of the Acceptable Macronutrient Distribution Range for carbohydrates (45-65%). Sugar consumption for the population averaged 131.08g/day (134.51g/day males and 126.07g/day females) and fiber consumption averaged 19.67g/day (20.3g/day males and 18.75g/day females). The sugar consumption was in excess of the Dietary Guidelines and the fiber consumption did not meet the guideline requirements, predisposing this population to chronic diseases including obesity and cardiovascular disease. [70] The average carbohydrate to saturated fat ratio for the sample population was 4.45.

The Pearson correlational data from the study that examined the relationships between the percent of carbohydrates consumed and cardiovascular biomarkers revealed statistically significant decreases in the total cholesterol and HDL cholesterol and an increase in the total cholesterol to HDL ratio. Looking at the carbohydrate to saturated fat ratio shows a reduction in HDL cholesterol, although to a lesser degree than the percent of total carbohydrates. These findings would be consistent with increased cardiovascular

risk. Examination of the correlation between the percent of fiber intake, the fiber to saturated fat ratio, and biomarkers showed an increase in the HDL cholesterol and a decrease in the total cholesterol to HDL ratio. These findings would be consistent with improved cardiovascular outcomes. The relationship between the percent of sugar consumption, the sugar to saturated fat ratio, and cardiovascular biomarkers shows reductions in the HDL cholesterol and increases in the total cholesterol to HDL ratio (the correlational consistent for the percentage of total sugar were larger than with the sugar to saturated fat ratio). These findings are consistent with an increase in cardiovascular risk.

Simple linear regression analysis of the percent of carbohydrate intake and the total cholesterol to HDL ratio showed a predictive model that allowed us to estimate how much of an increase to expect from increased dietary carbohydrates. Regression analysis of carbohydrate to saturated fat ratio allowed for the same model for the elevation in HDL cholesterol. Looking at the fiber to saturated fat ratio gave us models for predicting the reduction in total cholesterol to HDL ratio and the increase in HDL cholesterol. Looking at the sugar to saturated fat ratio gave us models for predicting the elevation in total cholesterol to HDL ratio and the decrease in HDL cholesterol. Even though these models are statistically significant, their primary use is to further demonstrate the linear relationship between these dietary variables and the cardiovascular biomarker outcomes.

The study participant data was separated into three groups by their carbohydrate to saturated fat ratio. These groups were low (<5), medium (5-10), and high (>10) ratios. Evaluating these three groups to look at their differences, as they related to the cardiovascular biomarkers, demonstrated no statistically significant differences between the groups. This may be due, in part, to the low correlation between the carbohydrate to

saturated fat ratios and cardiovascular outcomes. Based on this analysis of variance we would accept the null hypothesis that there is no difference in the ratio of carbohydrate to saturated fat ratio and cardiovascular biomarker outcomes.

Multivariate regression analysis looked at the percent of dietary intake of carbohydrates and saturated fat together as they related to cardiovascular biomarkers which allowed us to develop statistically significant models to predict the impact of alterations in these dietary variables and the total cholesterol to HDL ratio, HDL cholesterol, and total cholesterol. The low adjusted R^2 of these models indicated that their predictive strength is not robust, but it does help to further elucidate the relationship between these variables and outcomes.

Overall, the data presented as part of the evaluation of hypothesis four was consistent to some degree with the literature. The literature found that the replacement of saturated fat with carbohydrates would either not improve or even increase cardiovascular risk blood parameters and in this regard, the data does not disagree. Carbohydrate consumption and the carbohydrate to saturated fat ratio did show minimally negative to no impact on cardiovascular health, although some biomarkers like triglycerides, did not align with expectations from the literature. In total, the role of replacing saturated fat with carbohydrates failed to demonstrate any cardiovascular benefit and therefore the hypothesis is rejected.

Hypothesis 5

Hypothesis number five "A diet low in dietary fat is associated with reduced cardiovascular risk blood parameters" involved the examination of both the effects of the

114

percent of total fat consumption and total carbohydrate consumption as well as the impact of the ratio of carbohydrate to total fat in the diet and their effects on cardiovascular lipid biomarkers.

Evaluation of the descriptive statistics showed that the average total fat consumption of the sample population was 34.05% of dietary caloric intake (33.7% male and 34.6% female). This percentage is within the AMDR of 20-35% but on the higher end of the scale indicating that our sample population had a high normal percent of their caloric intake of total fat. [70] The ratio of carbohydrate to total fat consumption as a percentage of total calories was 1.43.

Pearson correlations performed to look at the correlations between the ratio of carbohydrate to total fat consumption and cardiovascular biomarkers showed a reduction in HDL cholesterol, elevation in triglycerides, and elevation in the total cholesterol to HDL ratio. These numbers would suggest that elevations in the total carbohydrate to fat ratio would be associated with increased cardiovascular risk blood parameters.

Separating the participants into four groups by their carbohydrate to total fat ratio, (0-1,1-1.5,1.5-2, and 2+) we see that there is an elevation in the triglycerides for the 1-1.5 group and an elevation in triglycerides, a reduction in the HDL cholesterol and an elevation in the total cholesterol to HDL ratio for the 2+ group. These findings indicate a negative impact on cardiovascular biomarker outcomes particularly in the 2+ carbohydrate to total fat group.

