Effects of Low and High Carbohydrate Meals on Postprandial Concentrations of Circulating Plasma Lipids

By

Sarah Elizabeth Bergman

A THESIS

Presented to the Faculty of the Graduate Programs in Human Nutrition

and the Oregon Health & Science University

School of Medicine

in partial fulfillment of the

requirements for the degree of

Master of Science in Clinical Nutrition

October 2009

School of Medicine
Oregon Health & Science University

CERTIFICATE OF APPROVAL

This is to certify that the Master's Thesis of

Sarah Elizabeth Bergman

has been approved

Melanie Gillingham, PhD, RD

Diane Stadler, PhD, RD

Dennis Koop, PhD

William Connor, MD

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AUC	Area Under the Curve
BMI	Body Mass Index
CDC	Center for Disease Control
CHO	Carbohydrate
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DASH	Dietary Approaches to Stop Hypertension
DXA	Duel Energy x-ray Absorptiometry
FFA	Free Fatty Acids
GC/MS	Gas Chromotography/ Mass Spectrometry
HC	High Carbohydrate
HDL-C	High Density Lipoprotein Cholesterol
IR	Insulin Resistance
IV	Intravenous
LC	Low Carbohydrate
LDL-C	Low density Lipoprotein Cholesterol
MUFA	Monounsaturated Fatty Acids
n-3	Omega 3 Fatty Acids
n-6	Omega 6 Fatty Acids
NEFA	Non-esterified Fatty Acids
NIH	National Institute of Health
OCTRI	Oregon Clinical and Translational Research Institute
OHSU	Oregon Health and Sciences University
PFB	Pentaflorabenzene
PUFA	Polyunsaturated Fatty Acids
SFA	Saturated Fatty Acids
TAG	Triacylglycerols
VLDL	Very Low density Lipoprotein

AKNOWLEDGEMENTS

Here at OHSU, I want to thank the OCTRI staff. This includes so many departments: Thanks to Bionutrition who made our test meals, trusted us with their indirect calorimetry equipment, and performed our DXA scans. Thanks to the nurses in 10D and in 2SE for screening and taking such great care of our participants. Thanks also to the Core Lab who kindly let us store all of our samples in their freezers and helped us run our analysis. A special thanks is definitely needed for Andy Bernards, thanks so much for running my NEFA samples. It made my life so much easier considering I was awful at it.

I would also like to thank Carol Marsh in the OHSU Lipid Lab for performing my TAG analysis. I really appreciate your patience with me.

Our participants were such sports! Thank you so much for giving up your normal diet and time for our study. Without you I wouldn't be graduating right now.

As many of you know I am a statistics master. Ok, not really. But Mike Lasarev is and he talked me through my analysis at least 100 times. Thanks for not quitting before I understood what you were talking about. I would still be trying to figure out numbers without your guidance.

I think it goes without saying that Julie Smith and Melissa Kumagai are amazing. My friends and co-investigators made this project go as smooth as possible. I would not have

been able to pull this off without each of their unique knowledge surrounding this project and research in general. I will be lost without you ladies.

I would like to thank my friends and family. Without your support through the entire experience of grad school I would not have made it. I appreciate you waiting for me to join life. I am so close, get ready to see more of me. I love you all.

I want to thank my committee members Dr. Connor and Dr. Koop. Your expertise and willingness to take part in my project was greatly appreciated. Thank you for your guidance.

I also need to thank Dr. Stadler, without your Energy Balance Study I wouldn't have had such a great project. Thank you for your dedication and attention to detail surrounding the sub-study. It was great working with you throughout the year. I will always remember Honduras, thank you for that experience.

And last but certainly not least, I would like to thank Dr. Melanie Gillingham. I don't think I could ever truly express what it meant to me when I came into your office and said, "Ok, um, I need a pep talk," and you so kindly obliged (Especially since I did that more than once). Thank you for your guidance, patience, and expertise. This experience would not have been the same without you.

Thanks to everyone who put up with my craziness this past 2 years!

ABSTRACT

Background: Postprandial hyperlipidemia is associated with increased risk of cardiovascular disease (CVD). Low carbohydrate diets are a popular choice for weight-loss, but acute effects of low carbohydrate diets on postprandial plasma fatty acid concentrations have not been tested.

Methodology: Ten healthy, normal weight adults participated in a randomized crossover feeding study. Subjects consumed a standard diet (51% carbohydrate, 14% protein, 35% fat) for 3 days. On day 4 participants consumed either low carbohydrate (4%) or high carbohydrate (58%) meals. Subjects then repeated the standard diet and alternate test meals after a minimum 3 day washout period. Blood samples were drawn at fasting (0800) and postprandially every hour for 9 ½ hours. Pre- and postprandial concentrations of plasma triacylglycerols, total non-esterified fatty acids and individual fatty acids were analyzed by modified Wahlefeld method, colormetric enzymatic assay, and gas chromatography/mass spectrometry, respectively. Differences between diets were compared by paired t-tests and contrast analyses.

Results: Triacylglycerol (TAG) concentrations were significantly higher after the low carbohydrate meals compared to the high carbohydrate meals (Area under the curve [AUC] for TAG: low carbohydrate 1097 ± 108 and high carbohydrate 843 ± 89 mg·h/dL, $p < 0.001$). Saturated fatty acid concentrations were significantly higher throughout the sampling period after the low carbohydrate meals compared to the high carbohydrate meals (AUC low carbohydrate 35882 ± 2588 vs. high carbohydrate 27748 ± 2475

μmol/L, p = 0.002). AUC of monounsaturated fatty acid concentrations was higher after the low carbohydrate meals compared to the high carbohydrate meals (8462 ± 1077 vs. 6867 ± 647 μmol·h/L, respectively, p = 0.049). There was no difference in AUC of total polyunsaturated fatty acids, or n-6 or n-3 polyunsaturated fatty acid concentrations between diets. Insulin concentrations were lower and non-esterified fatty acids concentrations were higher following the low carbohydrate meals compared to the high carbohydrate meals, (total insulin AUC 84 ± 9.9 vs. 240 ± 23 μIU·h/ml, respectively, p < 0.01); total non-esterified fatty (AUC 3.46 ± 0.259 vs. 1.850 ± 0.257 mM·h, respectively, p = 0.002). Insulin was negatively correlated to non-esterified fatty acid concentrations following the high carbohydrate meals but not the low carbohydrate meals (p=0.002).

Conclusions Plasma triacylglycerols, saturated fatty acids, monounsaturated fatty acids, and non-esterified fatty acid concentrations were higher and insulin concentrations were lower after the low carbohydrate meals compared to the high carbohydrate meals. Chronic elevation of postprandial lipids may contribute to the development of CVD.

Funding Disclosure: This sub-study of the Energy Balance Study was funded by the OHSU Graduate Programs in Human Nutrition and grants from the NIH: National Center for Complementary and Alternative Medicine (1 R21 AT002753-01) and the National Center for Research Resources (1U L1RR024140).

CHAPTER 1: SIGNIFICANCE, AIMS, AND HYPOTHESES

SIGNIFICANCE

Cardiovascular disease (CVD) continues to be the primary cause of death in the United States (1). In 1999, the American Heart Association (AHA) set a goal to decrease CVD related death by 25% by 2010. As of January 2008, this goal was met early. This success is attributed, at least inpart, to the advances in research and technology regarding medications, treatment procedures, and development of evidence-based practice guidelines (1). Although the death rate due to CVD has decreased 25%, the frequency of CVD risk factors remain high. The revised goal by the AHA is to decrease risk factors associated with CVD. The rate of people who smoke or have uncontrolled hypertension, and/or hypercholesterolemia decreased between 1999 and 2005. Yet, other risk factors have not decreased as much; physical inactivity only dropped 2.5%, while the prevalence of diabetes and obesity increased and are occurring in earlier age groups (1).

It is well known that obesity, metabolic syndrome, and diabetes mellitus are linked to increased risk for CVD. With the increased prevalence of obesity and occurrence in earlier age groups, many people are taking weight loss into their own hands through the use of fad diets, including carbohydrate restricted diets (2). This tactic is of concern as we do not fully understand the effects these weight loss strategies may have on long term CVD risk. Most studies investigating the effects of carbohydrate restriction on CVD risk factors have been performed in overweight and/or obese individuals who lose weight as a result of the dietary intervention. In these designs, understanding the impact of the diet on CVD risk may be confounded by weight loss (2-5). What is not well

established is the effect of continued use of low carbohydrate diets on CVD risk factors when weight loss ends and weight maintenance or weight regain occurs.

This study used a randomized, cross-over design to examine acute exposure of normal weight (BMI 18.5-25 kg/m^2), weight stable individuals to low carbohydrate (LC) and high complex carbohydrate (HC) meals on postprandial lipid concentrations. Circulating concentrations of postprandial lipids (non-esterified fatty acids [NEFA], fatty acid profiles, and triacylglycerols) were measured without the confounding effects from weight loss. Although NEFA and fatty acid profiles are not traditional CVD risk factors, recent research has focused on the acute postprandial period of lipemia as a newly recognized risk factor in the development of CVD, a chronic disease. By investigating the response of normal weight, weight stable individuals, our study measured the acute effects of diet on postprandial lipids without the confounding effects of obesity or change in body weight due to weight loss.

SPECIFIC AIMS

Study Objective: To investigate the acute physiological response of normal weight, weight stable individuals after consuming low vs. high carbohydrate meals. Circulating concentrations of postprandial lipids, a newly recognized class of risk markers associated with CVD, were measured using a randomized, cross-over study design.

Primary Aim #1: To measure circulating concentrations of lipids: total NEFA, total triacylglycerols (TAG), and individual plasma fatty acids; before and after consumption of low and high carbohydrate meals.

Hypothesis #1: Peak concentration and area under the curve (AUC) of triacylglycerols and saturated fatty acids will be higher after the low carbohydrate meals than the high carbohydrate meals.

Hypothesis #2: Postprandial insulin concentrations will be inversely correlated with postprandial NEFA concentrations. NEFA concentrations will be highest at admission (fasting) and decline postprandially after the high carbohydrate meals; NEFA concentrations will remain near fasting after consumption of the low carbohydrate meals.

Hypothesis #3: Postprandial plasma omega-3 fatty acid concentrations will be lower after the low carbohydrate meals compared to the high carbohydrate meals. There will be no difference in monounsaturated, polyunsaturated, or omega-6 fatty acid concentrations between the two intervention meal types.

CHAPTER 2: INTRODUCTION AND BACKGROUND

There are many risk factors for CVD. Some of the most strongly associated risk factors for CVD are preventable, such as obesity and prediabetes. In the United States, obesity alone was responsible for 13% of CVD related deaths in 2004 (6). An Australian study reported individuals with pre-diabetes after five years were more than twice as likely to die of CVD compared to those without (7). These two risk factors, obesity and pre-diabetes, effect an estimated 72 million and 57 million people in the United States, respectively (8).

According to the National Diabetes Information Clearinghouse, individuals with prediabetes can slow the progression and/or decrease the risk of developing diabetes mellitus and CVD with a 5-7% reduction of bodyweight (9). The AHA reports while "no prospective trials have convincingly shown changes in mortality rate with weight loss in obese patients, it has been reported that individuals who attempted intentionally to lose weight present significantly lower all-cause mortality, independent of weight change (10)."

There are many approaches to achieve weight loss in an effort to reduced risk of developing diabetes or CVD. Two popular dietary choices are very low carbohydrate diets and high, complex carbohydrate diets. Recently there has been a greater focus to assess long term effects of consuming these diets. Concern over the development of CVD with long term consumption of a very low carbohydrate diet was raised because the diet tends to be inherently high in fat. Similar concerns arise when following a high carbohydrate diet as excessive carbohydrate consumption has been associated with high circulating triacylglycerol concentrations possibly contributing to CVD risk. The

American Diabetes Association (ADA) has made the recommendation of using these types of diets for weight loss, "either low-carbohydrate or low-fat calorie-restricted diets may be effective in the short term (11)." In most cases, studies investigating the CVD risk associated with consumption of these types of diets are influenced by weight loss.

When the weight loss occurs, stored fat is mobilized and used to generate energy. First, adipose tissue breaks down stored triacylglycerol into glycerol and fatty acids. These compounds are then converted to glucose and ketones in the liver. Second, the body prioritizes protein use. As a result, non-essential proteins, such as HMG-CoA reductase, are synthesized at lower rates. When HMG-CoA reductase synthesis is limited, endogenous production of cholesterol is decreased. In addition, peripheral tissues use lipids in the blood to generate energy through fatty acid oxidation. All of these physiological responses reduce plasma lipid concentrations during weight loss. Thus active weight loss, regardless of diet composition, theoretically improves plasma lipids and lowers CVD risk. However, once weight loss is achieved and the dieter enters a period of weight loss maintenance and weight stability, the effects of continued consumption of high or low carbohydrate diets on CVD risk are not well established.

To date, the effects of low or high carbohydrate diets on plasma lipids and CVD risk in a healthy, normal weight, weight stable population has not been fully elucidated. This study was designed to assess the acute response of plasma lipids to low and high carbohydrate meals in healthy, normal weight, weight stable subjects.

CHAPTER 3: LOW CARBOHYDRATE DIET AND LIPID RESPONSE

The low carbohydrate diet used in this study was modeled after the Atkins philosophy (12). The Atkins diet is divided into four phases. The first phase, the induction phase, instructs dieters to consume no more than 20 grams of carbohydrate for two weeks. During the second phase, active weight loss occurs. In the third phase, carbohydrate intake is gradually increased by 5-10 grams a week until the desired weight is reached. The last phase is the maintenance phase where weight loss stops and carbohydrate intake remains at a level that promotes weight maintenance (12). The primary reason a dieter may be interested in the Atkins diet is the promise of a "metabolic advantage" over the traditional low fat diet. Dr. Robert Atkins explains that when a person restricts their carbohydrate intake, fat is used to generate energy as opposed to glucose derived from carbohydrate. Since burning fat takes more energy than burning carbohydrate, more energy is expended which induces weight loss without having to increase physical activity. Another benefit promised by the Atkins diet, is decreased use of insulin by the body; in his book, insulin is described as a contributor to obesity. Therefore with this logic, a person who follows the Atkins diet produces less insulin and burns more fat which results in reduced body mass. In addition to burning more calories and using less insulin, this diet advertises a reduced sense of hunger. Dr. Atkins states that after 48 hours on the diet the body enters a state of ketosis and "ketosis always induces appetite suppression (12)."

There are a number of studies that have investigated the effectiveness of low carbohydrate diets on cardiovascular risk response. Yancy et al. conducted a 24 week study to compare the effects of a low fat vs. low carbohydrate diet in treating obesity and

hyperlipidemia. A cohort of 120 adults were randomly assigned to a low fat (<30%) or low carbohydrate (<20g) intervention. Both interventions included group meetings, diet instruction, and exercise recommendations (4). This study demonstrated that compared to those assigned to the low fat diet group, those in the low carbohydrate diet group lost more weight (-12 kg [95% CI -13.8, -10.2 kg] vs. -6.5 kg [95% CI -8.4, -4.6 kg]; respectively), and demonstrated larger reductions in fasting triacylglycerol (TAG) concentrations (-74.2 mg/dl vs. -27.9 mg/dl; $p = 0.004$) and greater increases in high density lipoprotein cholesterol (HDL-C) (5.5 mg/dl vs. -1.6 mg/dl; $p < 0.001$). Changes in low density lipoprotein cholesterol (LDL-C) concentrations did not differ between the two intervention groups (1.6 mg/dl vs. -7.4 mg/dl; $p = 0.2$) (4).

A study done at the Philadelphia Veterans Administration Hospital, by Stern et al., investigated the effect of low carbohydrate and low fat diets on lipid markers and glycemic control after one year. Stern et al. found that subjects in the low carbohydrate diet group experienced greater weight loss of approximately 2 kg, although results were not statistically significant (5.3 ± 8.7 kg vs 3.1 ± 8.4 kg, respectively; $p = 0.2$). Triacylglycerol concentrations were found to be lower at fasting ($p = 0.04$), and HDL-C concentrations decreased less ($p = 0.01$) compared to the conventional low fat diet. No significant changes were seen in either intervention group for LDL-C and total cholesterol concentrations ($p= 0.3$, $p= 0.1$; respectively) (13).

Forsythe et al. looked at circulating fatty acid concentrations and markers of inflammation in individuals consuming low fat vs. low carbohydrate diets for 12-weeks. In the overweight sample of men and women studied, the very low carbohydrate diet resulted in significant changes in fatty acid profiles and decreased concentrations of

inflammation markers compared to the low fat diet (14). Ingestion of the very low carbohydrate diet resulted in a greater increase in circulating n-6 polyunsaturated fatty acid (PUFA) concentrations (25.18% ± 2.86 vs. 22.39% ± 3.13; p = 0.004) but no significant change in n-3 PUFA concentrations (2.05 ± 0.63 vs. 2.41 ± 0.72; p = 0.79). Although weight loss was not a primary outcome for this study, weight loss was seen in both groups and the very low carbohydrate group lost more weight compared to the low fat diet group (14).

Studies by Volek et al. attempted to feed eucaloric high and low carbohydrate diets to participants for four to six weeks to assess the effect of diet without weight loss (15, 16). This group studied men and women separately but derived similar conclusions for both normal weight, normolipidemic cohorts. In both cohorts, increases in total cholesterol, LDL-C, and HDL-C, and decreases in triacylglycerol concentrations and the total cholesterol/HDL-C ratio were observed (15, 16).

Table 1: Fasting Lipids in Normal Weight Women and Men (15, 16)

	Women (15)				**Men (16)**			
	LC (Wk 0)	LC (Wk 4)	LF (Wk 0)	LF (Wk 4)	LC (Wk 0)	LC (Wk 6)	LF (Wk 0)	LF (Wk 6)
Total Cholesterol (mmol/L)	4.6 ± 0.9	5.3 ± 0.9*	4.8 ± 0.9	4.5 ± 0.9	4.3 ± 0.8	4.5 ± 0.8	4.2 ± 1.0	4.1 ± 1.2
LDL-C (mmol/L)	2.9 ± 0.7	3.4 ± 0.6*	3.1 ± 0.7	3.0 ± 0.7	2.9 ± 0.8	3.0 ± 0.8	2.9 ± 0.9	2.7 ± 1.1
HDL-C (mmol/L)	1.3 ± 0.3	1.7 ± 0.4*	1.3 ± 0.3	1.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.4	1.2 ± 0.2	1.2 ± 0.5
TAG (mmol/L)	0.9 ± 0.3	0.6 ± 0.1*	0.8 ± 0.6	0.8 ± 0.3	1.1 ± 0.5	0.7 ± 0.3*	1.1 ± 0.3	1.1 ± 0.7
Total Cholesterol: HDL-C Ratio	3.7 ± 0.8	3.3 ± 0.5*	3.8 ± 0.7	3.9 ± 0.7	3.6 ± 0.9	3.5 ± 0.9	3.7 ± 0.7	3.6 ± 0.8

Mean ± SD; LC = Low Carbohydrate Diet; HC = High Carbohydrate Diet;
* Significant difference within diet between wk 0 and wk4, $p < 0.05$

Despite the increases in total and LDL cholesterol concentrations, the authors discussed that the decreases in the total cholesterol/HDL-C ratio were more important than the elevations in total and LDL cholesterol concentrations. The decreases seen in fasting and

postprandial triacylglycerol concentrations at the end of the intervention periods, for both men and women, may also be related to weight loss. Although there were efforts to maintain weight, both cohorts experienced small but significant ($p < 0.05$) amounts of weight loss on the low carbohydrate diets (15, 16); women on the control diet also experienced weight loss (15). Without the small but significant amounts of weight loss, it is possible that the beneficial effects of the very low carbohydrate diet would not be observed.

All studies of low carbohydrate diet which demonstrated beneficial changes in CVD risk markers were accompanied by significant weight loss. The most common change in lipid concentrations with low carbohydrate diets is a decrease in triacylglycerol concentrations and increases in HDL-C concentrations. None of the studies observed a decrease in LDL-C concentrations, one of the most important CVD risk markers. During active weight loss lipids can improve, yet the impact of diet on lipids during weight maintenance and weight stability are unknown.

CHAPTER 4: HIGH CARBOHYDRATE DIET AND LIPID RESPONSE

The high, complex carbohydrate diet used in this study was modeled after the DASH (Dietary Approaches to Stop Hypertension) diet (17). Scientists supported by the National Heart, Lung, and Blood Institute (NHLBI) found that elevated blood pressure was reduced with an eating plan low in saturated fat, cholesterol, and total fat; and that emphasized fruits, vegetables, and fat-free or low-fat milk and milk products (18). Other foods endorsed by this eating plan are whole grains, lean meats, fish, and nuts. In 2003, the DASH diet was reported to be the most successful diet in NIH studies to lower blood pressure and cholesterol (17).