Separation of the groups into either a low carbohydrate (<45%) and high fat (>35%) diet or a high carbohydrate (>50%) and low fat (<30%) diet showed that in a

high carbohydrate low fat diet the percentage of carbohydrates was associated with reduction in HDL cholesterol and elevation of C-reactive protein, a biomarker for inflammation. Additionally, in the high carbohydrate group the percentage of total fat was associated with elevations in HDL cholesterol, reductions in triglycerides, and reduction in the total cholesterol to HDL ratio. These findings suggest that in a high carbohydrate low fat environment elevation in carbohydrate are associated with poor cardiovascular and inflammatory biomarkers and elevations in total fat are associated with improved cardiovascular biomarkers.

Simple linear regression that analyzes the carbohydrate to total fat ratio as related to cardiovascular biomarker outcome allows for the development of a statistically significant model for the prediction of reduction in HDL cholesterol, elevation in the total cholesterol to HDL ratio, and elevation in triglycerides. The low r^2 for these models would indicate that the best use for this model is to demonstrate the relationship between this ratio based metric and cardiovascular biomarker outcomes.

The separation of the participants into four groups by their carbohydrate to total fat ratio (0-1, 1-1.5, 1.5-2, and 2+) and the evaluation of the differences between these groups allowed for us to test the hypothesis that there is a difference between the groups in relation to cardiovascular outcomes. The resulting ANOVA performed showed that there was a significant difference in the groups in respect to HDL cholesterol. Further analysis using Tukey's test showed that the differences were largely between the low (0-1) and medium/high (1.5-2) and low and high (2+) groups. This indicated that the ratios of carbohydrate to total fat consumption of 1.5 or higher would be most likely to result in statistically significant reductions in HDL cholesterol. It is interesting to note, that the

current dietary consumption of the sample population of 1.43 was nearing, but not quite, at this 1.5 group.

Multivariate regression analysis that examined the impact of both total fat and carbohydrates as a percentage of caloric intake with cardiovascular biomarkers, allowing us to develop a statistically significant predictive model between these nutritional variables and HDL cholesterol, as well as the total cholesterol to HDL ratio. The low adjusted R^2 of these models, however, indicate that the predictive strength of these models is not strong.

Evaluation of the data associated with the fifth hypothesis that "A diet low in dietary fat is associated with reduced cardiovascular risk blood parameters" showed that there is a relationship between dietary total fat consumption and cardiovascular biomarker outcome. This was seen from the point of view of carbohydrate as the replacement nutrient. Examination of the increase in carbohydrate as it related to total fat showed an increase in cardiovascular risk blood parameters, indicating that the reduction in dietary fat and replacement with carbohydrate may not be effective. Further evaluation looked at diets of differing ratios and compositions and showed that the reduction in total fat was not going to be beneficial to cardiovascular outcomes and in fact would, to some degree, increase cardiovascular risk blood parameters. Therefore, the fifth hypothesis is rejected and a diet lower in total fat is not recommended.

Hypothesis 6

Hypothesis number six "A diet low in dietary carbohydrate is associated with decreased cardiovascular risk blood parameters" involved the examination of the effects

of the percent of carbohydrates, percent of sugar, percent of fiber, as well as the impact that differing carbohydrate percentages will have on lipid biomarkers. According to the literature review, a low carbohydrate diet has been linked with improved overall cardiovascular biomarkers, particularly, as noted, with reduced or non-impacted LDL cholesterol, elevations in HDL cholesterol, and reduction in serum triglycerides.

Examination of the Pearson correlational research associated with the percentage of carbohydrate in the diet and cardiovascular biomarkers, as previously mentioned, showed a reduction in total cholesterol, reduced HDL cholesterol, and an increased total cholesterol to HDL ratio. Additionally, in looking at the percent of fiber we see elevations in the HDL cholesterol and reduction in the total cholesterol to HDL ratio. Looking at sugar, on the other hand, shows a reversal of this with reductions in HDL cholesterol and elevations in the total cholesterol to HDL ratio. These findings would indicate that a reduction in dietary carbohydrate would be associated with more favorable cardiovascular biomarkers. The only exception would be fiber, whose elevation would be associated with cardiovascular beneficial biomarkers.

Separating the study participants into three groups of differing dietary carbohydrate concentrations (<45%, 45-65%, and >65%) of caloric intake allowed us to further evaluate the impact of dietary carbohydrate consumption. There were no statistically significant correlations noted in either the high or low carbohydrate group. It is only when we looked at the 45-65% carbohydrate group where we saw statistically significant correlation, 45-65% is the current AMDR for carbohydrates. [70] It was in this group where the percent of carbohydrate consumed is associated with reduced HDL cholesterol and increased triglycerides. The percent of sugar consumed in the 45-65%

118

group was associated with decreased HDL cholesterol, increased triglycerides, and reduced total cholesterol to HDL ratio. Additionally, total fiber in this group was correlated to elevation in HDL cholesterol and reduction in the total cholesterol to HDL ratio. These numbers would indicate that there is a slight increase in risk for those whose carbohydrate consumption is in the 45-65% of daily calorie range.

As we have seen already, simple linear regression analysis showed that there is a statistically significant linear model that demonstrated the relationship between the total percentage of carbohydrate intake and reduction in total cholesterol, reduction in HDL cholesterol, and elevation in the total cholesterol to HDL ratio. The best use for these models remains to further elucidate the linear relationships that exist between the independent variables and cardiovascular outcomes.