In September of 1999 recruitment for one of the largest DASH studies began at four clinical centers across the United States (19). The PREMIER study had three intervention groups with a total of 810 participants. The three intervention groups were given either advice only, an established intervention with behavioral modification counseling, or the established intervention plus DASH diet teaching. The authors of this study reported that free living participants who followed the established intervention with or without the DASH component lost weight, and had lowered LDL-C and total cholesterol concentrations (20). However, the DASH intervention group had no greater improvement in overall outcomes compared to the established intervention group.

Harsha et al. investigated the effect of different sodium levels within either a DASH or "typical American" diet on various outcomes as part of the DASH-Sodium Trial (21). The diets provided were adjusted for energy intake throughout the study so that participants remained weight stable. Although they were researching the effects of sodium on these two diets, groups assigned to the DASH diet demonstrated lower

lipoprotein concentrations without increased triacylglycerol concentrations at all three levels of sodium intake compared to those assigned to the typical American diet. Among those assigned to the DASH diet, total cholesterol concentrations were lowered 0.4-0.5 mmol/L, LDL-C concentrations were lowered by 0.3-0.4 mmol/L, and HDL-C concentration were lowered by 0.1 mmol/L, compared to baseline (21). Another study, by Obarzanek et al., found similar results with their DASH intervention and weight stable subjects (22). The DASH diet lowered total and LDL-C concentrations, as well as HDL-C concentrations. There was no significant effect on triacylglycerol concentrations. A study performed at Johns Hopkins University looked at inflammation and the DASH eating pattern (23). Inflammation has been associated with increased risk for CVD. The authors concluded that subjects with low baseline C-reactive protein (CRP) concentrations, a marker of inflammation, demonstrated a greater reduction in serum lipid concentrations while consuming a DASH diet compared to those with high baseline levels of CRP. Triacylglycerols concentrations increased in both groups but there was a greater increase in triacylglycerols (+19.8% vs. +0.01%) among individuals with high baseline CRP concentrations compared to those with low baseline CRP concentrations (23). The results of this study suggest chronic inflammation may be a barrier to decreasing CVD risk markers through diet.

Within the published research, there exists conflicting results regarding consumption of a DASH eating pattern and blood lipid concentrations. Some studies show a beneficial change while other studies find no change with the addition of the DASH diet. Few studies using the DASH intervention used a eucaloric diet and most

studies using the DASH diet resulted in weight loss leaving the question of whether the lipid lowering effects of DASH are related to weight loss or the diet.

CHAPTER 5: FATTY ACID PHYSIOLOGY

Dietary fats are found in a variety of foods and include saturated, monounsaturated, polyunsaturated, and trans-fatty acids. Key risk factors for the development of CVD include elevated blood triacylglycerols and LDL-C concentrations, and low HDL-C concentrations. Dietary fats and their effect on plasma triacylglycerols, LDL-C and HDL-C concentrations have been extensively studied. It is well accepted that diets high in saturated fatty acids (SFAs) raise cholesterol; while diets high in polyunsaturated fatty acids (PUFAs) lower cholesterol; and diets high in monounsaturated fatty acids (MUFAs) are neutral. Although, recent studies of diets high in MUFAs report contradicting results; short and long-term studies show increases and decreases in HDL-C concentrations leaving the effect of MUFAs on HDL-C open to further investigation (24-27).

Circulating concentrations of triacylglycerols are affected by many things: rates of lipogenesis and lipolysis, hormones, eating, and fasting. Triacylglycerols (TAG) are mainly stored in adipose tissue and continually undergo lipolysis and re-esterification. These processes contribute to the level non-esterified fatty acids in the plasma. Insulin suppresses lipolysis and therefore suppresses release of free fatty acids from triacylglycerol stores in adipose tissue. In the fed state, carbohydrate induces the release of insulin. The surge in circulating insulin significantly reduces release of free fatty acids from adipose tissue, and promotes lipogenesis. In the typical postprandial state, circulating free fatty acid concentrations are negligible. When levels of circulating insulin are low, as in a fasting or “starved” state, lipolysis is activated and free fatty acids are

released from adipose tissue into the blood stream to be used as a source of energy (28, 29).

In contrast to free fatty acids, triacylglycerol concentrations tend to rise postprandially when a meal contains fat and excessive carbohydrate. Lipid absorption is more complex than carbohydrate absorption delaying peak lipid concentrations in the blood. Circulating triacylglycerol concentrations are affected within an hour of ingesting a meal with peak plasma concentrations occurring 6-8 hours later. Depending on the macronutrient composition of the meal, peak triglyceride concentrations may occur earlier or later in the postprandial period. The concern with delayed peaks in triacylglycerol concentrations is, in a typical day, a diet high in saturated fat will extend the hyperlipidemic state for a greater part of the day and lipid levels will be additive from one meal to the next. While elevated plasma saturated fatty acid concentrations are associated with increased CVD risk, certain unsaturated fatty acids may have cardioprotective effects.

Dietary omega-3 fatty acids have been researched for their antithrombotic and lipid lowering effects. Eicosapentaenoic acid, an omega-3 fatty acid, competes with arachidonic acid, an omega-6 fatty acid, for prostaglandin and leukotriene synthesis at the cyclooxygenase and lipoxygenase level (30). The prostaglandins and leukotrienes synthesized in both pathways interrupt blood clotting, assisting in lowering atherogenic and inflammatory responses within the body (30). Omega-6 fatty acids are known for their pro-inflammatory effect and are now recommended in lesser amounts to reduce potential CVD risk. Dietary intake of omega-6 and omega-3 fatty acids, as a result, are now recommended to be eaten in a ratio of less than 10:1 compared to the ratio of 16-

20:1, associated with the current Western diet (30, 31). Decreasing the omega-6:omega-3 polyunsaturated fatty acid ratio allows the cardioprotective properties offered by the omega-3 fatty acids to be observed (28, 29, 32).

CHAPTER 6: NON-ESTERIFIED FATTY ACIDS

High plasma non-esterified fatty acid (NEFA) concentrations have been associated with many cardiovascular events including: ventricular fibrillation, thrombosis, ventricular arrhythmias, insulin resistance, and sudden death (33-36). Unlike the other markers of CVD risk, elevated NEFA concentrations suggest mobilization and release of endogenous lipid stores for energy production as in a fasting situation. In 1970, elevated plasma NEFA concentrations were believed to lead to primary ventricular fibrillation (33). It was hypothesized that when acute myocardial ischemia occurs, the subsequent catecholamine response activates a sequence of physiological events. First, there is a decrease in pancreatic release of insulin and therefore a decrease in glucose utilization. Second, there is an increase of lipolysis in adipocytes that results in an increase in plasma non-esterified fatty acid concentrations. At the same time, oxygen consumption increases in the muscle. This combination of events leads to abnormal electrophysiological conduction in the myocardium, also known as ventricular arrhythmias (33).

Even earlier than the ventricular arrhythmia-NEFA hypothesis, elevated NEFA concentrations were observed to cause thrombosis and sudden death in dogs (34, 37). In the early 1960's it was demonstrated that infusion of long chain saturated fatty acids induced thrombosis but infusion of unsaturated fatty acids of the same chain length did not induce thrombosis (37). A study published in 1964 assessed saturated fatty acid infusion in combination with either warfarin or heparin anticoagulation therapies (34). Warfarin proved to be less effective than heparin in preventing thrombosis induced by saturated fatty acid infusion, yet the heparinized dogs still died suddenly from acute

myocardial infarction. However, when fatty acids were incubated with bovine albumin prior to infusion, generalized thrombosis and death did not occur (34). Circulating plasma NEFA's bind with various degrees of affinity to albumin. Saturation of albumin binding sites occurs at NEFA concentrations of about 1.2 μmol/L (33). When NEFA concentrations rise above 1.2 μmol/L, there is an increase in circulating free fatty acid concentrations. Circulating free fatty acids are rapidly taken up into tissues, including the myocardium, which increases the risk of ventricular fibrillation; as seen in the dog studies (33, 34).

Current research priorities have shifted away from ventricular arrhythmias/thrombosis and NEFA concentrations due to advances in medical technologies that allow control of the acute cardiac ischemia period with new procedures and medications. Now elevated NEFA concentrations are implicated in contributing to insulin resistance (35). In insulin resistance is a condition in which higher amounts of insulin are required to lower serum glucose levels during a fed state; this condition is known to be a precursor to non-insulin dependent diabetes mellitus. Insulin resistance affects approximately 57 million people in the United States, possibly more as this is not something commonly diagnosed due to a lack of diagnostic criteria (38). Many theories exist as to how elevated NEFA concentrations contribute to insulin resistance but mechanisms are not yet known (39, 40).

All of the mentioned connections implicate elevated plasma NEFA concentrations in cardiovascular related death and disease. Non-esterified fatty acid concentrations were once a well established risk factor for CVD that have been overshadowed by the focus on fasting total cholesterol and LDL-cholesterol concentrations today.

CHAPTER 7: LONG TERM EFFECTS OF AN ACUTE POSTPRANDIAL RESPONSE

More than 50 years ago Gofman and Lindgren suggested that a relationship between specific lipoprotein classes and cardiovascular disease may exist (41). Not until 1979 did the idea of postprandial lipemia, rather than fasting lipoprotein concentrations, become accepted as a potential cause for atherosclerosis (42). In a given day a typical, healthy individual eats 3-6 times. Consumption of frequent meals throughout the day results in an extended postprandial absorptive state lasting 6-8 hours. Therefore the next meal is most likely consumed before triglyceride levels return to baseline (43). This pattern of meal consumption creates a dynamic state with constant remodeling of lipoproteins throughout the body compared to stable fasted state (43). These sequential postprandial lipid excursions have been recently associated with the development of CVD because of the prolonged exposure of the media intima to elevated concentrations of circulating lipids (44-46).

High fasting triacylglycerol concentrations are becoming more recognized as an independent risk factor for CVD. The Third Report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III) instituted the use of lower cut points (Table 2) to categorize triacylglycerol concentrations compared to the ATP II guidelines (47). This recommendation demonstrates the increasing awareness that moderately elevated triacylglycerol concentrations are associated with increased risk of CVD.

Table 2: ATP III vs. ATP II Classifications for fasting plasma triacylglycerol levels (48, 49)

	ATP III	ATP II
Normal	< 150 mg/dL	< 200 mg/dL
Borderline-high	150-199 mg/dL	200-400 mg/dL
High	200-499 mg/dL	400 -1000 mg/dL
Very high	≥ 500 mg/dL	> 1000 mg/dL

With the increasing association of hyperlipidemia and CVD, studies of both indirect and direct effects of lipoproteins on CVD have been conducted (50). Researchers theorize that those who develop atherogenic CVD may improperly transport triacylglycerols creating a delay in processing and removal of circulating triacylglycerol (46, 50). Individuals with impaired triacylglycerol metabolism experience high concentrations of triacylglycerol rich lipoproteins for a prolonged period of time permitting increased enrichment with cholesteryl esters (46). The cholesterol ester pool increases postprandially and offers more esters to be taken up by the lipoproteins. These cholesterol ester enriched particles are rerouted from their preferential removal pathway and deposited at sites where they cause atherogenic plaques (46).

Hyperlipidemia has been demonstrated to be cytotoxic to cultured human endothelial cells. Speidel et al. incubated endothelial cells in a control serum, a lipolized hypertriacyglycerol serum or a triacylglycerol rich lipoprotein serum. The hypertriacylglycerol serum and the triacylglycerol rich lipoproteins caused cytotoxicity after 24 hours of incubation. The investigator added HDL-C to the cells and found a protective effect (51). These results indicate that the interaction of endothelial cells with lipolytic substances may play a role in the pathogenesis of atherosclerosis and that HDL may have a protective effect in endothelial cell injury produced by hyperlipidemia (51).

In human studies, it has been shown that elevated postprandial lipids create an influx of atherogenic particles that can lead to the development of CVD (46, 52). To support this theory, a study by Karpe et al. provided evidence that tolerance of an oral fat load, in the form of a mixed meal, was correlated with the progression of coronary heart disease in a 5 year time period ($r = 0.51$, $p = 0.01$) (44). The progression of CVD was assessed by coronary angiographies spaced 5 years apart. The coronary arteries were divided into segments and assessed for lesions with sharp edged, plaque-like or irregular indentations, often multiple, into the vessel lumen; these lesions were then given a global coronary atherosclerosis score. Progression was defined as an increase in the global coronary atherosclerosis score between the first and the second angiogram (44). The oral fat load was performed after the second angiography to determine if there was an association between any of the measured postprandial lipids. The overall conclusion was that postprandial plasma levels of small chylomicron remnants were found to relate to the progression of coronary lesions (44).

This background has discussed several studies examining the effects of a low carbohydrate diet and a high carbohydrate diet on plasma lipids when consumed over several weeks to months. The acute response among healthy weight, weight stable adults to these diets on plasma lipids is not known. The goal of this study was to examine the acute effects of low and high carbohydrate meals on postprandial lipids. This information will be used to identify the ability of the diet, independent of weight loss, to acutely induce postprandial hyperlipidemia in healthy, normal weight individuals.

CHAPTER 8: METHODOLOGY

GENERAL DESIGN

The main goal of this study was to examine the effect of an acute feeding intervention of low carbohydrate (LC) and high carbohydrate (HC) meals on postprandial plasma lipid concentrations**.** The study was conducted using a randomized, cross-over design to measure the response between two different meal compositions. Six healthy, normal weight subjects were recruited from the Portland, Oregon area through recruitment fliers posted throughout Portland, including the Oregon Health & Science University (OHSU) and Portland State University campuses. Data from four subjects from a previous pilot study were also used in the analysis. Each study participant completed two 4-day controlled dietary phases separated by at least a three day wash-out phase. All study related procedures were reviewed and approved by the OHSU Institutional Review Board and performed in the Oregon Clinical & Translational Research Institute (OCTRI).

SUBJECT SELECTION

Study participants included 4 men and 6 women, with BMIs between 18.5-26 kg/m^2 and age between 21-65 years. Inclusion and exclusion criteria are detailed in Table 2. All subjects were free-living and gave informed consent before participating in any screening or intervention related activities. A copy of the consent form and HIPAA research form is provided in Appendix A and B, respectively.

SCREENING

Pre-study screening visits were conducted for each potential participant to determine eligibility for the study. Participant height, weight, and blood pressure was

measured and recorded (Appendix C) at the OCTRI outpatient nursing station by trained research nursing staff. A fasting blood sample was obtained via finger-stick for point of care hemoglobin (HemoCue β-hemoglobin photometer, HemoCue AB, Angelholm, Sweden) and glucose assessment (Precision Xceed Pro Blood Glucose Monitor, Abbott Laboratories, Alameda, CA). A urine sample was provided by female participants to rule-out pregnancy (Aceava hCG Combo II test cartridges, Inverness Medical, Waltham, MA). Forms were administered at this time to assess physical activity (Baecke Activity Questionnaire, (53)), medical history (Cornell Medical Index, (54)), and food preferences. Subjects judged to be healthy by self-report, review of medical history, medication-use, and lab screening were considered eligible for participation. No subjects screened for this study required a second visit for a physical examination by the study physician for further determination of eligibility.

Table 3. Inclusion & Exclusion Criteria

Inclusion	**Exclusion**
- BMI 18.5-25 kg/m^2 - Age: 21-65 years - Good health - Willingness to eat both a high and low carbohydrate diet - Willingness to stop taking multivitamins or any other dietary supplements for the duration of the study	- Major debilitating mental or physical illness that would interfere with participation. - Pregnancy or lactation within the last 12 months - Weight instability (loss or gain of more than ± 5% within last 6 months) - Current participation in a self-directed or commercial weight loss plan - Any self imposed food restrictions (ex: kosher, vegetarian diet) that the participant would not be willing to stop for the duration of the study. - Any food allergies or food preferences that are not consistent with the research diets - Prescription medication use, with the exception of birth control and intermittent over the counter analgesics.

STANDARDIZATION PROTOCOL

On days 1-3 of the protocol each subject was fed a standard diet (50% carbohydrate, 35% fat, and 15% protein) designed to meet their individual energy needs to prevent weight loss or weight gain. Energy needs were calculated by the Harris Benedict equation (55) with activity factor. A table detailing the nutrient composition of the standard diet is provided in Appendix D. Each day, the participants arrived at the OCTRI Outpatient Unit between 0700 and 1000 to have their weight measured at the Bionutrition Unit. The participants then ate breakfast in the OCTRI dining room and completed study related forms. All other meals and snacks for the remainder of the day were prepared for each participant to take home. Participants were asked to consume all the food provided to them by the Bionutrition kitchen staff, and instructed to refrain from other food intake. Participants returned empty food and beverage containers from the previous day so that compliance to the diet could be monitored.

INTERVENTION PROTOCOL

At 0600 in the morning of the forth study day, subjects were admitted to the inpatient unit of the OCTRI. The total length of stay for each subject was approximately 12 hours. Subjects are instructed not to eat anything after 2200 the night before, or to have engaged in significant physical activity for at least 24 hours prior to admission. Upon admission blood pressure and vital signs were taken, and an indwelling catheter was placed in a peripheral vein in the subject's arm. Half-normal saline was infused by gravity through the catheter to keep the system open. A fasting blood sample was taken at 0800, after which the subject consumed either a low carbohydrate or high carbohydrate

breakfast. Postprandial blood samples were taken at 0830, with subsequent samples taken every hour after for nine hours and at 1300 according to the blood sample collection schedule shown in Appendix E. Subjects consumed a lunch meal of the same composition as breakfast between 1305 and 1325. Participants were discharged with orders to resume their usual diet and activity level and returned to repeat the 4 day procedure as scheduled. During the second phase of the study, participants completed the same measurements and blood sampling protocol, but consumed the alternative diet option of either low carbohydrate or high complex carbohydrate meals compared to their first admission.

INPATIENT INTERVENTION DIETS

During the inpatient admissions participants consumed either two low carbohydrate meals, modeled after the Atkins induction phase diet, or two high complex carbohydrate meals, modeled after the DASH diet. Macronutrient composition of the low carbohydrate meal was 66% fat, 30% protein, and 4% carbohydrate. Macronutrient composition of the high carbohydrate meal was 27% fat, 55% carbohydrate, and 18% protein. Each meal consumed during the inpatient admission provided 10 kcal/kg. A table detailing the nutrient composition of the low and high carbohydrate test meals is presented in Table 6.

ESTIMATED ENERGY REQUIREMENTS:

The energy intake necessary to maintain the body weight of participants during the standardization phase of the study was calculated using the Harris-Benedict energy

prediction equation (55) and multiplied by an activity factor. The activity factor was determined by the average amount of physical activity (1.3 for very inactive to 1.6 for very active) for each participant as estimated by the Baecke activity questionnaire (53) and participant interview. The Harris-Benedict equation takes into account a person's sex, age, height and weight.

Harris-Benedict equation:

Basal Energy Requirements (male)

= 66 + 13.7 (wt. in kg) + 5 (ht. in cm) – 6.8 (age in years)

Basal Energy Requirements (female)

= 665 + 9.6 (wt. in kg) + 1.8 (ht. in cm) – 4.7 (age in years)

BODY COMPOSITION:

Body weight was measured in light clothing with a digital scale (Scale-Tronix, Model 5002, Carol Stream, IL) in the Bionutrition Unit of the OCTRI. Height was measured by a wall mounted stadiometer (Holtain Ltd., UK) without shoes. Body composition was measured by total body duel energy x-ray absorptiometry (DXA) scan (Discovery Series Densitometer, Hologic Inc., Bedford, MA) before the 1st inpatient admission.

OUTCOME VARIABLE METHOD ANALYSIS

Blood samples were collected in pre-chilled phlebotomy tubes, except for serum samples that were collected in tubes maintained at room temperature. All plasma tubes were centrifuged under 4°C refrigerated conditions immediately after each collection for

12 minutes at a speed of 2600 rpms. The plasma was harvested and divided into polypropylene aliquot tubes and stored for batched analysis at the end of the study. Serum samples were centrifuged and processed for storage using the same process as plasma samples after 20-30 minutes of clotting time. All storage tubes were frozen at -20°C and then transferred within 24 hours to -80°C freezers until the time of analysis.

Total cholesterol and low density-lipoprotein cholesterol (LDL-C) are well recognized markers of CVD risk. Cholesterol concentrations take longer than an acute 12 hour period to undergo large changes. Cholesterol concentrations, at most, minimally respond to normal food intake within individuals of the general population (56). Total cholesterol and LDL-C fell slightly (1-5%) after a meal in a large cohort of 26,330 women (57). Due to the acute nature of this study, we did not assess cholesterol concentrations as large changes were not likely to be observed.