Separation of the study participants into three groups of differing dietary carbohydrate concentrations (<45%, 45-65%, and >65%) allowed us to perform an analysis of variance to test the hypothesis that there will be a difference in the means of the three groups and the cardiovascular biomarkers of interest. The ANOVA showed that, in relation to the HDL cholesterol, there was a significant difference noted and the post-hoc Tukey's analysis indicated that it was between the low carbohydrate (<45%) and medium (45-65%) carbohydrate groups. This finding would suggest that there is a significant reduction in the serum HDL as the carbohydrate percentage increased.

Multivariate analysis that looked specifically at the impact of the percent of fiber and percent of sugar (as sub classifications of carbohydrates) allowed us to develop a statistically significant model that allowed for the prediction of the effect of either elevating fiber or reducing sugar can have on the total cholesterol to HDL ratio. The low

adjusted R^2 indicates that although statistically valid the model is likely not strong enough to draw clinical inference.

The evaluation of the data associated with this hypothesis showed some strong answers. There is correlation between carbohydrate consumption and cardiovascular biomarkers with higher percentages of dietary consumption of carbohydrates associated with decreased total cholesterol, HDL cholesterol, and an increased total cholesterol to HDL ratio. Higher carbohydrate to total fat ratios were correlated with decreased HDL cholesterol, increased triglycerides, and an increase in the total cholesterol to HDL ratio. Regression analysis showed that higher carbohydrate consumption, as a percentage, showed a linear relationship with reduced HDL cholesterol and an increase in the total cholesterol to HDL ratio and higher percentages of carbohydrate to fat ratios are linearly associated with reduced HDL cholesterol, increased triglycerides, and an increase in the total cholesterol to HDL ratio. Added to this were similar correlations with the percent of sugar consumption indicating reductions in both total carbohydrates and sugar would be beneficial for cardiovascular biomarker risk improvement. Separation of carbohydrate consumption into groups of differing percentages did support that the lower carbohydrate groups did not carry the negative biomarker impact as the 45-65% group. The >65% group only contained 55 participants, so it was difficult to ascertain if there were increased or decreased risks associated with that group. Conversely as the sample population had a minimum percent carbohydrate consumption of 27%, an assessment of a ketogenic or extremely low carbohydrate diet could not be performed. Further research should be undertaken to look at these diets and their impact. Additionally, there is enough evidence to support that, while reducing carbohydrate may be of benefit, the carbohydrate

consumed should include more fiber, as it has been shown to reduce cardiovascular risk blood parameters. Overall, the data suggested that we should accept the hypothesis that "A diet low in dietary carbohydrate is associated with decreased cardiovascular risk blood parameters".

Summary

The objective of this study was to examine the role of macronutrient ratio manipulation on cardiovascular biomarkers and attempt to ascertain some clinical recommendations as to these macronutrient ratios. The overall scope of the studies' independent variables was limited to the macronutrients of fat and carbohydrates, including their sub classifications. Other nutritional variables were not examined, so as to keep the scope of the study focused and manageable. Dependent variables, in the form of cardiovascular lipid biomarkers, were limited to total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, apolipoprotein B, C reactive protein, and the total cholesterol to HDL ratio. This limitation was based on data availability from the NHAINES database.

Data collection and processing was a straightforward process that allowed for an assessment of the nutritional variables as they related to the laboratory outcomes. Initial assessment began with a review of the descriptive statistics. This allowed for us to gain some context of the standard diet of the sample population. It was from here that we made clinical recommendations. The most striking thing, from the standpoint of the descriptive statistics, was the fact that the average dietary consumption not only followed the Acceptable Macronutrient Distribution Range, but was lower in carbohydrate than expected before conducting the study. The only values that were out of range, when looking at average consumption, were the exceptionally high sugar intake, the very low

121

fiber intake, and the elevation in saturated fat consumption all three of which were outside of the associated dietary guidelines. [70] Interestingly, fiber consumption and sugar consumption became relevant factors for discussion for clinical implications.

Examination of the individual hypotheses did not go as expected prior to undertaking this study, but were revealing in that dietary trends, and their associated laboratory outcomes, do allow for the development of clinical recommendations.

The results suggested that saturated fat, which has been a point of contention in the nutritional community, was not associated with any outcomes in either a positive or negative manner. This would indicate that it is borderline irrelevant how much saturated fat is consumed, as it relates to lipid biomarkers. We did see that the specific saturated fats ex. caproic, caprylic, and capric did show beneficial effects on biomarkers. This clinically could be important as it shows that, despite a lack of significant effect of total saturated fat consumption on lipid biomarkers, an inclusion of foods containing these specific fatty acids may be particularly of benefit. Additionally, although saturated fat consumption was not relevant when viewed by itself, the addition of polyunsaturated fat to saturated fat consumption adds relevance to the total saturated fat consumption as part of the ratio. It is worth noting at this point, that cardiovascular risk in this study is discussed from the context of lipid biomarkers and the real risk and incidence of cardiovascular disease will be made up of many factors beyond biomarkers.

Polyunsaturated fat, as expected, was associated with improvement of almost all cardiovascular biomarker metrics. A reduction in LDL cholesterol was noted, although outside the level of significance for the study. Looking at the individual fatty acids showed that linoleic and linoleic acids are particularly beneficial and food sources rich in

these acids may present a good clinical recommendation for inclusion in the diet. Arachidonic acid, on the other hand, was not of benefit to cardiovascular health and, despite the overall beneficial impact of polyunsaturated fats, clinically excluding foods rich in arachidonic acid may provide a decrease in cardiovascular risk. In taking the ratio of polyunsaturated fat to saturated fat into account, we saw not only a good area for clinical recommendation but also an increased relevance to saturated fat recommendations. In taking the ratio of polyunsaturated to saturated fat into account, we see the inclusion of LDL cholesterol reduction into the clinically significant effects. Additionally, when we looked at groups that ate diets of differing polyunsaturated to saturated fat ratios, we saw that the greatest positive impact was seen at groups that had a ratio of 1.0 or higher. The clinical recommendation here is that a diet contains a ratio of 1.0 polyunsaturated fatty acids or higher is going to be more associated with decreased cardiovascular risk blood parameters. This recommendation is in line with the American Heart Association's recommendation that saturated fat in the diet be replaced with polyunsaturated fatty acids, as this replacement would increase the polyunsaturated to saturated fat ratio.[7]

Monounsaturated fat did not show any significant impact on cardiovascular health other than the potential for an increase in the monounsaturated fat to saturated fat ratio to increase serum triglycerides, particularly are ratios higher than 2.0. Additionally, the monounsaturated fatty acid palmitoleic acid was associated with significant negative impact. The clinical recommendation here would be to keep the monounsaturated fat to saturated fat ratio below 2.0 and to reduce the dietary consumption of foods rich in palmitoleic acid.

Carbohydrates showed to be an unsuitable replacement for saturated fat in the diet. First of all, the overall impact of carbohydrates on cardiovascular biomarkers is a negative one reducing the total cholesterol, the HDL cholesterol, and increasing the total cholesterol to HDL ratio. Looking specifically at carbohydrate to saturated fat consumption as a ratio, we see less of an effect on biomarkers but the effect, reducing HDL cholesterol, is still a negative one. Part of the issue would be that some of the saturated fat would be sugar which shows to have a larger impact on HDL cholesterol reduction by itself, and the sugar to saturated fat ratio compared to the overall percent of carbohydrate consumption or as part of the carbohydrate to saturated fat ratio. The percent of fiber intake and the fiber to saturated fat intake ratio had the opposite effect and elevated HDL cholesterol indicating a cardio protective element to fiber intake increases. Looking at the carbohydrate to saturated fat ratio did not yield clinical recommendations as to the ratio of carbohydrates to saturated fat, but did result in the recommendation to reduce sugar consumption and to increase fiber consumption.

Looking at the ratio of carbohydrate consumption as it relates to total fat consumption did allow for some more useful interpretations. As noted earlier, the total percent of carbohydrates consumed had negative impact on some cardiovascular biomarkers (total cholesterol, HDL cholesterol, and the total cholesterol to HDL ratio). Additionally, when we look at the percent of carbohydrate to the total fat ratio we not only saw an impact on reducing HDL and increasing the total cholesterol to HDL ratio but also increases in serum triglycerides. Subdividing these groups by ratio and by percentage would indicate the negative impact on cardiovascular biomarkers will increase as the ratio goes over a 1.5 ratio or as the percent of carbohydrate increases. Clinically the

resultant recommendation from this would be that the ideal ratio between carbohydrate consumption and total fat consumption would be below a 1.5 ratio. If we used the AMDR of 45-65% for total carbohydrates and 20-35% of fat, we see that their recommendations range from 1.28 to 3.25 which would put the clinical recommendations on the lower end of the AMDR range. [70]

Looking at a lower carbohydrate diet as a means of impacting cardiovascular risk falls in line with the above findings in regards to the total percentage of carbohydrate consumption and the carbohydrate to total fat recommendations previously laid out. The results of this study showed that there is an increase in biomarker impact when the dietary carbohydrates are kept between 45-65% of total calories. This would lead to a clinical recommendation that carbohydrate consumption should be kept below 45%, although the exact amount has yet to be determined due to limitations in the sample population data available.

The above mentioned ratios between both carbohydrates and fats, and their associated subcategories, show some consistencies as well as deviations, when compared to the literature review. Although the studies evaluated looked more at the impact of nutrient replacement rather than establishing determinations of individual ratios, there is some area of convergence. The American Heart Association, for example, did not specify the specific macronutrient ratio between polyunsaturated fatty acids and saturated fatty acids, nor did they make specific recommendations as to the total fat to carbohydrate ratios as this study has done. Regardless of this, the ratios suggested by this study do fall in line with the guidelines of the American Heart Association's position statement that the replacement of fat with carbohydrates was a poor approach and that polyunsaturated

fat should outweigh saturated fat. [7] The Acceptable Macronutrient Distribution Range is one area where we do see divergence, as the suggested amount of carbohydrate in the diet (45-65%) would be above the studies' carbohydrate to total fat recommendations, although if we used the total fat recommendations and the saturated fat recommendations we would likely be above the 1.0 polyunsaturated to saturated fat ratio suggested by this study. [70] The research regarding the use of Ketogenic diets can neither be corroborated nor refuted by the findings of this study, as there were no participants who met the definition of a ketogenic diet. That being said, the ratios defined by the study did support a lower carbohydrate approach, a ketogenic diet could in fact be designed to match the ratios identified in the study, and therefore it is still possible that future research could better associate this study's ratios with a ketogenic approach. As far as a low-fat high carbohydrate diet is concerned, the ratios identified as being most associated with cardiovascular health did not support a low-fat dietary approach. In this regard the study results did suggest that a low-fat dietary approach is not appropriate for reducing cardiovascular biomarker risk.