Plasma fatty acids: Analysis was conducted in the Bioanalytical Shared Resource Pharmacokinetics Core Lab in the Department of Physiology and Pharmacology at OHSU using the methods of Lagerstedt et al. (58, 59). Samples were analyzed at five time points: 0800, 1030, 1230, 1430, 1630. Analysis was run in singlet. Internal standards of deuterated free fatty acids, including d3C10:0, d3C14:0, d3C16:0, d3C18:0, d3C20:0 and d4C22:0, were added to each plasma sample prior to extraction. Fatty acids from EDTA plasma were hydrolyzed with hydrochloric acid (HCl) followed by neutralization with sodium hydroxide (NaOH). After neutralization, the free fatty acids generated were extracted with hexane. Free fatty acids were then derivatized to their pentaflurobenzene-esters (PFB-esters) and quantified by gas chromatography-mass spectroscopy (GC/MS) using a DSQ II Single Quadrupole GC/MS operating in the negative ion chemical

ionization mode with methane as the reagent gas. The fatty acid PFB-esters were separated on a DB-5ms capillary column (30m x 0.25mm x 0.25µm film thickness; ThermoFisher Scientific, Inc., Waltham, Massachusetts) with helium as the carrier gas. Fatty acids analyzed included C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:4, C20:5 and C22:6 and were detected using selected ion monitoring for the corresponding fatty acids resulting from the loss of pentaflurobenzene. Each fatty acid was matched to the deuterated internal standard closest in length and retention time. Peak area ratios of known amounts of standard fatty acids and the internal standards were used to generate calibration curves to quantify unknowns using Xcalibur software (ThermoFisher Scientific, Inc., Waltham, Massachusetts). Individual fatty acid peaks were compared to internal standards of known concentration and identified by molecular mass and retention time. The sums of saturated (C 14:0, C 16:0, C18:0), monounsaturated (C 14:1, C 16:1, C 18:1), polyunsaturated (C18:2, C18:3, C20:4, C20:5, C 22:6), omega-3 (C 18:3, C 20:5, C 22:6) and omega-6 fatty acids (C 18:2, C20:4) were calculated for each subject by adding together absolute concentrations of respective individual fatty acids within each summed group.

Total non-esterified fatty acids: Tetrahydrolipostatin was added to whole blood collected in EDTA tubes to inhibit lipase hydrolysis of triacylglycerols ex-vivo to prevent falsely elevate non-esterified fatty acid concentrations (60). Plasma was separated and free fatty acid levels were analyzed in duplicate by OCTRI Core Laboratory staff using a commercially available enzymatic colorimetric kit (Wako Chemicals USA, Inc, Richmond, VA). If the percent difference for a sample was >10% between duplicates, it was re-analyzed. The interassay percent coefficient of variance (%CV) was calculated for

a high and low concentration control samples. After 7 analysis, the high concentration control %CV was 2.41% and the low concentration control was 2.89%.

Triacylglycerols: Triacylglycerols were analyzed in singlet. Triacylglycerols in EDTA plasma were hydrolyzed by microbial lipase to glycerol and free fatty acids. The glycerol was then oxidized to dihydroxyacetene phosphate and hydrogen peroxide and quantified spectophotometrically - a modified version of the Wahlefeld method (Roche/Boehringer Mannheim Corp. Triglyceride/GPO Reagent; Hitachi 704 Chemistry Analyzer) (61). Analysis was completed by the OHSU Lipid Laboratory Staff.

Insulin: Insulin concentrations were measured in singlet in serum using a chemiluminescent immunoassay on the automated immulite system (Siemens Medical Solutions Diagnostics, Los Angeles, CA). The lowest concentration of insulin able to be detected by the assay is 2.0 µIU/ml. The average inter-assay %CV for insulin was 2.6, 4.8, 0.4, and 3.5% for four controls after four assays. Analysis was performed by Melissa Kumagai, study co-investigator, in the OCTRI Core Laboratory.

STATISTICAL ANALYSIS

Means, standard deviations, and standard error of the means were calculated for absolute circulating concentrations of saturated fatty acids (C14:0, C16:0, C18:0), monounsaturated fatty acids (C14:1, C16:1, C18:1), polyunsaturated fatty acids (C18:2, C18:3, C20:4, C20:5, C22:6), total non-esterified fatty acids, total triacylglycerols, and insulin at each time point.

Change in the concentrations of plasma fatty acids and triacylglycerols from baseline were analyzed using area under the curve (AUC) from breakfast to lunch, lunch

to discharge and breakfast to discharge (total) calculated by the trapezoidal method (62). Incremental AUC is another method that was considered for the change over time analysis. Incremental AUC subtracts out each analyte baseline to account for differences in fasting concentrations. The trapezoidal method was chosen because our fasting baseline values before each set of intervention meals were not different. If baseline values had been statistically different incremental AUC would have been used. Differences in AUC after ingestion of the low carbohydrate and high carbohydrate meals were compared using one sided paired t-tests.

Differences between concentrations for each analyte at pre-selected time points after low carbohydrate and high carbohydrate meal consumption were analyzed using Scheffe's method. This is generally performed when comparisons other than simple pairwise differences of means are of interest and when these more general comparisons are not planned a priori (63); the general comparisons used for this analysis were contrasts. Research questions were formulated for each analyte and specific contrasts were developed to test the effect associated with each specific question. One overall test statistic is provided by contrast analysis for each analyte when screening for the presence of the effect of interest among any of the involved time points. If the initial screening test was significant, then Bonferroni-adjusted t-tests were performed to assess differences at individual time points to identify the times at which the effect was significant. Contrasts between pre-selected time points were also analyzed for differences in changes over time and patterns of change after consumption of low carbohydrate and high carbohydrate meals.

The relationship between non-esterified fatty acids and insulin concentrations were tested two different ways. Prior to testing, the sampling period was split into two periods, 0800-1300 and 1300-1730, and compared between intervention meals. First, it was tested whether insulin and non-esterified fatty acid concentrations were inversely correlated during each time interval. For each individual person, a correlation coefficient was computed and then a Wilcoxon signed-rank test was performed to determine whether the collection of correlation coefficients significantly differed from zero for each sampling period. The second analysis examined whether the maximum insulin concentration occurred at the same time as the minimum NEFA concentration for both postprandial periods. This was done by fitting a line for each person for both analytes. From each fitted line a maximum and minimum concentration was determined and recorded; in some instances no reasonably smooth line could be fit and no unique maximum/minimum concentration could be estimated. Paired t-tests were performed for those analytes in which a unique maximum/minimum concentration could be identified to determine whether the maximum and minimum concentration occurred at the same time.

Data analyses was performed using Microsoft Excel 2007 for participant descriptive statistics, STATA (version 10.1; StataCorp LP, College Station, Texas) for AUC and paired t-test. Mike Lasarev, Research Associate for the Center for Research on Occupational and Environmental Toxicology at OHSU, performed trend and contrast analysis in SciLab (version 5.1.1; INRIA Paris-Rocquencourt Research Center, Le Chesnay Cedex, France), and R (version 2.9.0; R: A Language and environment for Statistical Computing 2007**)** for the insulin and non-esterified fatty acid correlation analysis.

Data Management

All data collected as a result of participation in this study was kept completely confidential. Paper forms, excluding one master copy, identified patients only by a study specific identification. All non-electronic forms were kept in a locked cabinet within a locked office in the OHSU Hatfield Research Building. Specific forms were developed for each data set including patient demographics and history, as well as each discrete outcome variable. Password protected computer databases were developed to store information; these were accessible only by select study staff.

CHAPTER 9: RESULTS

SUBJECT CHARACTERISTICS

The acute response of plasma lipids to the consumption of low carbohydrate and high carbohydrate meals was studied in healthy, weight stable male and female subjects. A total of 11 participants were enrolled in the study and all were Caucasian. Four male and six female participants completed the study (n=10). One participant dropped out due to time constraints. All participants met the inclusion criteria described in Table 3. The average age was 24.5 ± 2.9 yr (reported as the mean ± standard error of the mean; $\overline{x}$ ± SEM) and the average BMI was 22.8 ± 0.7 kg/m^2 (Table 4). The average percent body fat of participants, as determined by DXA, was 23.7 ± 4.5%. Body weight for all subjects remained stable throughout the three day standard diet phase and during the washout period between dietary phases (data not shown).

Table 4: Subject Demographics

Subject Characteristics	Women	Men	Total
	6	4	10
Age (years)	25.0 ± 1.5 [22 – 31]	23.8 ± 0.6 [22 – 25]	24.5 ± 2.9 [22 – 31]
Height (centimeters)	167.1 ± 2.02 [160.8 – 173.5]	183.1 ± 2.7 [178.2 – 190.5]	173.5 ± 9.6 [160.8 – 190.5]
Weight (kilograms)	61.4 ± 3.7 [49.8 – 72.3]	82.6 ± 1.6 [78.2 – 85.3]	69.9 ± 4.1 [49.8 – 85.3]
BMI (kg/m^2)	21.7 ± 0.8 [19.3 – 24.8]	24.5 ± 0.7 [23.3 – 26.4]	22.8 ± 0.7 [19.3 – 26.4]
Body Fat (Percent)	25.9 ± 1.67 [20.8 – 31.2]	20.2 ± 1.8 [16.8 – 24.5]	23.6 ± 1.5 [16.8 – 31.2]

Data presented as $\overline{x}$ ± SEM; range shown in brackets.
BMI = body mass index

DIETARY CHARACTERISTICS

The 3-day standard diet provided an average 2775 ± 43 kcal/d and was comprised of 51% carbohydrate, 14% protein, and 35% fat. All subjects tolerated meals served and compliance was high as assessed by visual inspection of food containers upon return to the OCTRI kitchen and patient report. The macronutrient composition of the low carbohydrate test meals was 4% carbohydrate, 30% protein, and 66% fat. The macronutrient composition of the high carbohydrate test meals was 55% carbohydrate, 18% protein, and 27% fat. The average amount of energy consumed by participants during the inpatient admissions was 1366 ± 241 kcal (10 kcal/kg at each meal) and was the same for both the low carbohydrate and high carbohydrate test meals. Complete consumption of test meals was verified by visual inspection of participant trays by study coordinators during each inpatient admission. Further description of the macro and micronutrients consumed by participants during the standardization diet and test meals is presented in Tables 5 and 6.

Table 5: Nutrients Consumed During the Standardization Phase

Component	Mean ± SD	mg or g/1000 kcal
Carbohydrate (g)	360 ± 57	130
Protein (g)	101± 16	36
Fat (g)	111 ± 18	40
Cholesterol (mg)	228 ± 51	104
Total Dietary Fiber (g)	32 ± 0.43	9
Sodium (mg)	4035 ± 709	1454
Potassium (mg)	3022 ± 646	1089
Calcium (mg)	1876 ± 86	557
Phosphorous (mg)	1601 ± 399	557

Table 6 - Nutrient Composition of High- and Low-Carbohydrate Test Meals

Dietary Component	High Carbohydrate Meals	Low Carbohydrate Meals
Carbohydrate		
g/1000 kcal	142	9
% of energy	55	4
Protein		
g/1000 kcal	46	75
% of energy	18	30
Fat		
g/1000 kcal	30	73
% of energy	27	66
Saturated Fatty Acids		
g/1000 kcal	6	32
% of energy	6	29
Monounsaturated Fatty Acids		
g/1000 kcal	10	24
% of energy	9	21
Polyunsaturated Fatty Acids		
g/1000 kcal	11	5
% of energy	10	4
n-3 Fatty Acids (g/1000 kcal)	4.9	1.0
n-6 Fatty Acids (g/1000 kcal)	24.6	8.9
n-6:n-3 Ratio	5.0	8.9
Cholesterol (mg/1000 kcal)	137	653
Total Dietary Fiber (g/1000 kcal)	12	2
Sodium (mg/1000 kcal)	1255	2044
Potassium (mg/1000 kcal)	1313	1077
Calcium (mg/1000 kcal)	480	533
Phosphorus (mg/1000 kcal)	808	952

Missing Data

At the 1430 time point during one admission, blood samples were not drawn for one participant due to loss of IV access. Missing data for those 1430 blood samples were interpolated by taking the average of the values at the time points before and after the missing data point.

Within the fatty acid analysis, 7 values out of 1100 data points, in addition to the missing 1430 tube previously mentioned, came back with either negative, diluted, or exponentially large values in comparison to the surrounding time points, all values were judged to be inaccurate. Six of the values were replaced by averages of the values at time points before and after. The 7^{th} value was a fatty acid baseline sample. Fasting values were tested for the other participants to see if they differed; there was no difference between fasting values at the two admissions for fatty acid samples ($p = 0.473$) and therefore the missing value was replaced with the subject's other fasting value.

For the NEFA analysis, three samples were completely missing; 2 from the previous participants in 2006 and one from the new set of participants. For the missing time points, values were imputed as the averages of the values of samples taken before and after the missing value.

Postprandial Analyte Concentrations and Area Under the Curve

Mean concentration of each analyte at each time point and AUC analysis for each analyte are presented in Tables 7a and b, and 8. All values are presented as mean ± the standard error of the mean ($\overline{x}$ ± SEM).

Table 7a: Fasting and Postprandial Concentrations of Fatty Acids before and after Low and High Carbohydrate Meals*

Analyte	Meal	0800	1030	1230	1430	1630
SFA µmol/L	HC	3346 ± 385	3313 ± 366	3491 ± 335	3662 ± 310	3245 ± 300
	LC	3115 ± 243	4065 ± 3315	4496 ± 343	5269 ± 539	4943 ± 341
MUFA µmol/L	HC	773 ± 103	804 ± 107	839 ± 105	794 ± 100	824 ± 80
	LC	710 ± 92	933 ± 138	1005 ± 135	1255 ± 199	954 ± 120
PUFA µmol/L	HC	2503 ± 378	2348 ± 276	2156 ± 131	2686 ± 263	2282 ± 263
	LC	2275 ± 603	2106 ± 289	2022 ± 157	2436 ± 189	2033 ± 123
n-3 µmol/L	HC	309 ± 20	319 ± 27	306 ± 20	296 ± 24	294 ± 31
	LC	291 ± 16	314 ± 24	313 ± 38	308 ± 22	298 ± 25
n-6 µmol/L	HC	2194 ± 378	2029 ± 268	1850 ± 124	2396 ± 258	1987 ± 245
	LC	1984 ± 197	1826 ± 278	1708 ± 126	2128 ± 192	1735 ± 109

*Mean ± SEM
LC = low carbohydrate
HC = high carbohydrate
SFA = saturated fatty acids
MUFA = monounsaturated fatty acids
PUFA = Polyunsaturated fatty acids
n-3 = omega-3 fatty acids
n-6 = omega-6 fatty acids

Table 7b Fasting Postprandial Concentrations Triacylglycerols, Insulin, Non-Esterified Fatty Acids*

Analyte	Meal	0800	0830	0930	1030	1130	1230	1300	1330	1430	1530	1630	1730
TAG mg/dL	HC	69±9.0	70±9.2	71±9.1	79±9.5	90±10.6	101±10.2	99±10.3	102±10.9	101±11.6	99±10.6	89±9.9	86.4±9.4
	LC	68±4.4	72±6.0	86±7.2	109 ± 8.7	129 ±11.3	126±11.8	132±16.9	126±15.0	140±20.5	137±15.9	129±17.2	109±12.1
NEFA µmol/L	HC	380±30	290±20	90±10	90±20	150±40	310±60	400±70	340±50	170±40	130±30	120±40	160±30
	LC	390±40	360±40	190±20	240±30	330±30	410±30	490±40	430±40	410±40	400±40	450±40	420±50
Insulin (µIU/ml)	HC	4±0.6	51±11	39±6	22±3	12±2	7±2	6±1	24±3	35±5	33±3	26±4	16±3
	LC	4±0.6	9±1	8±1	8±0.7	9±0.9	8±1	8±1	11±1	10±2	9.1±2	10±1	8±2

*Mean ± SEM TAG = triacylglycerols
LC = low carbohydrate NEFA = non-esterified fatty acids
HC = high carbohydrate

Table 8: Summary of Area Under the Curve Calculations

Analyte	AUC $1_{(0800\text{-}1300)}$			AUC $2_{(1300\text{-}1730)}$			Total $AUC_{(0800\text{-}1730)}$		
	HC	LC	Δ	HC	LC	Δ	HC	LC	Δ
SFA[§] (μmol· h/L)	15129 ± 1573	17535 ± 1175	-2407 ± 1205[a]	14061 ± 1212	19978 ± 1619	-5925 ± 925[b]	29190 ± 2685	37513 ± 2617	-8323 ± 1639[b]
MUFA[§] (μmol·h/L)	3615 ± 411	3992 ± 504	-378 ± 359	3252 ± 321	4469 ± 629	-1217 ± 613[a]	6867 ± 647	8562 ± 1077	-1595 ± 862[a]
PUFA[§] (μmol·h/L)	10567 ± 1025	9603 ± 849	964 ± 739	9809 ± 693	8928 ± 473	882 ± 752	20377 ± 1536	18531 ± 1123	1845 ± 854[a]
n-3[§] (μmol·h/L)	1410 ± 96	1383 ± 105	28 ± 43	1193 ± 74	1227 ± 102	-38 ± 60	2603 ± 157	2610 ± 202	-7 ± 68
n-6[§] (μmol·h/L)	9157 ± 1016	8297 ± 820	860 ± 698[a]	8629 ± 674	7700 ± 450	929 ± 723	17786 ± 1515	15997 ± 1081	1789 ± 824
TAG (mg·h/dL)	410 ± 47	522 ± 43	-112 ± 30[b]	483 ± 50	653 ± 79	170 ± 50[b]	843 ± 89	1110 ± 115	-267 ± 68[b]
NEFA (μmol·h/L)	989 ± 121	1550 ± 125	-560 ± 180[b]	862 ± 144	1912 ± 161	-1051 ± 260[b]	1850 ± 257	3461 ± 259	-1611 ± 430[b]
Insulin (μIU·h/ml)	118 ± 14	40 ± 4	77.4 ± 11[b]	125 ± 13	47 ± 7	78 ± 8[b]	240 ± 23	84 ± 9.9	156 ± 16[b]

*Mean ± SEM; LC = low carbohydrate;
HC = high carbohydrate; Δ ($AUC_{LC} - AUC_{HC}$)
[§]AUC 1= 0800-1230; AUC 2 = 1230-1630
[a]Significantly different between meal types ($p<0.05$)
[b]Significantly different between meal types ($p<0.01$)

SFA = Saturated Fatty Acids
MUFA = Monounsaturated Fatty Acids
PUFA = Polyunsaturated Fatty Acids
Δ = $AUC_{LC} - AUC_{HC}$

n-3 = omega-3 fatty acids
n-6 = omega-6 fatty acids
TAG = triacylglycerols
NEFA = non-esterified fatty acids

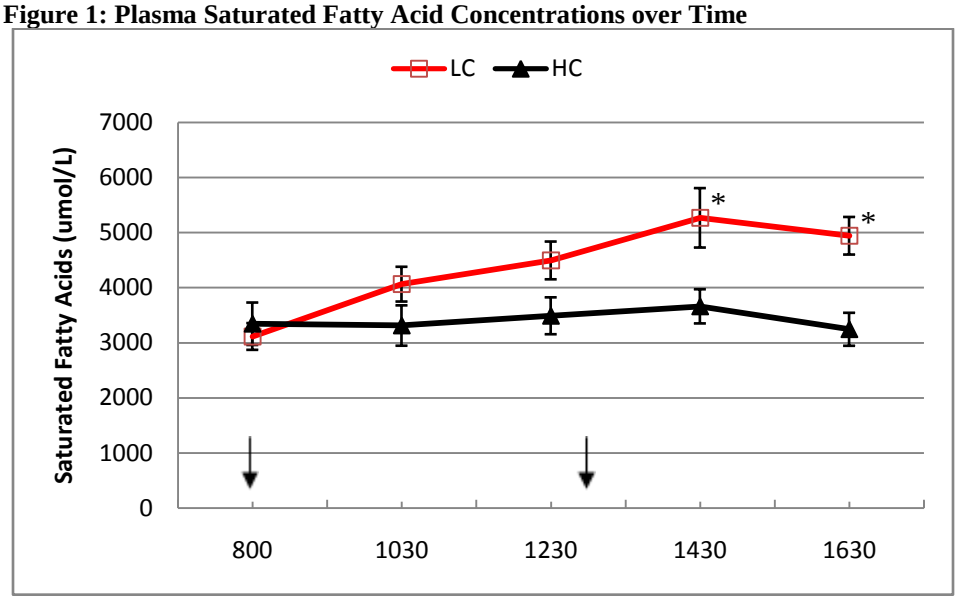
Contrast Analysis

Simple contrasts were used to test the difference between two means of chosen analytes at the same time point. Contrasts were also developed to test the pattern of change between diets. This method was used to determine if the change over time was parallel between diets or if the plasma response between diets followed different courses. Patterns were determined and described as having linear, quadratic, cubic, or quartic components. For example, if both diets have a strong positive linear component they could be described as increasing. However, this does not measure how strong the linear component is, simply that a linear component exists.

Postprandial Saturated Fatty Acid Concentrations and Area Under the Curve

Change in concentration over time for saturated fatty acids (SFA) after the HC and LC meals is displayed in Figure 1. Mean fasting SFA concentrations were similar at 3346 ± 385 and 3115 ± 243 μmol/L before the HC and LC meals, respectively ($p=0.47$). SFA concentrations rose after the LC breakfast and lunch meals until 1430, and remained stable until 1630. Circulating SFA concentrations after ingestion of the HC meals remained relatively stable throughout the day.