The data obtained in this study has been, as previously discussed, consistent with the literature and the expectations from the review of literature. There were some places where the data deviated from the expected, LDL cholesterol, for example, did not appear as a significant result for many of the analyses. Analysis of the data, within the context of the hypotheses, did allow for the determination of several clinical recommendations. The first recommendation should be to limit dietary carbohydrates to no more than 45% of the overall diet. It was above this percentage where we began to see detrimental impact on cardiovascular risk blood parameters. Additionally since the role of sugar was

significantly negative as an outcome for cardiovascular biomarkers, the second

recommendation would be to make sure that the majority of the calories from

carbohydrates come through increased fiber and more complex carbohydrates. Sugar

consumption should be reduced back to the Dietary guidelines of >10% of caloric intake.

Fiber should be consumed at a minimum of the dietary guidelines with the remainder of

carbohydrate in the form of more complex or lower glycemic carbohydrates. The third

recommendation in this respect, would be to keep the carbohydrate to total fat ratio below

1.5. If dietary carbohydrate is 45%, for example, than fat should be no less than 30% this

would allow us to maintain protein at 25% which is within the AMDR of 10-35% for

protein.[70] The fourth recommendation would be to make sure the polyunsaturated to

saturated fat ratio is kept at a minimum of 1.0 which would mean that if, for example, we

were to consume 10% or less of our calories from saturated fat, according to the Dietary

Guidelines, then we would need to have 10% be from polyunsaturated fat at a

minimum.[70] Saturated fat food sources should be rich in caproic, caprylic, and capric

acid. Polyunsaturated fat food sources should be rich in linoleic and linolenic acid and be

lower in arachidonic acid. The fifth recommendation would then be to make sure the

monounsaturated to saturated fatty acid ratios should be no higher than 2.0. In the above

example, 10% would fit within this model. Foods rich in palmitoleic acid should be

limited in this group.

     Many configurations of the above guideline, including a fully ketogenic diet, do

exist and should be studied and refined over time based on future research but the results

of this study would indicate that a diet that consists of a maximum 45% carbohydrate, a

minimum 30% fat (with a minimum 10% polyunsaturated and maximum 10% saturated

fat ratio), and 25% protein would be more associated with favorable cardiovascular biomarkers while remaining within the recommendations for the AMDR. [70]

Study Limitations

Limitations of this study are derived in several areas. First, the scope of the study looked at the interplay between two macronutrients but failed to consider all three macronutrients, as well as the micronutrients that may either impact cardiovascular health or may be required as cofactors in the metabolism and absorption of the examined macronutrients.

Another important limitation is from the data available through the NHANES database. The laboratory values examined were among the more commonly used laboratory values used for cardiovascular screening in the healthcare setting but may not be reflective of all potential biomarkers associated with cardiovascular health. These biomarkers were either not performed or not performed on all subjects, limiting the conclusions that can be extrapolated.

The data obtained through the nutritional 48-hour food diaries, while thorough, did not take certain important factors into account, such as the level of processing or the presence or absence of organic products in the diet. These factors may not have impact, but should be taken into consideration, particularly if we were to look deeper into individual fatty acid consumption.

The nature of the data lacked continuity and represented a single moment in time. The data consisted of a 48-hour food diary and a single blood draw. An examiner must assume the diet was representative of the patient's actual dietary intake, but this may not

be true as participants may either not be truthful or have altered their eating while recording (although these issues were asked as questions on the survey). In addition to pure error in the collection of data, the concern arises that the examiner does not see any trends associated with the data. If, for example, a participant was eating unhealthily for a long period of time but had recently started eating healthily three months prior, we may be judging laboratory values based off a non-representative eating history. Additionally, laboratory values do fluctuate both based on dietary trends and based on recent eating patterns. Repeated laboratory values may provide a more accurate picture of cardiovascular biomarker status when compared to a single lab value.

Finally, retrospective studies carry inherent limitations. The examination of a snapshot into a participant's past does not necessarily tell a whole story, nor does it truly encapsulate the effect that their current state will have on future outcomes. Therefore, all conclusions drawn from this study should be taken within the context that they are a representation of the sample population's health status as they relate to their eating habits at the time of recording. Further extrapolation into interventions should be accompanied by randomized control trials that have been informed by this data before widespread use in clinical practice or future guidelines.

Future research Directions

Future research should look to examine the data again with a look at protein as an included macronutrient factor. The review of the literature suggested that protein plays a role in the biomarker outcomes associated with cardiovascular disease, so future research that includes the evaluation of protein as part of the macronutrient ratio markup may shed further light of potential nutritional recommendations. Additionally, further research into

129

this area should include all lifestyle factors that may impact cardiovascular biomarkers, examples including smoking and alcohol consumption.

Genetic differences in individuals indicated that there may be polymorphisms associated with nutritional metabolism. These alterations in metabolism may have differing impacts on the impact of nutrients on lipid biomarkers and predisposition to cardiovascular risk. Two individuals who eat the same diet may then have two differing reactions to nutrients and this may lead do differing effects, either in total or in degree of effect. Examination into the genetic and even epigenetic factors associated with nutrient metabolism may prove helpful in defining the impact of recommendations on outcome.

As this research has been a correlational retrospective study, it is important to look at the potential impact that these findings may have with real patients. The concern here is that, while these findings may represent dietary trends that support cardiovascular health, they may also just be findings consistent with a healthy population and do not work on an interventional level. A prospective study that uses these recommendations as part of a randomized control trial may help to demonstrate if these findings have translational merit.