Figure 1: Plasma Saturated Fatty Acid Concentrations over Time



Mean ± SEM; Arrows indicated when meals were consumed (0805-0825, 1305-1325);
* indicates significant difference between diets ($p < 0.05$).

The AUC for the postprandial period after breakfast was significantly higher after the LC compared to the HC meal (17535 ± 1175 vs. 15129 ± 1573 μmol·h/L, respectively, $p = 0.038$). The AUC for the postprandial period after lunch was significantly higher after the LC meal compared to the HC meal (19978 ± 1619 vs. 14061 ± 1212 μmol·h/L, respectively, $p < 0.0001$). All calculated AUCs for SFA were significantly higher after the LC compared to the HC meals indicating consistent elevations in circulating plasma SFA following the LC meals (Total AUC 37513 ± 2617 vs. 29190 ± 2685 μmol·h/L, respectively, $p = 0.0003$)

Contrast analysis was used to determine differences in means between diets at individual time points, 0800, 1030, 1230, 1430, and 1630. Contrasts revealed a significant effect of diet on mean SFA concentrations for at least one of the time points ($p = 0.014$). Additional analysis revealed that means at 1430 and 1630 were significantly higher after the LC diet than the HC diet ($p<0.05$ Bonferroni adjusted).

Further contrast analysis was used to test differences in fasting plasma mean plasma SFA concentrations and the average of the four postprandial SFA concentrations within each diet. The mean postprandial SFA concentration was significantly higher than the mean fasting SFA concentration after consumption of the LC meals ($p = 0.001$). There was no significant difference between the mean fasting and mean postprandial concentrations after the HC meals. This evidence supports the hypothesis that sequential LC meals increase SFA plasma concentrations for a longer period of time compared to HC meals.

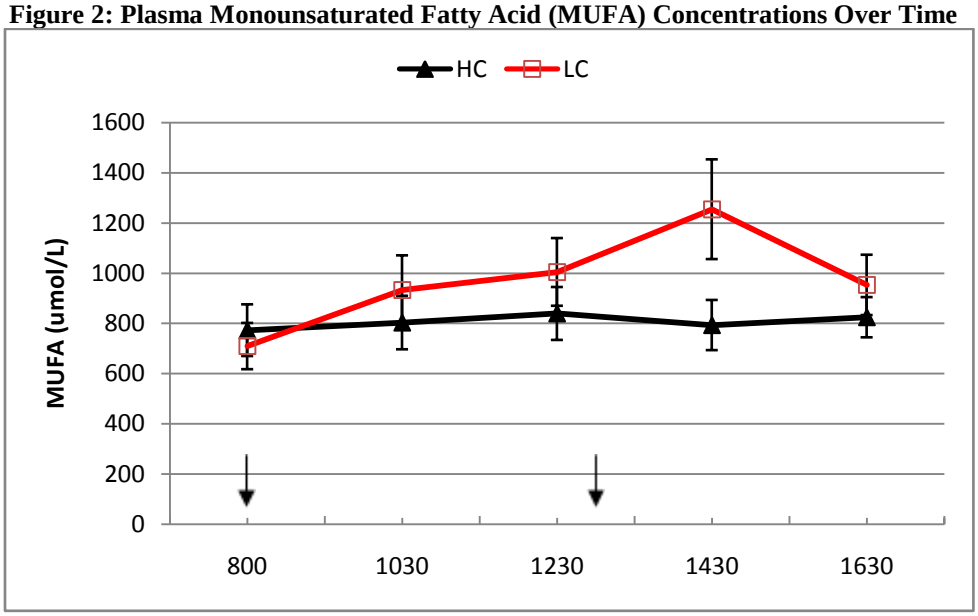
Postprandial Monounsaturated Fatty Acid Concentration and Area Under the Curve

Change in concentration over time for monounsaturated fatty acids (MUFA) after the HC and LC meals is displayed in Figure 2. Mean fasting MUFA concentrations were similar at 773 ± 103 and 710 ± 92 μmol/L before the HC and LC meals, respectively ($p=0.38$). MUFA also had a linear rise throughout the day following the low carbohydrate breakfast meal and continued to increase after the lunch meal. The only decrease in the low carbohydrate curve occurred between the 1430 and 1630 blood draw. The MUFA concentrations after ingestion of the HC meals remained relatively stable throughout the intervention period.

There was no significant difference in AUC of MUFA for the postprandial period after the HC or LC breakfast meals (3615 ± 411 vs. 3992 ± 504 μmol·h/L, respectively, $p = 0.16$). The AUC for the postprandial period after lunch was significantly higher after the LC meal compared to the HC meal (4469 ± 629 vs. 3252 ± 321 μmol·h/L, respectively, $p =0.039$). The total AUC for MUFA was also significantly higher after the

LC compared to the HC meals (8462 ± 1077 vs. 6867 ± 647 µmol·h/L, respectively, p = 0.0487).

Figure 2: Plasma Monounsaturated Fatty Acid (MUFA) Concentrations Over Time



Mean ± SEM; Arrows indicated when meals were consumed (0805-0825, 1305-1325).

Contrast analysis was used to determine differences in means between diets at individual time points, 0800, 1030, 1230, 1430, and 1630. This analysis demonstrated no difference at any of the time points (p=0.6). Contrasts testing the average difference in mean MUFA concentrations between the two diets determined that the average postprandial MUFA concentration was approximately 164 umol/L higher after the HC meal compared to the LC meal. There was a large amount of variability in the MUFA concentrations so this trend was not statistically significant (p = 0.107) and the 95% CI for the difference between diets crossed the zero (-43 to 372 umol/L).

The AUC results provide support for the conclusion that MUFA concentrations are significantly higher after ingestion of the LC intervention meals compared to the HC

meals over time. Therefore, the null hypothesis that MUFA concentrations do not differ between diets was rejected.

Postprandial Polyunsaturated Fatty Acid Concentration and Area Under the Curve

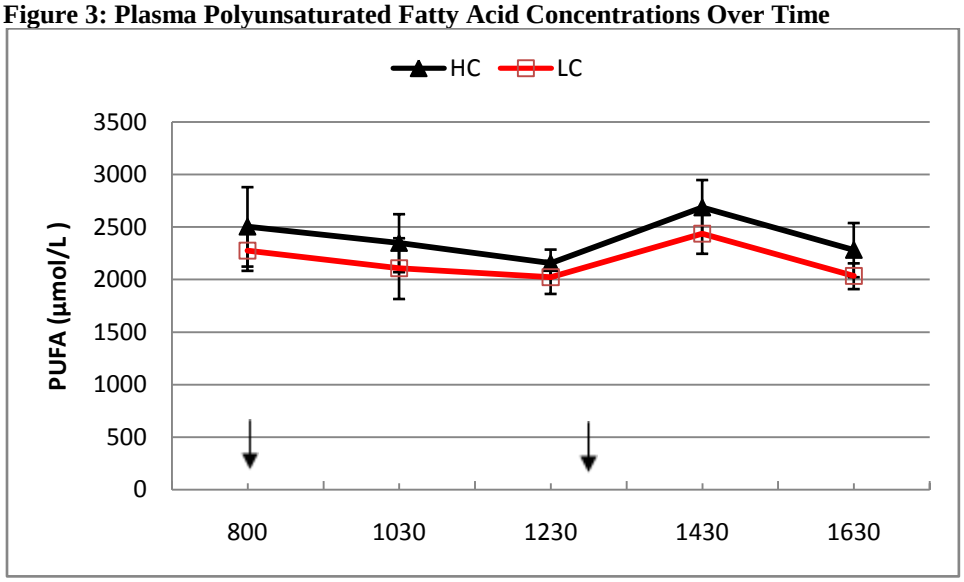
Circulating polyunsaturated fatty acids (PUFA) concentrations are a measure of omega 3 and omega 6 fatty acids. The change in concentration over time for polyunsaturated fatty acids (PUFA) after HC and LC meals is depicted in Figure 3. Mean fasting PUFA concentrations were similar at 2503 ± 378 and 2275 ± 191 μmol/L before the HC and LC meals, respectively (p=0.36). The concentrations run parallel to one another throughout the intervention period. First, PUFA concentrations decrease below fasting concentrations after the breakfast meal and rise slightly above baseline values after the lunch meal for both dietary interventions. By the end of the testing period concentrations fell to slightly below baseline.

Contrasts testing the difference in mean postprandial PUFA concentrations between the two meal types revealed that PUFA concentration were on average 220 μmol/L higher after the LC meal compared to the HC meal. There was large person to person variability as evidenced by the SEM, this variability contributed to the non-significant result with a large confidence interval crossing 0 (-18 to 458 umol/L). Although PUFA concentrations demonstrated a trend to be higher after the LC meals, this finding was not statistically significant (p = 0.07).

There was no significant difference in AUC of PUFA for the postprandial period after the HC or LC breakfast meals (10567 ± 1025 vs. 9603 ± 849 μmol·h/L, respectively, p = 0.11) or the HC or LC lunch meals (9809 ± 693 vs. 8928 ± 473 μmol·h/L,

respectively, p = 0.14). However, the total AUC for PUFA was significantly higher after the HC compared to the LC meals (20377 ± 1536 vs. 18531 ± 1123 µmol·h/L, respectively, p = 0.0295). This difference in total AUC could be due to the difference at baseline (228 µmol /L) despite values being statistically similar.

Figure 3: Plasma Polyunsaturated Fatty Acid Concentrations Over Time



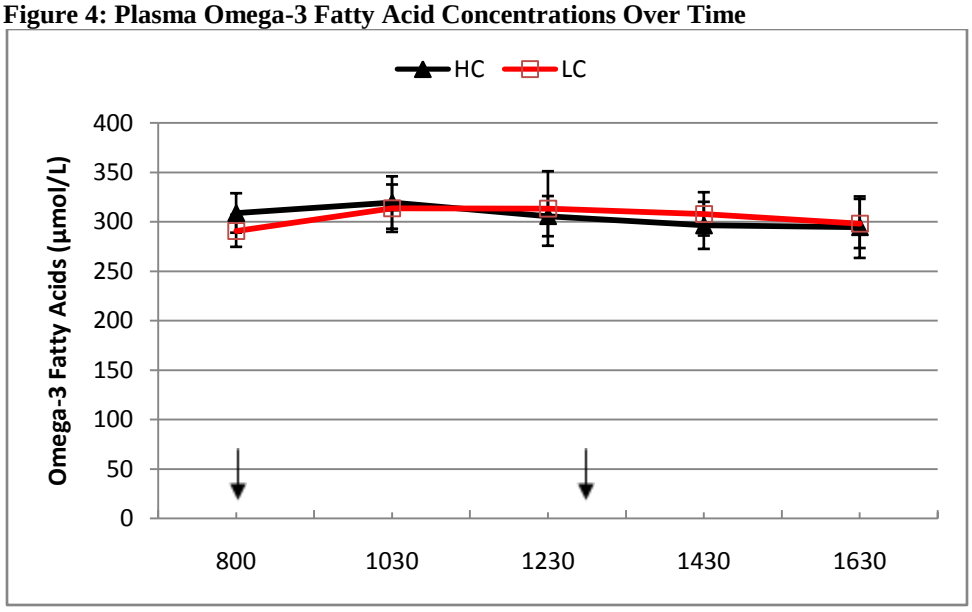
Mean ± SEM; Arrows indicated when meals were consumed (0805-0825, 1305-1325).

Contrast analysis was used to determine differences in means between diets at individual time points, 0800, 1030, 1230, 1430, and 1630. No difference was determined between the means at any of the time points between diets (p=0.59). Further contrast analysis was used to test differences in baseline plasma means and the average of the four postprandial PUFA concentrations within each diet. Postprandial PUFA concentrations did not change from baseline after either diet (p=0.964). These results support the hypothesis that there is no difference in PUFA concentrations after the consumption of low or high carbohydrate meals.

POSTPRANDIAL OMEGA-3 FATTY ACID CONCENTRATION AND AREA UNDER THE CURVE

Change in concentration over time for omega-3 (n-3) fatty acids after HC and LC meals is shown in Figure 4. Mean fasting n-3 concentrations were similar at 309 ± 20 and 291 ± 16 μmol/L before the HC and LC meals, respectively (p=0.111). Contrast analysis was used to determine differences in means between diets at individual time points, 0800, 1030, 1230, 1430, and 1630; no differences were identified (p=0.72). Four contrasts including baseline omega-3 fatty acid concentration mean and each postprandial mean were created to test changes over time; there were no changes over time indicated by this analysis (p=0.207). Furthermore, there was no difference in AUC between diets for n-3 fatty acids (p=0.46).

Figure 4: Plasma Omega-3 Fatty Acid Concentrations Over Time



Arrows indicated when meals were consumed (0805-0825, 1305-1325).

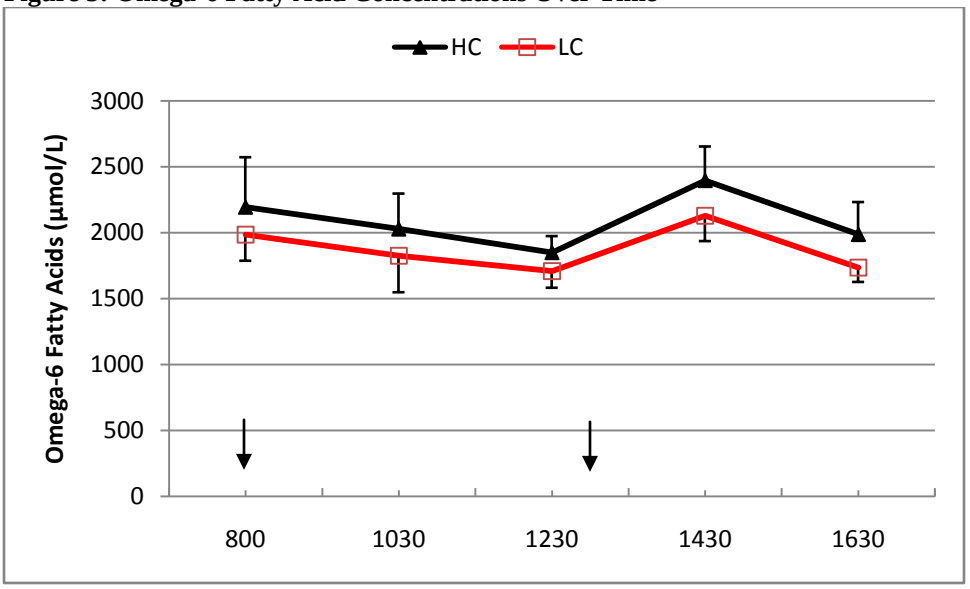
These results contradict the hypothesis that n-3 fatty acid concentrations are lower after consumption of the LC meals compared to the HC intervention meals. These results

indicate that plasma n-3 concentrations are relatively stable before and after consumption of the high and low carbohydrate intervention meals.

Postprandial Omega-6 Fatty Acid Concentration and AUC

The change in plasma concentration over time for omega-6 (n-6) fatty acids after HC and LC meals is shown in Figure 5. Mean fasting n-6 fatty acid concentrations were similar at 2194 ± 378 and 1984 ± 197 µmol/L before the HC and LC meals, respectively (p=0.3856). The concentrations are similar throughout the sampling period. Concentrations in response to both intervention meal patterns were parallel. The omega-6 fatty acid concentrations, after low and high carbohydrate breakfast meals, slightly decreased below baseline until the lunch meal and then returned below baseline by the end of the sampling period. Both plasma values fall by the end of the intervention period returning to baseline.

Figure 5: Omega-6 Fatty Acid Concentrations Over Time



Arrows indicated when meals were consumed (0805-0825, 1305-1325).

There was no difference in AUC for n-6 fatty acid concentrations after breakfast or lunch meals ($p = 0.124$ and $p=0.116$, respectively). The total AUC for n-6 fatty acids was significantly higher after the HC compared to the LC meals (17786 ± 1515 vs. 15996 ± 1081 μmol·h/L, respectively, $p = 0.0290$). The difference in total AUC could be due to the difference at baseline (210 μmol /L) despite values being statistically similar.

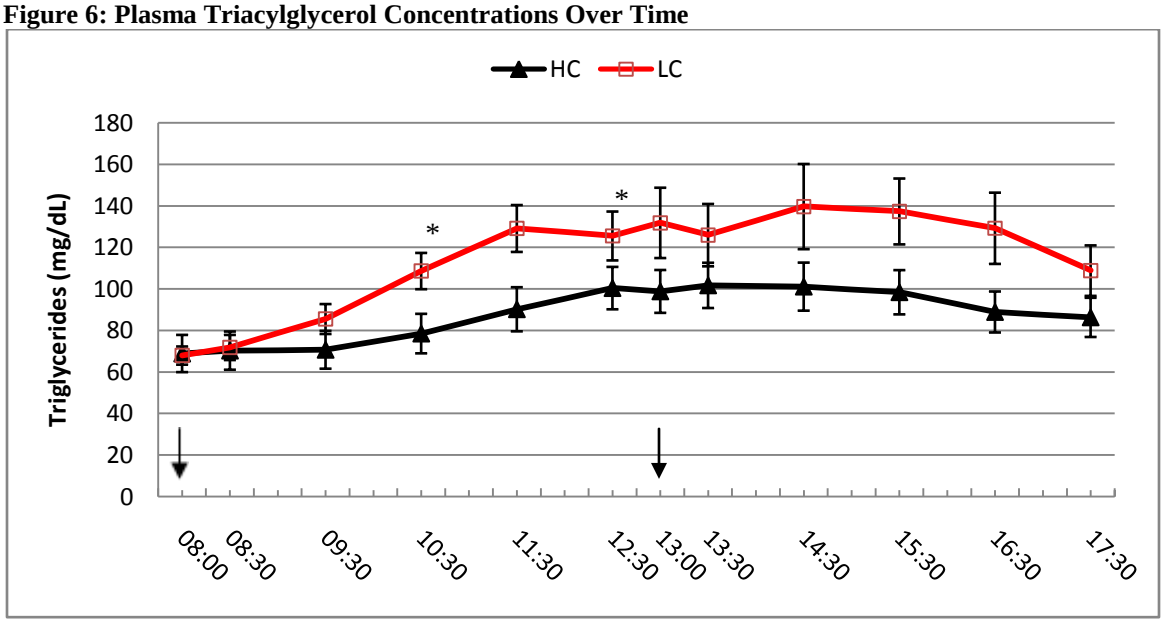
Contrast analysis was used to determine differences in means between diets at 0800, 1030, 1230, 1430, and 1630. No differences in means between meal types means were observed ($p=0.686$). Four contrasts including baseline omega-6 fatty acid concentration mean and each postprandial mean were created to test changes over time; there were no differences in changes over time indicated by this analysis ($p=0.80$). These results support the hypothesis that changes in n-6 fatty acid concentrations do not differ after consumption of the LC meals compared to the HC meals.

Postprandial Triacylglycerol Concentrations and Area Under the Curve

Change in concentration over time for triacylglycerols (TAG) after HC and LC meals is depicted in Figure 6. Mean fasting TAG concentrations were similar at 69 ± 9 and 68 ± 4 mg/dL before the HC and LC meals, respectively ($p=0.90$). Contrast analysis was used to assess whether TAG concentrations follow a similar pattern of change after consumption of the two meal types. Triacylglycerol concentrations followed as negative quadratic pattern suggesting that the pattern changed direction once. After both the HC and LC meals, patterns demonstrate a peak and decrease toward the end of the intervention period ($p< 0.05$ level of significance with Bonferroni-adjustment). The analysis also demonstrated a significant linear trend ($p< 0.05$ levels of significance with

Bonferroni-adjustment) indicating that the TAG concentration at the end were higher concentration compared to baseline.

Figure 6: Plasma Triacylglycerol Concentrations Over Time



Mean ± SEM; Arrows on axis indicated when meals were consumed (0805-0825, 1305-1325).
* Indicated difference between diets ($p < 0.05$)

The AUC for TAG concentration in the postprandial period after breakfast was significantly higher after the LC compared to the HC meal (522 ± 43 vs. 410 ± 47 mg·h/dL, respectively, $p = 0.0045$). Concentrations in the postprandial period after lunch were, again, significantly higher after the LC meal compared to the HC meal (653 ± 79 vs. 483 ± 50 mg·h/dL, respectively, $p= 0.0038$). The total AUC for TAG was significantly higher after the LC compared to the HC meals (1110 ± 115 vs. 843 ± 89 mg·h/dL, respectively, $p = 0.0018$).

Contrast analysis was used to determine differences in means between diets at 0800, 1030, 1230, 1430, and 1630. Contrasts revealed a non-significant effect of diet on mean TAG concentrations ($p = 0.078$). The resulting p value is a compilation of the five

contrasts created for this test. The overall non-significance and value could be a result of a very non-significant p value in combination with one or more significant p values. Therefore, this composite p-value shows potential for significance at, at least, one time point. Because of this potential difference, additional tests for significance between diets were run. Contrasts of means at individual time points were tested and it was determined that mean TAG concentrations at 1030 and 1230 were significantly different between diets.