References

1. CDC - National Center for Health Statistics-Age Age-adjusted death rates for selected populations: United States, 2015 and 2016. https://www.cdc.gov/nchs/products/databriefs/db293.htm. Accessed March 20, 2018

2. CDC - National Center for Health Statistics-Heart Disease Facts https://www.cdc.gov/heartdisease/facts.htm Accessed February 18, 2020.

3. World Health Organization-Top 10 Global Causes of Death 2016 http://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death Accessed March 20, 2018

4. World Health Organization-Top 10 Global Causes of Death 2000 http://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death Accessed March 20, 2018

5. CDC - National Center for Health Statistics- Cost of Care for Medicare Beneficiaries Diagnosed with Heart Disease - Total Costshttps://www.cdc.gov/dhdsp/maps/quick-maps/costs-total.htm Accessed June 05, 2018

6. CDC - National Center for Health Statistics- Heart Disease Death Rates, Total Population Ages 65+https://www.cdc.gov/dhdsp/maps/national_maps/hd65_all.htm Accessed June 05, 2018

7. Sacks F, Lichtenstein A, American Heart A, et al. Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. *Circulation* [serial online]. July 18, 2017; 136(3):e1-e23. Available from: CINAHL Plus with Full Text, Ipswich, MA. Accessed July 29, 2018.

8. Keys, A. Seven countries : a multivariate analysis of death and coronary heart disease. Cambridge, Mass. Harvard University Press. 1980.

9. Philip C. C. LIPIDS: A hole in the diet-heart hypothesis? *Nature Reviews Cardiology* [serial online]. 2016; Available from: Journals@OVID, Ipswich, MA. Accessed July 29, 2018.

10. Brassard D, Arsenault B, Lamarche B, et al. Saturated Fats from Butter but Not from Cheese Increase HDL-Mediated Cholesterol Efflux Capacity from J774 Macrophages in Men and Women with Abdominal Obesity. *Journal Of Nutrition* [serial online]. April 2018;148(4):573-580. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

11. Davidson M. Is LDL-C passed its prime? The emerging role of non-HDL, LDL-P, and ApoB in CHD risk assessment. *Arteriosclerosis, Thrombosis, And Vascular Biology* [serial online]. September 2008;28(9):1582-1583. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

12. Pagana K, Deska, Pagana, James T. Mosby's diagnostic and laboratory test reference 13th ed. St. Louis, MO. Elsevier, 2017.

13. Ridker P, Hennekens C, Buring J, Rifai N. C-Reactive Protein and Other Markers of Inflammation in the Prediction of Cardiovascular Disease in Women. *New England Journal Of Medicine* [serial online]. March 23, 2000;342(12):836-843. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

14. Ann F. La B. How the Ideology of Low Fat Conquered America. *Journal Of The History Of Medicine & Allied Sciences* [serial online]. April 2008;63(2):139-177. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

15. Page H, Allen V, Chamberlain L, et al. Dietary Fat and Its Relation to Heart Attacks and Strokes. *Circulation* 1961:23(1), 133–136.

16. CDC - National Center for Health Statistics- Trends in Intake of Energy and Macronutrients in Adults From 1999-2000 Through 2007-2008 https://www.cdc.gov/nchs/products/databriefs/db49.htm Accessed April 10, 2018

17. National Cancer Institute- Fat intake as a percentage of total calories, 1989-2014 https://progressreport.cancer.gov/prevention/fat_consumption Accessed April 14, 2018

18. Gropper S, Smith J, Carr T. Advanced nutrition and human metabolism 7th ed. Boston, Mass. Cengage Learning, 2018.

19. Brown A. Understanding food : principles and preparation 2nd ed. Belmont, CA. Thomson/Wadsworth, 2004.

20. Beinortas T, Mahtani K, Nunan D. Revisiting the diet-heart hypothesis: critical appraisal of the Minnesota Coronary Experiment. *BMJ (Clinical Research Ed.)* [serial online]. June 1, 2017;357:j2108. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

21. Bill S. Testing the boundaries of recommended carbohydrate intakes: Macronutrient exchanges, carbohydrate quality and nutritional adequacy. *Nutrition & Dietetics* [serial online]. 2013;(1):59. Available from: Journals@OVID, Ipswich, MA. Accessed July 29, 2018.

22. Givens D. Saturated fats, dairy foods and health: A curious paradox?. *Nutrition Bulletin* [serial online]. September 2017;42(3):274-282. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

23. Dominguez-Lucas A, Pichler G, Chaves F, et al. LDL particle size and composition and incident cardiovascular disease in a South-European population: The Hortega-Liposcale Follow-up Study. *International Journal Of Cardiology* [serial online]. August 2018;264:172-178. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

24. Rutgers Libraries-Web of Science https://www.libraries.rutgers.edu/indexes/web_of_science Accessed January 12, 2018

25. Shortliffe E.H, Cimino J.J, Biomedical Informatics: Computer Application in Health care and Biomedicine. 4[th]ed, London; Springer-Verlag; 2014.

26. Siri-Tarino P, Krauss R, Sun Q, et al. Saturated Fatty Acids and Risk of Coronary Heart Disease: Modulation by Replacement Nutrients. *Current Atherosclerosis Reports* 2010:12(6).