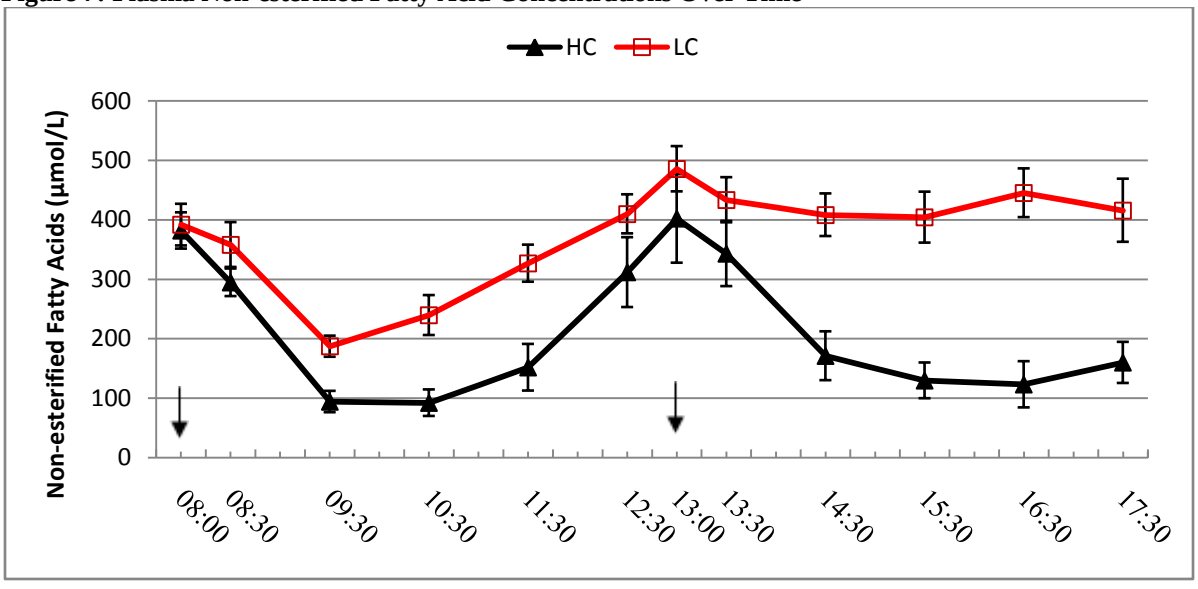
Contrast analysis was used to test differences in mean fasting concentrations and the mean of the average of the four postprandial TAG concentrations within each diet. The average of the postprandial TAG concentrations were significantly higher than the mean baseline concentration after consumption of the LC and HC meals ($p< 0.05$, $p<0.05$, respectively). The change from baseline was greater after consumption of the LC meals compared to the HC meals ($p=0.007$). This evidence supports the hypothesis that sequential consumption of LC meals have a greater impact on plasma TAG concentrations compared to HC meals.

Postprandial Non-esterified Fatty Acid Concentrations and AUC

Change in concentration over time for non-esterified fatty acids (NEFA) after HC and LC meals is shown in Figure 7. Mean fasting NEFA concentrations were similar at 382 ± 30 and 392 ± 35 μmol/L before the HC and LC meals, respectively ($p=0.71$). The response curve following the HC meals follows the predicted pattern. After consumption of each HC meal the NEFA concentrations fall as the body readily uses available carbohydrate for fuel.

To evaluate the effect of each meal on postprandial concentrations of NEFAs, analysis for patterns of change was broken into four segments, LC breakfast, LC Lunch, HC breakfast and HC lunch and each segment was tested for its own descriptive pattern of change. This analysis is similar to the analysis discussed previously for patterns of change in TAG concentrations. The pattern of change for both, HC and LC intervention meals have a strong quadratic component in the first postprandial period (0800-1230) ($p=0.009$, $p=0.001$, respectively). After lunch the LC pattern of change stabilizes and does not decrease as it did following the breakfast meal similar to a "fasting" response. There was no strong descriptive result (linear, quadratic, cubic, or quartic) to describe the pattern of change after the second LC meal ($p=0.190$). In contrast, the HC postprandial period after lunch has a strong linear and quadratic pattern of response ($p=0.028$) which can be seen in Figure 7. By the end of the sampling period after the second HC meal, the NEFA concentrations remain suppressed providing evidence of a negative linear trend.

Figure 7: Plasma Non-esterified Fatty Acid Concentrations Over Time



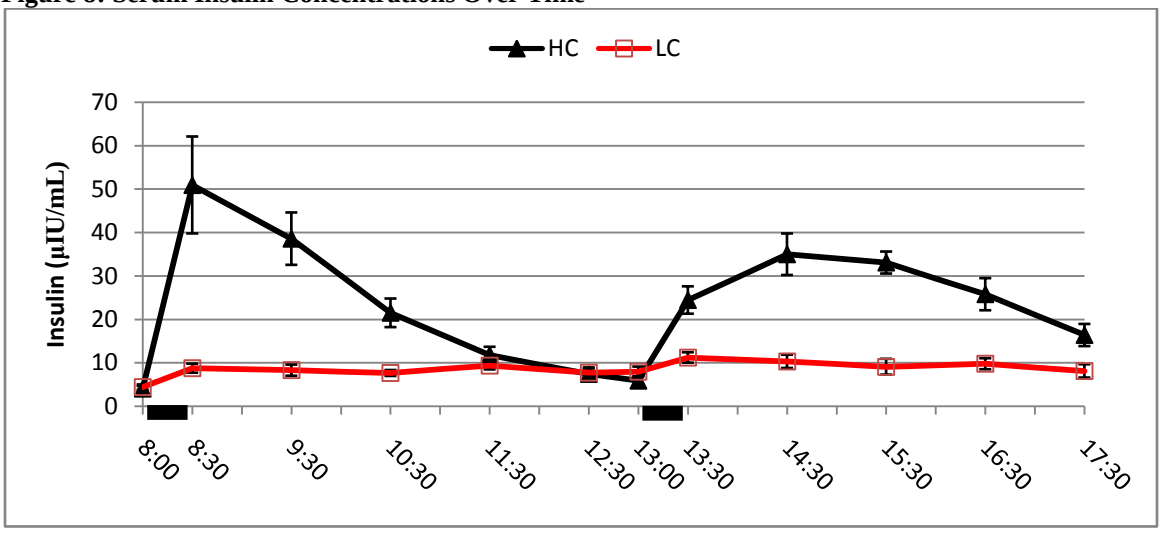
Mean ± SEM; Arrows on axis indicate when meals were consumed (0805-0825, 1305-1325).

The AUC for NEFA concentration in the postprandial period after breakfast was significantly higher after the LC compared to the HC meal (1550± 125 vs. 9890 ± 122 μmol·h/L, respectively, p = 0.0059). AUC concentrations in the postprandial period after lunch were, again, significantly higher after the LC meal compared to the HC meal (1913 ± 161 vs. 862 ± 144 μmol·h/L, respectively, p= 0.0015). The total AUC for NEFA was significantly higher after the LC compared to the HC meals (3460 ± 259 vs. 1850 ± 257 μmol·h/L, respectively, p = 0.0023).

Postprandial Insulin Concentration and Area Under the Curve

The postprandial patterns of change in insulin concentration were also different after consumption of the LC and HC meals (Figure 8). Mean fasting insulin concentrations were similar before the LC and HC meals (4.4 ± 0.6 vs. 4.0 ± 0.6 μIU/ml, respectively; p=0.4). To assess the pattern of change for mean insulin concentrations after consumption of each meal type, four segments were created to describe the pattern of change (same as for NEFA, described in previous section). After the HC breakfast meal, mean insulin concentrations rose sharply and returned to baseline before consumption of the lunch meal following a quadratic pattern ($p < 0.05$, Bonferroni-adjusted). After breakfast, the LC meal generated a significantly different pattern than after HC breakfast meal (p=0.008). The LC pattern of change is linear barely rising above baseline ($p < 0.05$ Bonferroni corrected). For the postprandial lunch period, the mean plasma insulin response resembled the postprandial breakfast period with a quadratic pattern after the HC meal and, again, a linear pattern following the LC meal ($p > 0.05$ Bonferroni corrected).

Figure 8: Serum Insulin Concentrations Over Time



Boxes on axis indicate when meals were consumed (0805-0825, 1305-1325).

The post breakfast and post lunch AUC, as well as total AUC, for insulin were significantly lower after the LC than the HC meals ($p<0.01$).

Non-esterified Fatty Acid and Insulin Relationship Analysis

The first analysis to determine if a relationship existed between the pattern of change for insulin and non-esterified fatty acid (NEFA) concentrations was to identify the maximum and minimum concentrations of insulin and NEFA, respectively. The sampling period was divided in to HC morning, HC afternoon, LC morning, and LC afternoon segments, as previously described, to separate each of the postprandial periods.

To test if the maximum insulin concentration occurred at the same time as the NEFA minimum concentration, time to peak/trough was calculated for each of the four postprandial segments. On average, minimum NEFA concentrations were reached 52 minutes after maximum insulin concentrations were reached (95% CI: 33, 70 minutes). indicating these two events happen at different times. After the HC lunch meal, the

minimum NEFA concentration occurred approximately 73 minutes after the maximum insulin concentrations peaked (95% CI: -10, 156 minutes). The results indicate there is no difference in when these two occurrences happened yielding an inverse relationship. Due to large variability among the subject's plasma NEFA and insulin concentrations in the postprandial HC lunch interval, the inverse relationship was found despite the maximum and minimum concentrations occurring father apart than in the postprandial HC breakfast period.

A distinct pattern was not observed following the LC meals. For the postprandial period after the LC breakfast meal, plasma NEFA concentrations reached their minimum concentration approximately 23 minutes before insulin concentrations peaked (95% CI: -49, 7). The insulin maximum and NEFA minimum statistically occurred at the same time, yielding an inverse relationship after the LC breakfast meal. However, insulin concentrations did not significantly rise after the LC breakfast making this finding statistically accurate but clinically irrelevant. The postprandial period following the LC lunch was less distinctive and correlation analysis could not be run due to large variability. Overall, only the postprandial period after the HC breakfast showed a significant difference for when the NEFA minimum and the insulin maximum were reached. The postprandial period after the HC lunch and LC breakfast were more variable demonstrating that the time at which NEFA concentrations reach its minimum concentration and insulin reaches its maximum concentration do not differ ($p= 0.12$ and $p= 0.17$, respectively). For the postprandial period after the LC lunch meal, there was so much variability no specific relationship between the two variables could be concluded from the data. (Graphs of variability can be seen in Appendix F).

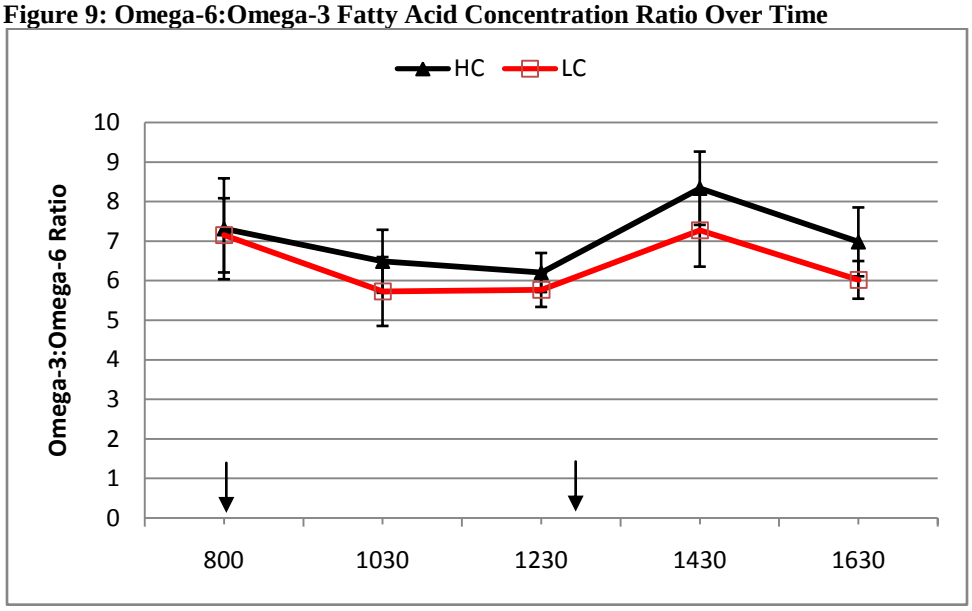
The relationship between circulating insulin and NEFA concentrations were further tested through correlational analysis. Again, the sampling period was divided into HC, LC morning and HC, LC afternoon intervals. Correlations were calculated for each individual and tested together by Wilcoxon Sign Rank test. This test calculates whether a correlation exists, it does not determine the strength of the correlation. For the postprandial periods after the HC breakfast and lunch meals, negative correlations were determined ($p=0.002$ and $p= 0.002$, respectively). For the postprandial periods after the LC breakfast and lunch meals, no correlations between insulin and NEFA concentrations were observed ($p= 0.11$ and $p= 1$, respectively). The absence of a correlation between insulin and NEFA concentrations is related to the relatively small changes in both analytes following the consumption of the LC meals.

Our findings support the hypothesis that there is a negative correlation between circulating insulin and NEFA concentrations, but only after the HC meals. The hypothesis is negated in relation to the LC meals as no correlation was observed.

Postprandial Omega-6: Omega-3 Fatty Acid Concentration Ratio Changes with Diet

Changes in the omega-6/omega-3 concentration ratio over time for after HC and LC meals are shown in Figure 9. Mean fasting concentration ratios were similar at 7.31 ± 1.27 and 7.15 ± 0.94 before the HC and LC meals, respectively ($p=0.768$). The visual pattern of response closely followed that of the PUFA curve, indicating the response is derived from changes in PUFA concentrations.

Figure 9: Omega-6:Omega-3 Fatty Acid Concentration Ratio Over Time



Arrows on axis indicate when meals were consumed (0805-0825, 1305-1325).

AUC for the ratio values were not significantly different between intervention meal types following breakfast (HC 30 ± 3.4 vs. LC 28 ± 3.2; p=0.077) or lunch (HC 30 ± 2.5 vs. LC 26 ± 2.5; p=0.094). Total AUC was significantly different between intervention meals with a higher change over time following the HC meals compared to the LC meals (HC 60 ± 5.3 vs. LC 54 ± 5.2; p=0.018). These results also match the PUFA response.

When comparing the plasma n-6: n-3 ratio response to the n-6:n-3 ratio of the intervention meals, the response does not seem to be driven by the difference in the meal n-6: n-3 ratio. There was no difference between fasting and postprandial ratios after either diet, except at the 1030 time point immediately following the LC breakfast meal (p=0.045). The plasma n-6:n-3 concentration ratio dropped after the LC breakfast meal from 7.15 ± 0.94 to 5.7 ± 0.87 despite the meal containing a n-6: n-3 ratio of 7.45. Meal ratios and plasma responses are reported in Table 9 (below).

Table 9: Meal and Plasma Omega 6: Omega 3 Fatty Acid Ratio

Breakfast Meal	Meal n-6:n-3 ratio	Plasma Ratio Before Meal (0800 or 1230)	Plasma Ratio After Meal (1030 or 1430)
High Carbohydrate	4.3	7.3 ± 1.3	6.5 ± 0.8
Low Carbohydrate	20.1	7.1 ± 0.9	5.7 ± 0.4
Lunch Meal			
High Carbohydrate	6.5	6.2 ± 1.6	8.3 ± 0.9
Low Carbohydrate	7.0	5.8 ± 0.4	7.3 ± 0.9

Mean ± SEM; n-6 = omega-6 fatty acids; n-3 = omega 3 fatty acids

CHAPTER 10: DISCUSSION

Despite public health efforts to educate the US population on the health hazards of being overweight and obese, the prevalence of overweight and obese continue to rise (64). The Akins dietary philosophy is a popular choice by the public as is promises quick weight loss results. The primary issue with fad diets, such as the Atkins, is there is limited scientific evidence on overall, long term health effects, especially cardiovascular disease risk (64-66).

Of the research that has been performed regarding the efficacy of low carbohydrate diets versus conventional low fat diets, weight loss and CVD risk are the primary outcomes. In the physiological state of weight loss, plasma cholesterol concentrations (total, HDL, LDL) improve due to the utilization of lipids for energy resulting in decreased concentrations of markers of cardiovascular disease risk. A study completed at the University of Connecticut assessed plasma lipids with a LC diet in healthy, normal weight women (15). The authors concluded overall cardiovascular risk was decreased after the LC intervention and an oral fat test; HDL cholesterol increased, and there was a decrease in fasting and postprandial triacylglycerol (15). However, the women lost weight even with efforts to maintain weight (data not shown). The decrease in triacylglycerol could be due to weight loss or decreased carbohydrate intake. Our study assessed acute feeding in healthy, normal weight individuals without weight loss. Subjects demonstrated postprandial hyperlipidemia throughout the day following the LC meals. If the diet is consumed long term without accompanied weight loss, such as in the post weight loss, weight maintenance phase, the diet may be associated with increased CVD risk. Plasma SFA, MUFA, TAG, and NEFA total area under the curve values were significantly higher following the LC meals compared to the HC meals.

Ever since Zilversmit in 1979 (42) introduced the idea of atherosclerosis as a postprandial occurrence, research has focused more on the postprandial response to a diet rather than fasting values when assessing risk for CVD (67). Humans spend the majority of their day, up to 17 hours (68), in a postprandial state, which consists of continual fluctuation in the degree of lipemia throughout the day (43, 67). The postprandial state is dynamic with rapid remodeling of lipoproteins compared to the stable fasting condition in which CVD risk markers are traditionally assessed (43). This study utilized the consumption of two consecutive meals to study daytime postprandial responses. Using two meals allowed analysis of the "building effect" described in a study conducted in the United Kingdom that demonstrated the first meal effects the results of the second meal. Silva et al. demonstrated that the second triacylglycerol peak following lunch occurred sooner than after the breakfast meal and that part of the rise was associated with the fat in the breakfast meal (68). Because of the building effect, our design using two meals is a better representation of the true postprandial response (68, 69).

Use of two consecutive mixed meals in healthy, weight stable participants with no confounding effects of weight loss to evaluate postprandial lipemia makes our study novel. The study design compared postprandial values to fasting values. Although fasting values are the accepted measure for clinical assessment of CVD risk, postprandial hypertriglyceridemia is more strongly associated with certain CVD risk factors compared to fasting triacylglycerol levels (70). An increasing body of literature associates postprandial lipemia with CVD risk.

This study demonstrated that circulating SFA, MUFA, and triacylglycerol concentrations were higher after consumption of low carbohydrate meals versus high

carbohydrate meals. We saw greater elevations in SFA, MUFA, and triacylglycerol after the consumption of the LC meals compared to the HC meals. It is well established that diets higher in SFAs are associated with higher total and low density lipoprotein cholesterol levels (71). Elevated LDL-C is one of the strongest risk factors associated with CVD risk. Epidemiologic studies have found that MUFA concentrations are inversely correlated with CVD after adjusting for dietary SFA and cholesterol (71). In our study, the LC meals did increase plasma MUFA concentrations. According to Grundy and Mattson if the MUFA replaced the SFA intake, long-term consumption of this diet would lower LDL-C. However, the LC meals were high in both SFA and MUFA, and raised postprandial plasma SFA and MUFA concentrations so the overall long term effect is still not known.

Some studies have demonstrated that consumption of PUFA is associated with reduced CVD mortality, but other studies have shown no such association (71). Our study showed that circulating PUFA concentrations were not altered by acute consumption of either intervention meal composition. This finding suggests that plasma PUFA concentrations are not acutely affected by diet and may represent a more long-term marker of dietary intake.

The inverse relationship between NEFA and insulin was more prominent following the HC meals compare to the LC meals. Our results found a negative correlation between insulin and NEFA following the HC meals that was not observed following the LC meals. This is most likely due to the fact that insulin is a potent suppressor of lipolysis and NEFA release. Plasma insulin was higher following the HC meals compared to the LC meals (Figure 8). Each participant reacted differently to the

LC meal leading to a wide variability in the NEFA concentrations. Brynes et al. had similar results following their high fat meal intervention. They saw lower insulin response and less NEFA suppression on the intervention with high fat, low carbohydrate meals (72). Although lower postprandial insulin and glucose concentrations are associated with decreased disease risk, when recommending a low carbohydrate intervention, atherogenic and thrombogenic consequences should be considered in individuals already at risk. Higher postprandial TAG and NEFA concentrations have been proposed as an independent risk factor for coronary heart disease (73). The risks of elevated postprandial TAG and NEFA concentrations may outweigh the benefits of lower glucose and insulin.

Elevated NEFA concentrations have been widely accepted as a contributor to insulin resistance. Insulin resistance is a state where the body does not use insulin properly resulting in high plasma glucose, insulin and NEFA concentrations. This metabolic condition places individuals at risk for diabetes, further hyperlipidemia, and CVD. Elevated plasma and muscle triacylglycerol content, as well as plasma NEFA concentrations are associated with insulin resistance, but the exact mechanism is not known (74, 75). The challenge researchers now face is to determine which of the theorized mechanisms dominate in the insulin sensitive tissues and under what circumstances these mechanisms cause insulin resistance. The determination of whether lipid induced insulin resistance is caused by the same mechanism, or if there are different mechanisms of lipid induced insulin resistance, such as acute elevations in free fatty acids versus longstanding obesity (76), has not been elucidated. In diseases such as obesity, metabolic syndrome, and non-insulin dependent diabetes mellitus, there is a chronic

elevation in free fatty acids which can induce or aggravate an insulin resistant state. Therefore weight loss diets that knowingly elevate postprandial plasma SFA, TAG, and NEFA concentrations in a weight stable population should be recommended with caution. When losing weight, CVD risk markers will theoretically improve regardless of diet. When weight loss slows and weight maintenance is the new goal of a patient, continued use of a LC diet should be reconsidered because of the possible increased CVD risk from elevated SFA, TAG, and NEFA concentrations; although long term consumption was not tested in this study. This is especially a concern in populations already at risk for insulin resistance, diabetes, or CVD.

LIMITATIONS OF THE STUDY

Despite a sample size of 10 participants, the primary analysis of this study reached statistical significance. A greater sample may have shown significant results in the MUFA and insulin-NEFA correlation analysis. There was a potential ascertainment bias associated with the subjects we enrolled in the study. Although our subject recruitment pool was open to the greater Portland area, all of our subjects except one were either medical professionals or students from OHSU. There was also a lack of blinding of research staff to aspects of the intervention phase, such as the composition of study meals consumed during the intervention phase. Although participants were not told which diet they were consuming during each inpatient admission, blinding to the type of meal was not achieved due to the stark contrast in food items served for the LC and HC meals. One source of bias associated with the data analysis portion of our study included secondary analysis of data collected in the previous pilot study.

STRENGTHS OF THE STUDY

The study design contributed many strengths to this project. The cross-over design of this study allowed each subject to act as their own control minimizing confounding variables generally found between cohorts, such as age, race, BMI, and activity level. The resultant sample of young, healthy subjects offers a model that has fewer confounding variables compared to an older or diseased population.

The three day standardization diet consumed prior to the intervention meals helped to minimize differences in nutrient intake within and between subjects eliminating confounding variables of previous macro- and micro-nutrient intake from each subject's personal diet.

The inpatient protocol of consuming 2 intervention meals was a strength due to its simulation of a more normal eating pattern. This allowed the investigators to study a more "real-life" daily pattern of physiological response to either HC or LC meals (68). The whole meals used in this study are a strength because it increases the generalizability and real-life effectiveness of the results.

The postprandial analysis was important to see because, as mentioned previously, the postprandial response may be more indicative of CVD risk than fasting values (42-44, 77). Postprandial lipemia is one aspect of the study that makes it novel. The analysis of fatty acids categorized into SFA, MUFA, and PUFA is unique to this study and provides a greater understanding to the postprandial response to these two types of meals and possibly to better understand hyperlipidemia risk of CVD. The method used to measure the fatty acids was also a strength. Previous studies (2) utilize a weight percent method as a measure of the fatty acid which give a relative number in comparison to the other fatty

acids within the sample. In this protocol fatty acid concentrations were measured quantitatively in μmol/L, providing an absolute concentration for each individual fatty acid analyzed.

CLINICAL IMPLICATIONS

Our study measured fatty acids in absolute concentrations, not in weight percent. The results indicate SFA and MUFA concentrations increase but PUFA concentrations do not change dramatically following dietary intake. If the weight percent method had been used to assess fatty acid postprandial changes, the increases is plasma SFA and MUFA concentrations after the LC meals would have decreased the percentage of PUFA concentrations when in reality the PUFA concentrations stayed relatively stable throughout the postprandial sampling period.

The fatty acid profile technique used in this study is also used to assess essential fatty acid status in at risk populations (58). When essential fatty acid deficiency is suspected, diagnosis was traditionally accomplished by gas chromatography-flame ionization detection (GC/FID) and reported as weight percent of the total amount of fatty acids. This method of analysis may not be ideal when it comes to diagnosing a deficiency. Lagerstadt et al. (58) did a comparison study looking at EFA deficiency diagnosis measured by weight percent and in absolute concentrations of μmol/L. It was found that with the weight percent analysis there were more false positives for deficiency when compared to the absolute concentrations analysis (58). When there is a postprandial rise in lipids or a dramatic change in fat intake, the percent of circulating C18:2 may decrease if there is a significant increase in saturated fatty acids such as C16:0. The

perceived decrease in C18:2 would be due to the rise in SFA when in actuality there was no change in the C18:2 concentration.

This study demonstrated that the essential fatty acid concentrations, included within polyunsaturated fatty acid concentrations, are relatively stable after the ingestion of a meal. Recommendations for essential fatty acid assessment have traditionally been to measure fatty acids in fasting samples. Populations most at risk for essential fatty acid deficiency tend to be fed frequently and/or continuously: growing babies on skim milk formulas, total parenteral nutrition patients and many patients with inherited metabolic disorders (31). The frequent feedings and energy demands make collecting a fasting sample difficult in these patient populations. It was believed non-fasting conditions may not allow accurate assessment of essential fatty acid status. The results of this study suggest the use of non-fasting blood draws for evaluation of essential fatty acid concentrations and may be valid in assessing essential fatty acid deficiency status.

CHAPTER 11: SUMMARY AND CONCLUSIONS

This prospective randomized crossover clinical trial characterized the postprandial lipid response in ten healthy, weight stable adults following consumption of low versus high complex carbohydrate meals.

The first hypothesis was accepted: low carbohydrate meals significantly increased circulating concentrations of plasma TAG and SFA postprandially compared to the high, complex carbohydrate meals. Both meal types raised TAG and SFA levels over time. However, the rise in plasma TAG and SFA concentrations following the high carbohydrate meals was smaller than the rise following the low carbohydrate meals.

The second hypothesis, that postprandial insulin levels would be inversely correlated with postprandial NEFA levels, was not universally accepted. There was a negative correlation between these two metabolic markers associated with the postprandial period after the HC meals but not after the LC meals. This finding suggests the insulin and NEFA responses are diet dependant. The data suggest that in the presences of low insulin concentrations, as after the LC meals, NEFA concentrations are more variable between subjects than when insulin concentrations are higher, as after the HC meal.

The third hypothesis proposed that plasma omega-3 fatty acid concentrations would be lower after the LC meals compared to the HC meals, with no difference in MUFA, PUFA, or omega-6 fatty acid concentrations. This hypothesis was not universally accepted. Omega-3fatty acid concentrations were not different between intervention meals rejecting the hypothesis. In addition, there was no substantial change from baseline after the HC or LC meals indicating that n-3 plasma concentrations were relatively stable

over time. MUFA concentrations were higher based on AUC in the postprandial period following the LC lunch and overall for the LC meals. MUFA concentrations following the HC meals did not differ from baseline. Although AUC indicates a higher MUFA concentration following the LC meals, contrast analysis suggests no difference in the plasma concentrations between HC and LC meals most likely due to the large interperson variability. This data in inconclusive and neither supports or negates the hypothesis that MUFA concentrations do not differ between diets.

The third hypothesis also states there will be no difference in PUFA or omega-6 fatty acid concentration after the consumption of high or low carbohydrate meals. Changes in PUFA concentrations were similar after both meal types. Contrast analysis determined PUFA concentrations after the HC meals tended to be higher compared to the LC meals but overall there was no significant effect. Total AUC was higher for the PUFA concentrations after the HC meals compared to the LC meals suggesting increased concentrations over time. The PUFA pattern of change was predominantly determined by the n-6 fatty acid concentration. Thus, the trend for concentration over time for omega-6 fatty acids was similar to that of total PUFA. Total AUC was statistically greater following the HC meals compared to the LC meals over time. However, contrast analysis determined there was no difference between diets suggesting no effect from diet. The higher AUC for PUFA and omega-6 fatty acids may be due to the difference in fasting values. Statistically, baseline values were the same but the actual HC values were higher concentrations compared to the LC plasma concentrations for PUFA and omega-6 fatty acids. This slightly higher baseline concentration may explain the higher AUC observed

following the HC meals. This conclusion is supported by the contrast analysis that suggests the pattern of change was not different between diets.

Both diets effected postprandial lipid concentrations. The LC meals increased plasma SFA and TAG concentrations, which may be associated with increasing CVD risk. There was no difference in total PUFA, n-6 or n-3 fatty acid concentrations between diets. This suggests that the LC diet could be detrimental when consumed by weight stable individuals, especially in the long-term.

CHAPTER 12: FUTURE DIRECTIONS

During the study period the methodology for quantifying plasma ketones by GC/MS was not validated. The method we were going to attempt to carry out was first validated at Saint Louis University Medical Center. Plasma lactate, pyruvate and ketone concentrations will be determined by stable-isotope dilution. Keto acids will then be extracted and derivatized to their trimethlysilyl (TMS) esters. Analysis will be completed with GC/MS. This validation project will be tested in the Bioanalytical Shared Resource Pharmacokenetics Core Lab at OHSU, directed by Dr. Dennis Koop. Formulating this method is the next step for this project in order to compare postprandial ketone concentrations with insulin and NEFA concentration after the consumption of a very LC meal.

Another aspect that could be analyzed in the future is the potential insulin resistance caused by the intervention meals. Knowing that elevated NEFA levels are strongly associated with insulin resistance, an equation developed by Marsuda and DeFronzo (78) can be used to calculate fasting and postprandial insulin resistance for both intervention diets. This equation (Figure 10) is highly correlated with whole body insulin sensitivity measured by euglycemic insulin clamp test ($r = 0.73$, $p < 0.0001$). By looking at this we can more confidently recommend the proper weight loss diet for individuals at risk for diabetes and CVD.

$$\frac{10{,}000}{\sqrt{(FPG \; x \; FPI) X \; (\bar{G} \; x \; \bar{I})}}$$

Figure 10: Matsuda and DeFronzo composite equation for measuring fasting and postprandial whole body insulin sensitivity. FPG: fasting plasma glucose, FPI: fasting plasma insulin, $\bar{G}$: mean glucose, $\bar{I}$: mean insulin

This project measured plasma lipids in the postprandial period in healthy, normal weight individuals. This information helps to understand the physiological response to two intervention diets without confounding variables such as weight loss or disease. Future directions in the research of postprandial hyperlipidemia and CVD risk could proceed in several directions. Two concepts I would like to see developed in the future are postprandial chylomicron response and atherosclerotic plaque composition testing.

It has long been thought the postprandial period is partially responsible for the development of atherosclerosis (42). Through the digestion of fat, chylomicrons are formed by the enterocytes of the small intestine following a meal. As chylomicrons are metabolized in the plasma they give up their triacylglycerol content to become chylomicron remnants. The major apolipoprotein of chylomicrons and chylomicron remnants is apoB-48. It has recently been theorized that high circulating concentrations of chylomicrons and their remnants may be the postprandial component that directly imbeds in the intima wall and leads to the development of atherosclerosis (77, 79, 80). This was not hypothesized before because it was thought the large particle size of chylomicrons were unable to penetrate the endothelial cell space of the intima (77). It is likely that the small remnants, not the larger nascent chylomicrons are atherogenic since their small size can facilitate passage into the intima (79).

A study published in 2007 used a newly developed method to detect apolipoprotein within plaques taken post-mortem from human bodies (77). Nakano et al. was the first study to describe apoB-48 in human atherosclerotic plaques. Most of the apoB-48 lipid particles were found to be smaller in size than high density lipoproteins indicating chylomicron remnants as a larger contributor to atherogenic plaque compared

to nascent chylomicrons. However, the authors noted that most apoB-48 lipoproteins synthesized in the intestine become absorbed by and circulate in LDL-C. The LDL-C in circulation likely forms larger VLDL-sized particles in the late postprandial period (79). The significance of circulating remnant particles with increased VLDL leading to atherosclerosis is currently unknown.

As it has been discussed, this is an interesting and developing area of interest. The further investigation in the development of an oral fat tolerance test for clinical assessment would be a good next step for evaluation of postprandial triglyceridemia. Postprandial triglyceridemia would be indicative of abnormal plasma chylomicron metabolism, reflecting either intestinal chylomicron overproduction and or delayed plasma chylomicron clearance (80). Both situations would expose the vasculature for a prolonged period of time adding to CVD risk. There are three problem areas for the development of a fat tolerance test according to a review article by Cohn: first, there is no specific fat load or test meal recognized as being the most appropriate; second, there is no ideal way in which to monitor a subject's postprandial response; and third, there are no current standards for quantifying a subjects fat tolerance (80). These problem areas should be the immediate areas of future research to provide more methods to assess and quantify postprandial hyperlipidemia. In addition, more plaque studies are necessary to provide further evidence for the new methods introduced in Nakano's research.

REFERENCES

1. AHA. Heart and Stroke death rates steadily decline; risks still too high.: American Heart Association News, January 22, 2008.
2. Tay J, Brinkworth GD, Noakes M, Keogh J, Clifton PM. Metabolic Effects of Weight Loss on a Very-Low-Carbohydrate Diet Compared With an Isocaloric High-Carbohydrate Diet in Abdominally Obese Subjects. Journal of the American College of Cardiology 2008;51:59-67.
3. Seshadri P, Iqbal N, Stern L, Williams M, Chicano KL, Daily DA, McGrory J, Gracely EJ, Rader DJ, Samaha FF. A randomized study comparing the effects of a low-carbohydrate diet and a conventional diet on lipoprotein subfractions and C-reactive protein levels in patients with severe obesity. The American Journal of Medicine 2004;117:398-405.
4. Yancy WS, Jr., Olsen MK, Guyton JR, Bakst RP, Westman EC. A Low-Carbohydrate, Ketogenic Diet versus a Low-Fat Diet To Treat Obesity and Hyperlipidemia: A Randomized, Controlled Trial. Annals of Internal Medicine 2004;140:769-777.
5. Wood RJ, Volek JS, Davis SR, Dell'Ova C, Fernandez ML. Effects of a carbohydrate-restricted diet on emerging plasma markers for cardiovascular disease. Nutrition & Metabolism 2006;3.
6. Llyod-Jones D, Adams R, Carnethon M, Simone GD, Ferguson B, Flegl K, Ford E, Furie K, Go A, Greenlund K, Haase N, Hailpern S, Ho M, Howard V, Kissela B, Kittnew S, Lackland D. Heart Disease and Stroke Statistics--2009 Update: a report fromt eh American Heart Association Statistical Committee and Stroke Statistics Subcommittee. Circulation 2009;119:e21-e181.
7. Barr ELM, Zimmet PZ, Welborn TA, Jolley D, Magliano DJ, Dunstan DW, Cameron AJ, Dwyer T, Taylor HR, Tonkin AM, Wong TY, McNeil J, Shaw JE. Risk of Cardiovascular and All-Cause Mortality in Individuals With Diabetes Mellitus, Impaired Fasting Glucose, and Impaired Glucose Tolerance: The Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). Circulation 2007;116:151-157.
8. NIH. Insulin Resistance and Pre-Diabetes. US Department of Health and Human Services, 2008.
9. Diabetes Prevention Program Research G. Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin. New England Journal of Medicine 2002;346:393-403.
10. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH. Obesity and Cardiovascular Disease: Pathophysiology, Evaluation, and Effect of Weight Loss: An Update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease From the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 2006;113:898-918.

11. ADA. Nutrition Recommendations and Interventions for Diabetes. Diabetes Care 2008;31:S61-S78.

12. Atkins RC. Dr. Atkins' New Diet Revolution. 3rd ed: M.Evans, 2003.

13. Stern L, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams M, Gracely EJ, Samaha FF. The Effects of Low-Carbohydrate versus Conventional Weight Loss Diets in Severely Obese Adults: One-Year Follow-up of a Randomized Trial. Annals of Internal Medicine 2004;140:778-785.

14. Forsythe C, Phinney S, Fernandez M, Quann E, Wood R, Bibus D, Kraemer W, Feinman R, Volek J. Comparison of Low Fat and Low Carbohydrate Diets on Circulating Fatty Acid Composition and Markers of Inflammation. Lipids 2008;43:65-77.

15. Volek JS, Sharman MJ, Gomez AL, Scheet TP, Kraemer WJ. An Isoenergenic Very Low Carbohydrate Diet Improves Serum HDL Cholesterol and Triacylglycerol Concentration, the Total Cholesterol to HDL Cholesterol Ratio and Postprandial Lipemic Responses Compared with a Low Fat Diet in Normal Weight, Normolipidemic Women. Journal of Nutrition 2003;133:2756-2761.

16. Sharman M, Kraemer W, Love D, Avery N, Gomez A, Scheett T, Volek J. A ketogeneic diet favorably affects serum biomarkers for cardiovascular disease in normal-weight men. Journal of Nutrition 2002;132:1879-1885.

17. Moore T, Svetkey L, Lin P-H, Karanja N, Jenkins M. The DASH Diet for Hypertension. 1st ed. New York: Pocket Books, 2003.

18. NHLBI. Your Guide to Lowering Your Blood Pressure With DASH. Paperback ed. Bethesda: National Heart, Lung, and Blood Institute, 2006.

19. Svetkey LP, Harsha DW, Vollmer WM, Stevens VJ, Obarzanek E, Elmer PJ, Lin P-H, Champagne C, Simons-Morton DG, Aickin M, Proschan MA, Appel LJ. Premier: a clinical trial of comprehensive lifestyle modification for blood pressure control: rationale, design and baseline characteristics. Annals of Epidemiology 2003;13:462-471.

20. Lien LF, Brown AJ, Ard JD, Loria C, Erlinger TP, Feldstein AC, Lin PH, Champagne CM, King AC, McGuire HL, Stevens VJ, Brantley PJ, Harsha DW, McBurnie MA, Appel LJ, Svetkey LP. Effects of PREMIER lifestyle modifications on participants with and without the metabolic syndrome. Hypertension 2007;50:609-16.

21. Harsha DW, Sacks FM, Obarzanek E, Svetkey LP, Lin P-H, Bray GA, Aickin M, Conlin PR, Miller ER, III, Appel LJ. Effect of Dietary Sodium Intake on Blood Lipids: Results From the DASH-Sodium Trial. Hypertension 2004;43:393-398.

22. Obarzanek E, Sacks FM, Vollmer WM, Bray GA, Miller ER, III, Lin P-H, Karanja NM, Most-Windhauser MM, Moore TJ, Swain JF, Bales CW, Proschan MA. Effects on blood lipids of a blood pressure-lowering diet: the Dietary Approaches to Stop Hypertension (DASH) Trial. American Journal of Clinical Nutrition 2001;74:80-89.

23. Erlinger TP, Miller ER, III, Charleston J, Appel LJ. Inflammation Modifies the Effects of a Reduced-Fat Low-Cholesterol Diet on Lipids: Results From the DASH-Sodium Trial. Circulation 2003;108:150-154.

24. Mata P, Garrido JA, Ordovas JM, Blazquez E, Alvarez-Sala LA, Rubio MJ, Alonso R, de Oya M. Effect of dietary monounsaturated fatty acids on plasma lipoproteins and apolipoproteins in women. American Journal of Clinical Nutrition 1992;56:77-83.

25. Ruiz-Gutierrez V, Muriana FJG, Guerrero A, Cert AM, Villar J. Plasma lipids, erythrocyte membrane lipids and blood ressure of hypertensice women after ingestion of dietary oleic acid from two different sources. . Journal of Hypertension 1996;14:1483-90.

26. Mata P, Alvarez-Sala LA, Rubio MJ, Nuno J, De Oya M. Effects of long-term monounsaturated- vs polyunsaturated-enriched diets on lipoproteins in healthy men and women. American Journal of Clinical Nutrition 1992;55:846-850.

27. Nydahl MC, Gustafsson IB, Vessby B. Lipid-lowering diets enriched with monounsaturated or polyunsaturated fatty acids but low in saturated fatty acids have similar effects on serum lipid concentrations in hyperlipidemic patients. American Journal of Clinical Nutrition 1994;59:115-122.

28. Gropper SS, Smith JL, Groff JL. Advanced Nutrition and Human Metabolism. 4th ed: Wadsworth Publishing, 2004.

29. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Illustrated Biochemistry. 26th ed: McGraw-Hill Medical, 2003.

30. Simopoulos AP. Omega-3 Fatty Acids in Inflammation and Autoimmune Diseases. Journal of the American College of Nutrition 2002;21:495-505.

31. IOM, FNB. Total Fat and Fatty Acids. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington DC: National Academy of Sciences, 2005:422-541.

32. Mohan LK, Escott-Stump S. Krause's Food, Nutrition, and Diet Therapy. 11th ed. Philadelphia: Saunders, 2004.

33. Oliver MF. Sudden cardiac death: the lost fatty acid hypothesis. QJM: An International Journal of Medicine 2006;99:701-709.