27. Woodside J, McKinley M, Young I. Saturated and trans fatty acids and coronary heart disease. *Current Atherosclerosis Reports* December 2008:10(6): 460–466.

28. Zong G, Li Y, Sun Q, et al. Intake of individual saturated fatty acids and risk of coronary heart disease in US men and women: two prospective longitudinal cohort studies. *BMJ (Clinical Research Ed.)* [serial online]. November 23, 2016;355:i5796. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

29. Virtanen, K, Jyrki M, Jaakko T, et al. Dietary Fatty Acids and Risk of Coronary Heart Disease in Men: The Kuopio Ischemic Heart Disease Risk Factor Study. *Arteriosclerosis, Thrombosis, and Vascular Biology* December 2014:34(12): 2679–2687.

30. Praagman J, Beulens J, van der Schouw Y, et al. The association between dietary saturated fatty acids and ischemic heart disease depends on the type and source of fatty acid in the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort. *The American Journal Of Clinical Nutrition* [serial online]. February 2016;103(2):356-365. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

31. Lichtenstein A. Thematic review series: patient-oriented research. Dietary fat, carbohydrate, and protein: effects on plasma lipoprotein patterns. *Journal Of Lipid Research* [serial online]. August 2006;47(8):1661-1667. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

32. Fernandez M, West K. Mechanisms by which dietary fatty acids modulate plasma lipids. *The Journal Of Nutrition* [serial online]. September 2005;135(9):2075-2078. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

33. Boateng L, Ansong R, Owusu W, Steiner-Asiedu M. Coconut oil and palm oil's role in nutrition, health and national development: A review. *Ghana Medical Journal* [serial online]. September 2016;50(3):189-196. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

34. Coulston A. The role of dietary fats in plant-based diets. *American Journal Of Clinical Nutrition* [serial online]. September 2, 1999;70:512S. Available from: Complementary Index, Ipswich, MA. Accessed July 29, 2018.

35. Brassard D, Tessier-Grenier M, Lamarche B, et al. Comparison of the impact of SFAs from cheese and butter on cardiometabolic risk factors: a randomized controlled trial. *The American Journal Of Clinical Nutrition*[serial online]. April 2017;105(4):800-809. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

36. Månsson H. Fatty acids in bovine milk fat. *Food & Nutrition Research* [serial online]. March 2008;52:1-3. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

37. Daley C, Abbott A, Doyle P, Nader G, Larson S. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal* [serial online]. March 10, 2010;9:10. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

38. Stanley W, Dabkowski E, Ribeiro R, O'Connell K. Dietary fat and heart failure: moving from lipotoxicity to lipoprotection. *Circulation Research* [serial online]. March 2, 2012;110(5):764-776. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

39. Dehghan M, Mente A, Mapanga R, et al. Articles: Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *The Lancet* [serial online]. November 4, 2017;390:2050-2062. Available from: ScienceDirect, Ipswich, MA. Accessed July 29, 2018.

40. Kris-Etherton P, Fleming J. Emerging nutrition science on fatty acids and cardiovascular disease: nutritionists' perspectives. *Advances In Nutrition (Bethesda, Md.)* [serial online]. May 15, 2015;6(3):326S-337S. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

41. Nakamura Y, Ueshima H, Stamler J, et al. Relationship of three different types of low-carbohydrate diet to cardiometabolic risk factors in a Japanese population: the INTERMAP/INTERLIPID Study. *European Journal Of Nutrition* [serial online]. June 2016;55(4):1515-1524. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

42. Misra A, Sharma R, Pandey R, Khanna N. Adverse profile of dietary nutrients, anthropometry and lipids in urban slum dwellers of northern India. *European Journal Of Clinical Nutrition* [serial online]. September 2001;55(9):727-734. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

43. Brunner E, Wunsch H, Marmot M. What is an optimal diet? Relationship of macronutrient intake to obesity, glucose tolerance, lipoprotein cholesterol levels and the metabolic syndrome in the Whitehall II study. *International Journal Of Obesity And Related Metabolic Disorders: Journal Of The International Association For The Study Of Obesity* [serial online]. January 2001;25(1):45-53. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

44. Baum, Seth J., Kris-Etherton, Penny M., Willett, Walter C., Lichtenstein, Alice H., Rudel, Lawrence L., Maki, Kevin C., Whelan, Jay, Ramsden, Christopher E., and Block, Robert C. "Fatty acids in cardiovascular health and disease: A comprehensive update." Journal of Clinical Lipidology 6, no. 3 (2012): 216–234.

45. Li Y, Hruby A, Hu F, et al. Saturated Fats Compared With Unsaturated Fats and Sources of Carbohydrates in Relation to Risk of Coronary Heart Disease: A Prospective Cohort Study. *Journal Of The American College Of Cardiology* [serial online]. October 6, 2015;66(14):1538-1548. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

46. Harris C, Buyken A, Standl M, et al. Dietary Fatty Acids and Changes in Blood Lipids during Adolescence: The Role of Substituting Nutrient Intakes. *Nutrients* [serial online]. February 11, 2017;9(2)Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

47. Jakobsen M, Overvad K. Macronutrient advice for ischemic heart disease prevention. *Current Opinion in Lipidology* Februaruy 2011:22(1): 33–36.

48. Sacks F, Katan M. Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. *The American Journal of Medicine* 2002 113(9): 13–24.