34. Hoak JC, Connor WE, Wckstein JW. Fatty Acid-Induced Thrombosis and Death: Mechanisms and Prevention. The Journal of Laboratory and Clinical Medicine 1964;63:791-800.

35. Delarue J, Magnan C. Free fatty acids and insulin resistance. Curr Opin Clin Nutr Metab Care 2007;10:142-148.

36. Jouven X, Charles M-A, Desnos M, Ducimetiere P. Circulating Nonesterified Fatty Acid Level as a Predictive Risk Factor for Sudden Death in the Population. Circulation 2001;104:756-761.

37. Connor WE, Hoak JC, Warner ED. Massive Thrombosis Produced by Fatty Acid Infusion Journal of Clinical Investigation 1963;41:860-866.

38. NIH. Insulin Resistance and Pre-Diabetes. Bethesda, MD National Diabetes Information Clearinghouse, National Institute of Diabetes and Digestive and Kidney Diseases, 2009.

39. Galgani JE, Moro C, Ravussin E. Metabolic flexibility and insulin resistance. Am J Physiol Endocrinol Metab 2008;295:E1009-1017.

40. Muoio DM, Newgard CB. Molecular and metabolic mechanisms of insulin resistance and [beta]-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol 2008;9:193-205.

41. Gofman JW, Lindgren F, Elliott H, Mantz W, Hewitt J, Strisower B, Herring V, Lyon TP. The Role of Lipids and Lipoproteins in Atherosclerosis. Science 1950;111:166-186.

42. Zilversmit DB. Athrogenesis: A Postprandial Phenomenon. Circulation 1979;60:473-483.

43. Hyson D, Rutledge JC, Berglund L. Postproandial Lipemia and Cardiovascular Disease. Current Atherosclerosis Reports 2003;5:437-444.

44. Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A. Postprandial lipoproteins and progression of coronary atherosclerosis. Atherosclerosis 1994;106:83-97.

45. Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. Dietary fatty acids make a rapid and substantial contribution to VLDL-triacylglycerol in the fed state. American Journal of Physiology: Endocrinology and Metabolism 2007;292:E732-E739.

46. Miesenbock G, Patsch JR. Postprandial hyperlipidemia: the search for the atherogenic lipoprotein. Current Opinion in Lipidology 1992;3:196-201.

47. Jacobson TA, Miller M, Schaefer EJ. Hypertriglyceridemia and Cardiovascular Risk Reduction. Clinical Therapeutics 2007;29:763-777.

48. NHLBI. Second Report of the Expert Panel on Detection , Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatmetn Panel II). . Circulation 1994;89:1333-1445.

49. NHLBI. Executive Summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-2497.

50. Lopez-Miranda J, Perez-Martinez P, Marin C, Moreno JA, Gomez P, Perez-Jimenez F. Postprandial lipoprotein metabolism, genes and risk of cardiovascular disease. Current Opinion in Lipidology 2006;17:132-138.

51. Speidel M, Booyse F, Abrams A, Moore M, Chung B. Lipolyzed hypertriglyceridemic serum and triglyceride-rich lipoprotein cause lipid

accumulation in and are cytotoxic to cultured human endothelial cells. High Density lipoporteins inhibit this cytotoxicity. Thrombosis Research 1990;58:251-264.

52. Groot PHE, Stiphout WAHJv, Krauss XH, Jansen H, Tol Av, Ramshorst Ev, Chin-On S, Hofman A, Cresswell SR, Havekes L. Postprandial lipoprotein metabolism in normolipideamic men with and without coronary artery disease. Arteriosclerosis and Thrombosis 1991;11:653-662.

53. Baecke J, Burema J, Frijeters J. A short questionnaire for themeasurement of habitual physical activity in epidemiological studies. American Journal of Clinical Nutrition 1982;36:936-942.

54. Pendelton N, Clague J, Horan M. Concordance of Cornell medical index self-reports to structured clinical assessment for th identification of physical health status. . Archives of Gerontology and Geriatrics 2004;38:261-269.

55. Harris J, Benedict F. A Biometric Study of Human Metabolism. Proceedings of the National Academy of Sciences of the United States of America 1918;4:370-373.

56. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and Nonfasting Lipid Levels: Influence of Normal Food Intake on Lipids, Lipoproteins, Apolipoproteins, and Cardiovascular Risk Prediction. Circulation 2008;118:2047-2056.

57. Mora S, Rifai N, Buring JE, Ridker PM. Fasting Compared With Nonfasting Lipids and Apolipoproteins for Predicting Incident Cardiovascular Events. Circulation 2008;118:993-1001.

58. Lagerstedt SA, Hinrichs DR, Batt SM, Magera MJ, Rinaldo P, McConnell JP. Quantitative Determination of Plasma C8-C26 Total Fatty Acids for the Biochemical Diagnosis of Nutritional and Metabolic Disorders. Molecular Genetics and Metabolism 2001;73:38-45.

59. Gillingham M, Connor W, Matern D, Rinaldo P, Burlingame T, Meeuws K, Harding C. Optimal dietary therapy of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. Molecular Genetics and Metabolism 2003;79:114-123.

60. Krebs M, Stingl H, Nowotny P, Weghuber D, Bischof M, Waldhausl W, Roden M. Prevention of in Vitro Lipolysis by Tetrahydrolipstatin. Clinical Chemistry 2000;46:950-954.

61. Duell P, Marsh C, Merkens L. Lipid-Atherosclerosis Laboratory Services Guide. Portland: OHSU, 2009.

62. Atkinson K. An Introduction ro Numerical Analysis. 2nd ed. New York: John Wiley & Sons, 1989.

63. Kleinbaum DG, Kupper LL, Nizam A, Muller KE. Applied Regression Analysis and Other Multivariable Methods. 4th ed. Belmont, CA: Duxbury, 2008.

64. Ohlson MS. The Skinny on Low-Carbohydrate Diets: Should they be Recommended to patients with the Metabolic Syndrome? Journal of Cardiovascular Nursing 2006;21:314-321.

65. Kennedy E, Bowman S, Spence J, Freedman M, King J. Popular diets: correlation to health, nutrition, and obesity. J Am Diet Assoc 2001;101:411-420.

66. Bravata D, Sanders L, Huang J, Krumholz H, Olkin I, Gardner C. Efficacy and safety of low-carbohydrate diets: a systematic review. JAMA 2003;289:1837-1850.

67. Lopez-Miranda J, Perez-Martinez P, Marin C, Moreno JA, Gomez P, Perez-Jimenez F. Postprandial lipoprotein metabolism, genes, and risk of cardiovascular disease. Current Opinion in Lipidology 2006;17:132-138.

68. Silva K, Wright J, Williams C, Lovegrove J. Meal Ingestion provokes entry of lipoprotein containing fat from the previous meal: possible metabolic implications. European Journal of Nutrition 2005;44:377-383.

69. Brighenti F, Benini L, Rio DD, Casiraghi C, Pellegrini N, Scazzina F, Jenkins D, Vantini I. Colonic fermentation of indigestible carbohydrates contributes to the second-meal effect. American Journal of Clinical Nutrition 2006;83:817-822.

70. Alssema M, Schindhelm RK, Dekker JM, Diamant M, Nijpels g, Teerlink T, Scheffer PG, Kostense PJ, Heine RJ. Determinants of postprandial tryglyceride and glucose responses after two consecutive fat-rich or carbohydrate rich meals in normoglycemic women and in women with type 2 diabetes mellitus: The Hoorn Prandial Study. Metabolism Clinical and Experimental 2008;57:1262-1269.

71. Griel AE, Kris-Etherton PM. Beyond Saturated Fat: The Importance of the Dietary Fatty Acid Profile on Cardiovascular Disease. Nutrition Reviews 2006;64:257-262.

72. Brynes AE, Edwards CM, Ghatei MA, Dornhorst A, Morgan LM, Bloom SR, Frost GS. A randomised four-intervention crossover study investigating the effect of carbohydrates on daytime profiles fo insulin, glucose, non-esterified fatty acids, and triacylglycerols in middle-aged men. British Journal of Nutrition 2003;89:207-218.

73. Williams C. Postprandial lipid metabolism: effects of dietary fatty acids. . Proceedings of the Nutrition Society 1997;56:679-692.

74. Holland WL, Knotts TA, Chavez JA, Wang L-P, Hoehn KL, Summers SA. Lipid Mediators of Insulin Resistance. Nutrition Reviews 2007;65:S39-S46.

75. Kelley DE, Goodpaster B, Wing RR, Simoneau J-A. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol Endocrinol Metab 1999;277:E1130-1141.

76. Kraegen EW, Cooney GJ. Free fatty acids and skeletal muscle insulin resistance. Current Opinion in Lipidology 2008;19:235-241.

77. Nakano T, Nakajima K, Niimi M, Fujita MQ, Nakajima Y, Takeichi S, Kinoshita M, Matsushima T, Teramoto T, Tanaka A. Detection of apolipoproteins B-48 and B-100 carrying particles in lipoprotein fractions extracted from human aortic atherosclerotic plaques in sudden cardiac death cases. Clinica Chimica Acta 2008;390:38-43.

78. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462-1470.

79. Campos H, Khoo C, Sacks FM. Diurnal and acute patterns of postprandial apolipoprotein B-48 in VLDL, IDL, and LDL from normolipidemic humans. Atherosclerosis 2005;181:345-351.

80. Cohn JS. Are we ready for a prospective study to invetigate the role of chylomicrons in cardiovascular disease? Atherosclerosis 2008;Supplements 9:15-18.

Appendix A: Consent Form

IRB# e1867
Protocol Approval Date: 8/29/2008

OREGON HEALTH & SCIENCE UNIVERSITY
Consent Form and HIPPA Authorization- Student Research Project-2006

Study Title: Energy and Appetite Regulation by High and Low CHO Diets (a.k.a., The Energy Balance Study) Graduate Student Research Project sub-study

Principal Investigator:	Diane Stadler, PhD, RD	(503) 494-0168
Co-Investigators:	Melissa Kumagai, RD	(503) 494-0156
	Julia Jordan, MS, RD	(503) 494-6234
	Melanie Gillingham, PhD, RD	(503) 494-1682
	Bart Duell, MD	(503) 494-8986

Sponsor: National Institutes of Health, National Center for Complimentary and Alternative Medicine

PURPOSE:

Very low carbohydrate diets and high-complex carbohydrate, low-fat diets are popular weight-loss methods in the United States. The purpose of this study is to compare the impact of these diets on factors that influence energy balance. You are invited to participate in this research because you are healthy and between 21 and 65 years of age and have met the screening criteria established for this study. We plan to enroll up to 10 subjects into this study over the next six months. All study related procedures will take place at the Oregon Clinical and Translational Research Institute (OCTRI) at OHSU.

PROCEDURES:

Summary of Procedures:

One or two pre-study screening visits are required to determine eligibility for this research project.

The first screening visit will take up to 60 minutes to complete and will involve:

- Having your weight, height, and blood pressure measured.
- Completing a medical history questionnaire and other study related forms.
- Providing a fasting blood sample of less than 2 tsp. [Note: You will need to stop eating or drinking any food or beverages (except for water) after 7 p.m. the night before the blood sample is drawn.]
- Reviewing your medical history and having a brief physical examination with the study physician.
- Meeting with the study dietitian to review food preference and activity patterns. This part of the screening may be performed at a separate visit if you prefer.

You will be offered a complimentary breakfast in the OCTRI after all the procedures for the screening visit are complete.

The main study will involve two 4-day controlled dietary phases separated by at least three days.

During days 1-3 of the first controlled dietary phase you will be asked to:

- Have your weight measured, complete study related forms, and eat breakfast in the OCTRI outpatient unit between 7 and 10 a.m.
- Take prepackaged meals and snack foods prepared for you by the OCTRI kitchen staff to eat during the rest of the day (arrangements can be made to eat other meals at the OCTRI if preferred).
- Eat all of the food provided and nothing else so that you do not gain or lose weight.
- Return all of your food containers (and any uneaten food) to the OCTRI the day after the food was to be eaten.
- Wear an activity monitor for up to 7 days.

On Day 3 of either the first or the second controlled dietary phase you will also be asked to:

- Have your body composition (the amount of fat and muscle tissue you have) measured by:
  - Bioelectrical Impedance Analysis (BIA): The BIA procedure passes a very small, unnoticeable electrical current between electrode pads attached to your hands and feet.
  - DXA scan: A DXA scan passes a very small amount of X-rays through your body while you are lying on your back on a scanning bed. You will be asked to take off any jewelry or metal items that are part of your clothing during this measurement; hospital gowns will be available for you to use. Because of the exposure to X-rays <u>all</u> women will be asked to provide a urine sample for a pregnancy test on the day of the measurement.

A subgroup of participants will have internal body temperature measured:

On day 3 of both controlled dietary phases you will swallow a single-use, disposable, sensor capsule (about the size of a vitamin tablet). The capsule moves through your stomach and intestines in about 1 to 3 days. Every 15 seconds, the capsule sends information to a pager-sized monitor that you wear at your waist. You will need to wear the monitor for about 3 days until the capsule passes through your intestinal tract. The monitor can be placed within 3 feet of your body while you sleep. You will need to wear a wrist-band that states "MRI Risk: DO NOT Perform MRI; a metal-containing thermometer was swallowed on Day-MO-YR for research purposes. Contact Dr. Diane Stadler at 503-706-2074 in an emergency" until the capsule has passed from your intestinal tract.)

Early in the morning of Day 4 (by 6 am) you will be admitted to the OCTRI Inpatient Unit. When you arrive in the morning it should be before eating or drinking foods (except water) or performing any significant exercise.

During your visit to the Inpatient Unit you will:

- Be asked to start a 24-hour urine collection.
- Have your blood pressure, pulse, heart rate, and temperature measured.
- Have a blood sampling tube placed in your arm vein so that blood samples can be drawn 12 times (each sample will contain about 1 1/3 TB of blood for a total of about 1 cup of blood) over a period of about 10 hours.
- Have your resting energy (calorie) use measured. This process involves placing a lightweight, clear, Plexiglas canopy, with an adjustable air flow rate, over your head and chest to collect samples of the air that you breathe out while you rest on a hospital bed for 45 minutes.
- Eat very-low carbohydrate breakfast and lunch meals or high complex carbohydrate breakfast and lunch meals. The very low carbohydrate meals will include foods like meat, poultry, fish, eggs, cheese, small amounts of vegetables but no fruits, cereals or bread products. The high carbohydrate/low fat meals will include foods like fruits, vegetables, cereal, bread, and low-fat meat and dairy products.
- Have your calorie use associated with meals measured for 45 minutes each hour for 10 hours. This process involves placing the same breath-collection canopy over your head and chest after you eat the research meals and while you rest on a hospital bed. You will need to remain awake during this process but you will not be allowed to engage in any activities other than quiet pursuits such as listening to music or watching TV. You will only be allowed to get up for very light activity (stretch, walk to the bathroom, etc) for 15 minutes each hour.
- Select a dinner meal from the OHSU hospital menu to eat in the OCTRI or to take with you.

You will then be discharged from the OCTRI Inpatient Unit to follow your typical diet and activities.

After the activity monitoring period is finished, you will return the physical activity monitor and core body temperature monitor.

At least three days later you will repeat the study procedures described for days 1-3 except that you will not have your body composition measured by DXA if you did so during the first controlled dietary phase.

The following day you will be readmitted to the OCTRI Inpatient Unit and you will repeat the procedures described for day 4 except that you will eat the other combination of breakfast and lunch meals (very low carbohydrate or high complex carbohydrate meals).

<u>Sample Storage</u>:

- Blood and urine will be stored for other analyses if additional funds become available.
- Potential measurements will include heart, kidney, bone, and indicators of weight regulation.
- No samples will be used for genetic testing.

Study Visits and Procedures														
Screening Visit	**1**	**2 optional**												
Weight	X													
Height	X													
Blood Pressure	X													
Medical history form	X													
Other study forms	X													
Fasting blood sample	X													
Discussion with physician		X												
Discussion with dietitian		X												
Controlled Dietary Phase														
	Dietary Phase 1							**Dietary Phase 2**						
Day	**D 1**	**D 2**	**D 3**	**D 4**	**D 5**	**D 6**	**D 7**	**D 1**	**D 2**	**D 3**	**D 4**	**D 5**	**D 6**	**D 7**
Weight	X	X	X	X				X	X	X	X			
Self-reports	X	X	X	X				X	X	X	X			
Visual Analog Scales				X							X			
Standard Diet	X	X	X					X	X	X				
Accelerometer	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample (24-hour collection)				X-------X							X----------X			
Bioelectrical Impedance (BIA)			X							X				
DXA Body Scan			X							X (if not done before)				
Core Body Temperature Measurement (subgroup)			X-------X-------X							X-------X------X				
Repeated Blood Sampling				X							X			

High or Low Carbohydrate Research Meals				X							X			
Vital signs (BP, pulse, HR, temperature)				X							X			
Resting Energy Expenditure (calorie use)				X							X			
Meal Related Energy Expenditure (calorie use)				X							X			

If you have any questions about this study, now or in the future, please contact Dr. Stadler at (503) 494-0168.

RISKS AND DISCOMFORTS:

Very low carbohydrate meals: Risks associated with consuming a very low carbohydrate diet for a day are very low. Because the very low carbohydrate diet is low in fiber, you may experience transient changes in your bowel movement frequency and/or consistency that may result in constipation or diarrhea. Because this diet has a very low water content you will be provided with non-carbohydrate containing beverages and reminded to consume adequate fluid during the inpatient admissions.

High complex carbohydrate/low fat diet: Risks associated with consuming a high carbohydrate/low fat diet for a day are very low. This diet may have a higher fiber content than your typical diet and you may notice changes in your bowel movement pattern and/or consistency. You will be encouraged to consume water with and between meals throughout each inpatient admission.

Whole Body DXA Measurement: The procedure takes about 5 minutes to complete. You will be exposed to a small amount of radiation (x-rays) from the whole body DXA scans. While no amount of radiation has been proven safe, there is no direct evidence that small doses of radiation, similar to those used in the body scan, cause harmful effects in the persons who are exposed. Before each whole body DXA scan, every female subject must have a urine pregnancy test because of the exposure to x-rays. The reason we do this is to be as careful as possible to not scan a woman who is pregnant. The results of the urine pregnancy test will remain private. We will inform you of the results and, if positive, refer you to your regular doctor or health care provider for ongoing care.

Internal Body Temperature Measurement: There are minimal risks associated with measuring internal body temperature. Internal temperature information will be transferred by radio frequency transmission from the capsule to the external monitor. The capsule will be administered by the CTRC nursing staff. The capsule may be swallowed with water or other beverages. The capsule must be swallowed without chewing. There is a small chance that choking may occur when the capsule is swallowed. Ingestion of the capsule may result in gastrointestinal discomfort including nausea, vomiting, or pain. To minimize these risks, you will be screened for abnormalities in swallowing, esophageal or bowel strictures, fistulas, or gastrointestinal obstructions. If you have any one or combinations of these conditions, you will not be allowed to participate in this procedure . If medically necessary for non-study related purposes, an MRI should not be conducted until the capsule has passed from the digestive system. Study participants will be asked to wear a "MRI Warning" wristband until the capsule has passed through the digestive system. The study physician will provide on-going oversight and follow-up throughout this procedure.

Repeated Blood Samples: You will have 13 blood samples of about 1 tablespoon each drawn from a catheter (tube) placed in an arm vein two times during the study. Approximately 7/8th cup of blood will be collected during each inpatient admission. If the catheter stops working at any time during the inpatient admission, you may need to have a new catheter placed in your other arm. You may get an infection where the tube is placed. This would cause swelling, redness, and pain. You may bleed or get a bruise. There is a small chance your blood stream or heart valves might get a serious infection. You may get a blood clot that could go to your lungs. These problems are very rare. If you have these problems, you will need hospital care. Your blood-drawing catheter will be in place in your arm for about 11 hours.

Single Fasting Blood Samples: You will have a single fasting blood sample of about 2 teaspoons drawn from an arm vein or a few drops drawn by fingerstick once during the screening phase of the study. You may feel some pain when your blood is drawn. There is a small chance the needle will cause bleeding, a bruise, or an infection. This process will take about 5 minutes to complete.

Estimation of Resting and Meal-Related Energy (Calorie) Use: There are no risks associated with having resting or meal-related calorie use measured by the proposed methods. Some people may feel "closed-in" while lying under the plastic canopy or the air may feel "stuffy". This procedure takes about an hour to complete. These measurements will be performed over about 11 hours during each inpatient admission.

Bioelectrical Impedance Measurement: The electrical conductivity tests are painless to the extent that you will not feel any procedure taking place other than having the electrode pads placed on and removed from your ankles and wrists. The electrical conductivity test takes less than 1 minute to complete.

BENEFITS:
You may or may not notice any health or personal benefits from your participation in this study. However, by serving as a subject in this study, you may contribute new information that may benefit other patients in the future. You will be informed of any clinically significant abnormalities and the safety monitoring blood test results will be provided to your physician upon request and discussed with you at the conclusion of the study.

ALTERNATIVES:
You may choose not to be in this study.