49. Schwab U, Lauritzen L, Becker W, et al. Effect of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: a systematic review. *Food & Nutrition*

Research [serial online]. July 10, 2014;58Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

50. Hu F, Willett W. Optimal Diets for Prevention of Coronary Heart Disease. *JAMA: Journal Of The American Medical Association* [serial online]. November 27, 2002;288(20):2569. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

51. Wang D, Hu F. Dietary Fat and Risk of Cardiovascular Disease: Recent Controversies and Advances. *Annual Review Of Nutrition*[serial online]. August 2017;37:423-446. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

52. Jakobsen M, O'Reilly E, Ascherio A, et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *American Journal of Clinical Nutrition* [serial online]. 2009:1425. Available from: Health & Wellness Resource Center, Ipswich, MA. Accessed July 29, 2018.

53. Hamley S. The effect of replacing saturated fat with mostly n-6 polyunsaturated fat on coronary heart disease: a meta-analysis of randomised controlled trials. *Nutrition Journal* [serial online]. May 19, 2017;16(1):30. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

54. Virtanen J. Randomized trials of replacing saturated fatty acids with n-6 polyunsaturated fatty acids in coronary heart disease prevention: Not the gold standard? *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)* June 2018:133 8–15.

55. Tanasescu M, Cho E, Manson J, Hu F. Dietary fat and cholesterol and the risk of cardiovascular disease among women with type 2 diabetes. *American Journal of Clinical Nutrition* [serial online]. 2004:999. Available from: Health & Wellness Resource Center, Ipswich, MA. Accessed July 29, 2018.

56. Kris-Etherton P, Kris-Etherton P. AHA science advisory: monounsaturated fatty acids and risk of cardiovascular disease. *Journal Of Nutrition*[serial online]. December 1999;129(12):2280-2284. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

57. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. *BMJ (Clinical Research Ed.)* [serial online]. January 11, 1997;314(7074):112-117. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

58. Astrup A, Astrup A, Buemann B, Flint A, Raben A. Low-fat diets and energy balance: how does the evidence stand in 2002?. *The Proceedings Of The Nutrition*

Society [serial online]. May 2002;61(2):299-309. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

59. Parker B, Noakes M, Luscombe N, Clifton P. Effect of a high-protein, high-monounsaturated fat weight loss diet on glycemic control and lipid levels in type 2 diabetes. *Diabetes Care* [serial online]. March 2002;25(3):425-430. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

60. Song X, Kestin M, Kratz M, et al. A low-fat high-carbohydrate diet reduces plasma total adiponectin concentrations compared to a moderate-fat diet with no impact on biomarkers of systemic inflammation in a randomized controlled feeding study. *European Journal Of Nutrition* [serial online]. February 2016;55(1):237-246. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 27, 2018.

61. Vafeiadou K, Weech M, Lovegrove J, et al. A review of the evidence for the effects of total dietary fat, saturated, monounsaturated and n-6 polyunsaturated fatty acids on vascular function, endothelial progenitor cells and microparticles. *The British Journal Of Nutrition* [serial online]. February 2012;107(3):303-324. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 29, 2018.

62. Schwingshackl L, Hoffmann G. Comparison of Effects of Long-Term Low-Fat vs High-Fat Diets on Blood Lipid Levels in Overweight or Obese Patients: A Systematic Review and Meta-Analysis. *Journal Of The Academy Of Nutrition & Dietetics* [serial online]. December 2013;113(12):1640-1661. Available from: Academic Search Premier, Ipswich, MA. Accessed July 30, 2018.

63. Paoli A, Rubini A, Volek J, Grimaldi K. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European Journal Of Clinical Nutrition* [serial online]. August 2013;67(8):789-796. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 27, 2018.

64. Hu T, Mills K, Bazzano L, et al. Effects of low-carbohydrate diets versus low-fat diets on metabolic risk factors: a meta-analysis of randomized controlled clinical trials. *American Journal Of Epidemiology*[serial online]. October 1, 2012;176 Suppl 7:S44-S54. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 27, 2018.

65. Bazzano L, Hu T, He J, et al. Effects of low-carbohydrate and low-fat diets: a randomized trial. *Annals Of Internal Medicine* [serial online]. September 2, 2014;161(5):309-318. Available from: MEDLINE Complete, Ipswich, MA. Accessed July 27, 2018.

66. Forthofer r, Lee E, Hernandez M. Introduction to Biostatistics: A Guide to Design, Analysis, and Discovery 2nd Edition. Burlington, MA Elsevier Academic Press 2007.

67. Caldwell S. Statistics Unplugged 3rd Edition. Belmont, CA. Wadsworth 2010.

68. NHANES- National Health and Nutrition Examination Survey About https://www.cdc.gov/nchs/nhanes/about_nhanes.htm Accessed Nov 3rd 2018.

69. SAS- Base SAS Software Factsheet https://www.sas.com/content/dam/SAS/en_us/doc/factsheet/base-sas-101198.pdf Accessed October 09, 2018.

70. Office of Disease Prevention and Health Promotion(Health.gov)-Daily Nutritional Goals for Age-Sex Groups Based on Dietary Reference Intakes and *Dietary Guidelines* Recommendations Accessed September 20th 2019 from https://health.gov/dietaryguidelines/2015/guidelines/appendix-7/

71. American Heart Association (heart.org)- The American Heart Association Diet and Lifestyle Recommendations Accessed November 1st 2019 from https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/nutrition-basics/aha-diet-and-lifestyle-recommendations