CONFIDENTIALITY:
We will not use your name or your identity for publication or publicity purposes. Research records may be reviewed and/or copied by all investigators listed on page one of this consent form, others at OHSU who are participating in the conduct of this research protocol, the OHSU Institutional Review Board, and the Oregon Clinical and Translational Research Institute.

COSTS:
There will be no cost to you for participating in this study. The study will pay for all study-related examinations and laboratory procedures. In addition, the study will pay for the costs of your food and its preparation.

LIABILITY:
It is not the policy of the U.S. Department of Health and Human Services, or any federal agency funding the research project in which you are participating to compensate or provide medical treatment for human subjects in the event the research results in physical injury.

You have not waived your legal rights by signing this form. If you are harmed by the study procedures, you will be treated. Oregon Health & Science University does not offer to pay for the cost of the treatment. Any claim you make against Oregon Health & Science University may be limited by the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

PARTICIPATION:
Dr. Diane Stadler (503) 494-0168) has offered to answer any questions you may have about this study. If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887.You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. If you refuse to join or withdraw early from the study, there will be no penalty or loss of any benefits to which you are otherwise entitled.

The investigators may withdraw you from this research study at any time if they believe it is in your best interest. You may be asked to withdraw from the study at the investigator's discretion, sponsor's discontinuation, or because of pregnancy or serious side effects, or because of your failure to comply with instructions or unwillingness to participate in study procedures. If you decide to withdraw from this study, we will ask you to complete one final follow-up and discharge visit. We will inform you of any new findings that may affect your willingness to continue or to withdraw from this research study. We will give you a copy of this consent form.

The participation of OHSU students or employees in OHSU research is completely voluntary and you are free to choose not to serve as a research subject in this protocol for any reason. If you do elect to participate in this study, you may withdraw from the study at any time without affecting your relationship with OHSU, the investigator, the investigator's department, or your grade in any course.

SIGNATURES: Your signature below indicates that you have read this entire form and that you agree to be in this study.

OREGON HEALTH & SCIENCE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
PHONE NUMBER (503) 494-7887
CONSENT/AUTHORIZATION FORM
APPROVAL DATE

Sep. 4, 2008

Do not sign this form after the Expiration date of: 8/28/2009

Signature of Subject Date

Signature of Person Obtaining Consent Date

Signature of Investigator Date

APPENDIX B: HIPAA Research Authorization Form Version: _____

OHSU

Oregon Health & Science University

HIPAA RESEARCH AUTHORIZATION

AUTHORIZATION FOR THE CREATION, USE, AND DISCLOSURE OF PROTECTED HEALTH INFORMATION FOR INSTITUTIONAL REVIEW BOARD APPROVED RESEARCH

Instructions: This authorization should be attached to each Consent Form. Investigators please complete information fields below and questions 2-4, 8, 9. Leave subject name and signature areas blank.

Title of Study:	Energy and Appetite Regulation by High and Low CHO Diets (a.k.a., The Energy Balance Study): Graduate Student Research Project Sub-study
Name of Investigator:	Diane Stadler, PhD, RD
Phone Number:	494-0168
Sponsor:	NIH: National Center for Complementary and Alternative Medicine
IRB Number:	1867
Protocol Approval Date:	8/29/2008

This authorization is voluntary, and you may refuse to sign this authorization. If you refuse to sign this authorization, your health care and relationship with OHSU will not be affected. However, you will not be able to enter this research study.

1. This form authorizes Oregon Health & Science University (OHSU) to use and disclose (release) certain protected health information about _____ (name of subject)

   that we will collect and create in this research study. The description of the information to be used or disclosed and the purposes of the requested use or disclosure are indicated in item number 8 of the authorization form.

2. The persons who are authorized to use and disclose your protected health information are:
   - ☒ All investigators listed on page one of the Research Consent Form
   - ☒ Others at OHSU who are participating in the conduct of this research protocol
   - ☒ The OHSU Institutional Review Board
   - ☒ Others: Oregon Clinical and Translational Research Institute

3. The persons who are authorized to receive this information are:
   - ☒ The sponsor of this study: NIH: National Center for Complementary and Alternative Medicine
   - ☒ Federal or other governmental agencies as required for their research oversight and public health reporting in connection with this research study:
     ☒ OHRP ☐ FDA ☒ Other: _____

☒ Others: non-OHSU Laboratories performing analysis, National Center for Research Resources, Primary Care Provider.

4. We may continue to use and disclose protected health information that we collect from you in this study until:
   - ☐ HIPAA Research Authorization expiration date _____
   - -OR-
   - ☐ The study is completed _____
   - ☒ Indefinitely
   - ☐ Other: _____

5. While this study is still in progress, you may not be given access to medical information about you that is related to the study. After the study is completed and the results have been analyzed, you will be permitted access to any medical information collected about you in the study.

6. You have the right to revoke this authorization and can withdraw your permission for us to use your information for this research by sending a written request to the Principal Investigator listed on page one of the research consent form. If you do send a letter to the Principal Investigator, the use and disclosure of your protected health information will stop as of the date he/she receives your request. However, the Principal Investigator is allowed to use and disclose information collected before the date of the letter or collected in good faith before your letter arrives. If you withdraw any tissue or blood samples that were collected from you, they either will be destroyed or stored without any information that identifies you. Revoking this authorization will not affect your health care or your relationship with OHSU.

7. The information about you that is used or disclosed in this study may be re-disclosed and no longer protected under federal law. However, Oregon law restricts re-disclosure of HIV/AIDS information; mental health information; genetic information; and drug/alcohol diagnosis, treatment, or referral information.

8. Description of the information to be used or disclosed and the purposes of the requested use or disclosure:

HEALTH INFORMATION *(Check as applicable)*	PURPOSE(S) *(Enter corresponding letter(s) from Purpose Categories)*
☐ **Your complete existing health record ****	_____
☐ **Limited information from your existing health record** (specify):** _____	_____
**** If we are requesting existing health records that are located outside of OHSU, you will need to complete an additional authorization to release these records to OHSU.**	
THE FOLLOWING CHECKED ITEM(S) WILL BE GENERATED/COLLECTED DURING THE COURSE OF THIS STUDY:	
☒ **History and physical examinations**	**a,c,d,e**
Reports: ☒ **Laboratory** ☐ **Operative** ☐ **Discharge** ☒ **Progress**	**a,c,d,e**
☐ **Photographs, videotapes, or digital or other images**	_____
☒ **Diagnostic images/X-ray/MRI/CT**	**a,d,e**
☒ **Bioelectric Output (e.g., EEG, EKG)**	**a,d,e**
☒ **Questionnaires, interview results, focus group survey, psychology survey, behavioral performance tests (e.g., memory & attention)**	**a,c,d,e**
☒ **Tissue and/or blood specimens**	**a,c,d,e**
☒ **Other: Urine specimens**	**a,c,d,e**

PURPOSE CATEGORIES

a. To learn more about the condition/disease being studied
b. To facilitate treatment, payment, and operations related to the study
c. To comply with federal or other governmental agency regulations
d. For teaching purposes
e. To place in a repository or information/tissue "bank."
f. Other _____

9. If the information to be used or disclosed contains any of the types of records or information listed just below, additional laws relating to use and disclosure of the information may apply. You understand and agree that this information will be used and disclosed only if you **place your INITIALS** in the applicable space next to the type of information.

N/A Acquired immunodeficiency syndrome (AIDS) or human immunodeficiency virus (HIV) infection information
N/A Drug/alcohol diagnosis, treatment, or referral information
N/A Mental or behavioral health or psychiatric care
N/A Genetic testing information

You will receive a copy of this authorization form after you sign it.

OREGON HEALTH & SCIENCE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
PHONE NUMBER (503) 494-7887
CONSENT/AUTHORIZATION FORM APPROVAL DATE

Sept. 4, 2008

Do not sign this form after the Expiration date of: 8/28/2009

Printed name of Research Subject

Signature of Subject

Date

APPENDIX C: GSRP Screening Form

Participant ID __ __ __ __ __ __ __ __ __

Date __ __ / __ __ / __ __ __ __

Form 01: GSRP Screening Form

1. When was your last snack, beverage or meal? . . . .___ ___ : ___ ___ AM or PM

2. Fasting time sufficient (NPO after 12:00 am?) . . . . .YES or NO
   (proceed with collection regardless of fasting time)
3. Collection time . . . . . .___ ___ : ___ ___ AM or PM

4. Samples collected (listed in order of priority)

Measurement	Results
Height	
Weight	
Temperature	
Heart Rate	
Pulse	
Blood Pressure	
POC Glucose Fasting: 50-100 (mg/dL)	
POC Hemoglobin Male: 14-18 (g/dL) Female: 12-16 (g/dL)	
Calculated BMI Weight (kg) / Height^2 (m)	
Calculate Hematocrit Multiply Hemoglobin by 3 Male: 40% - 50% Female: 37% - 47%	

Collected by (staff signature):_____Entered by (staff ID): ____ ____ ____

APPENDIX D - Nutrient Composition of Standard Diet*

Dietary Component	Days 1-3
Carbohydrate	
g/1000 kcal	130
% of energy	51
mean ± SD (grams)	360 ± 57
Protein	
g/1000 kcal	36
% of energy	14
mean ± SD (grams)	101 ± 16
Fat	
g/1000 kcal	40
% of energy	35
mean ± SD (grams)	111 ± 18
Saturated Fatty Acids	
g/1000 kcal	13
% of energy	11
mean ± SD (grams)	35 ± 9
Monounsaturated Fatty Acids	
g/1000 kcal	14
% of energy	13
mean ± SD (grams)	44 ± 9
Polyunsaturated Fatty Acids	
g/1000 kcal	9
% of energy	8
mean ± SD (grams)	28 ± 14
Cholesterol (mg/1000 kcal)	104
mean ± SD (mg)	228 ± 51
Total Dietary Fiber (g/1000 kcal)	9
mean ± SD (grams)	32 ± 0.43
Sodium (mg/1000 kcal)	1454
mean ± SD (mg)	4035 ± 709
Potassium (mg/1000 kcal)	1089
mean ± SD (mg)	3022 ± 646
Calcium (mg/1000 kcal)	577
mean ± SD (mg)	1876 ± 86
Phosphorus (mg/1000 kcal)	577
mean ± SD (mg)	1601 ± 399

* The amount per 1000 kcal and percent of total energy for each nutrient was calculated from the average amount consumed during the standardization phase.

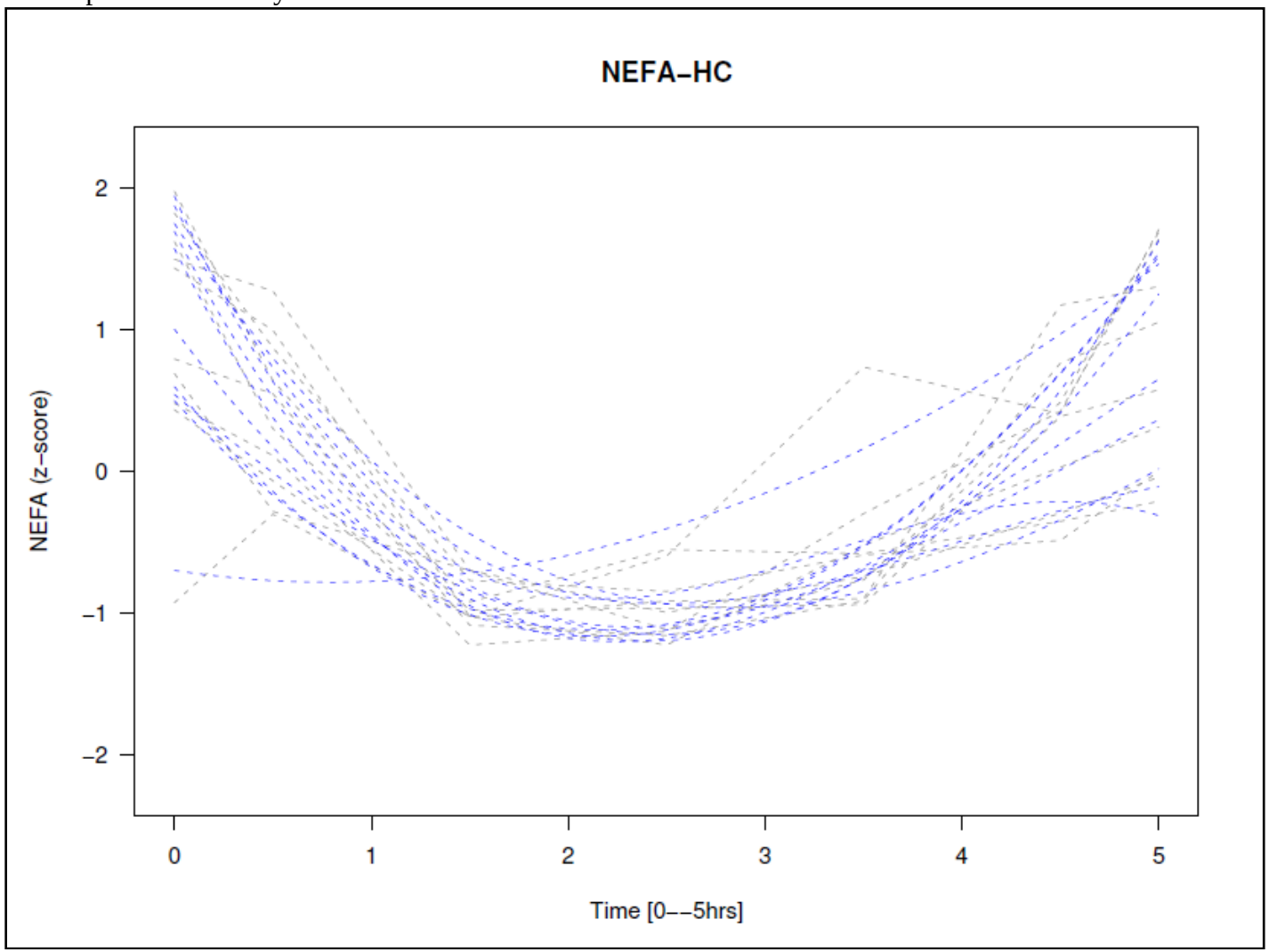
APPENDIX E. Blood Sampling and Processing Schedule

Table 4. Plasma biomarker analysis													
Collection Tube	**Analyte**	**Aliquot Vol (μl)**	**Time Points (hr)**										
In order of priority	**In order of priority**	**2 sets/ analyte if possible**	**0800**	**0830**	**0930**	**1030**	**1130**	**1230**	**1300**	**1330**	**1430**	**1530**	**1630**	**1730**
10 mL red top, deliver 6.0 mL whole blood	TSH*	500	X											
	hsCRP	100	X			X		X			X		X	
	C-peptide	500	X	X	X	X	X	X	X	X	X	X	X	X
	Insulin	500	X	X	X	X	X	X	X	X	X	X	X	X
	Leptin	500	X	X	X	X	X	X	X	X	X	X	X	X
2 mL green top (heparin)	Osteocalcin	500	X	X	X	X	X	X	X	X	X	X	X	X
	Carboxylated osteocalcin	500	X	X	X	X	X	X	X	X	X	X	X	X
2-mL grey top (NaFl/K-oxalate)	Glucose	500	X	X	X	X	X	X	X	X	X	X	X	X
6-mL purple top (K3-EDTA)	Total Triglyceride	500	X	X	X	X	X	X	X	X	X	X	X	X

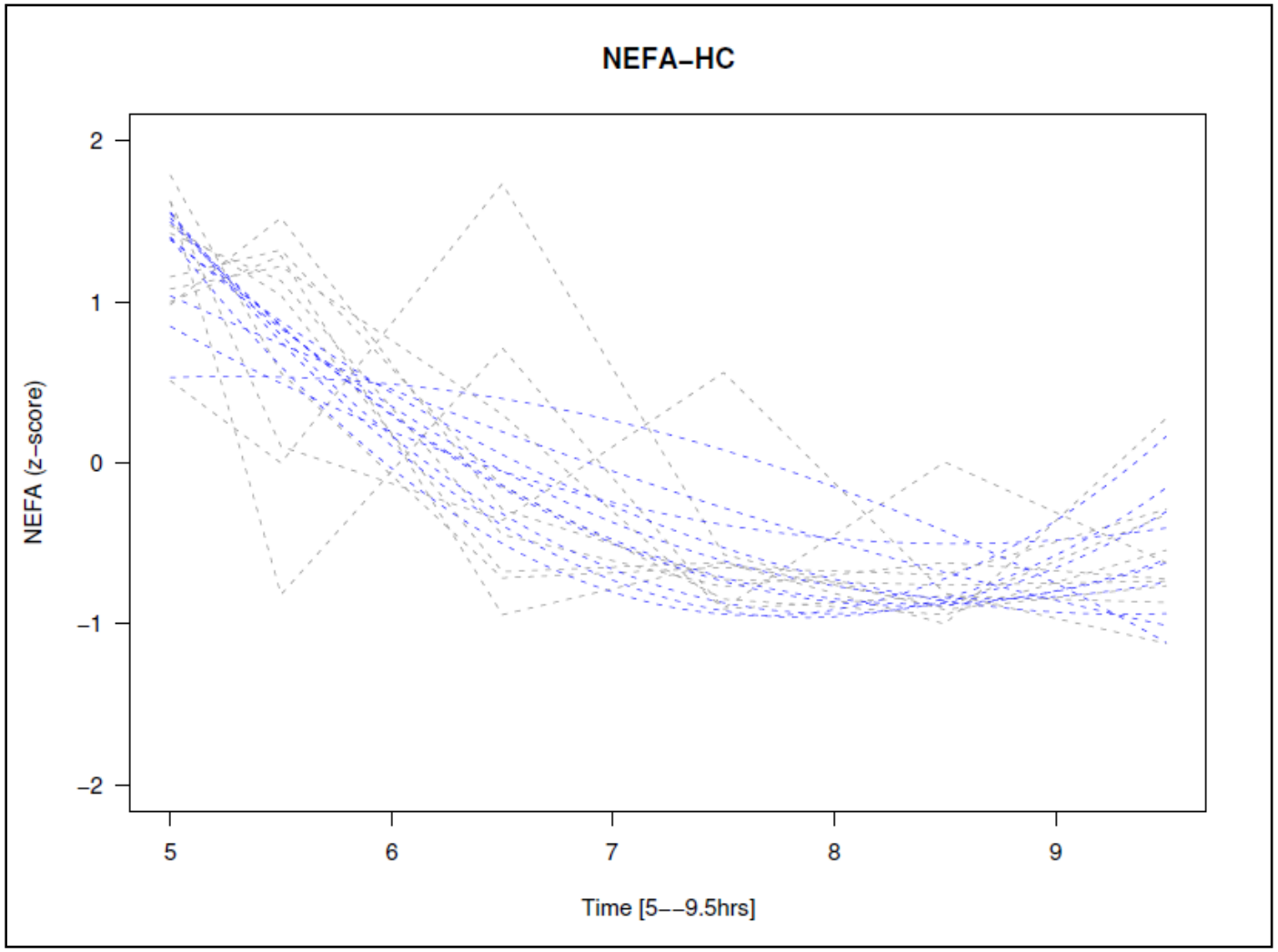
	Total ghrelin	500	X	X	X	X	X	X	X	X	X	X	X	X
	Active ghrelin	500	X	X	X	X	X	X	X	X	X	X	X	X
	TNF-α	500	X			X		X			X		X	
	IL-6	500	X			X		X			X		X	
	Fatty acid profile	100	X			X		X			X		X	
3-mL purple top (K3-EDTA) no vacuum, pretreated with 15 µl DPP-IV and 90 µl aprotinin deliver 1.5 mL whole blood	Active PYY (3-36)	300	X	X	X	X	X	X	X	X	X	X	X	X
3-mL purple top (K3-EDTA) no vacuum, pretreated with 15 µl DPP-IV, deliver 1.5 mL whole blood	Active GLP-1	300	X	X	X	X	X	X	X	X	X	X	X	X
3-mL purple top (K3-EDTA) no vacuum, pretreated with 20 µl THL, deliver 2.0 mL whole blood	Non-esterified Free Fatty Acid (NEFA)	400	X	X	X	X	X	X	X	X	X	X	X	X
Total volume drawn per time point (mL)			19	19	19	19	19	19	19	19	19	19	19	19

NOTE: All vacutainers, except for red top, should be pre-chilled on ice before collecting blood samples. Once blood is collected, all tubes (except red top) should be returned to ice and spun in a refrigerated centrifuge within 15 minutes. Red top tube should be allowed to sit at RT for 15-20 minutes before spinning. Make second set of aliquots if additional serum/plasma is available. Aliquot tubes for total and active ghrelin should be treated with HCl and PMSF. Aliquots should be frozen immediately at -20 C for up to 72 hours and then transferred to a -80 C freezer. *TSH is drawn with fasting sample during first inpatient admission, only. All samples were stored at -80 C at the GCRC Core Lab for EOS analysis. Total blood Volume = 228 mL (~1 cup total).

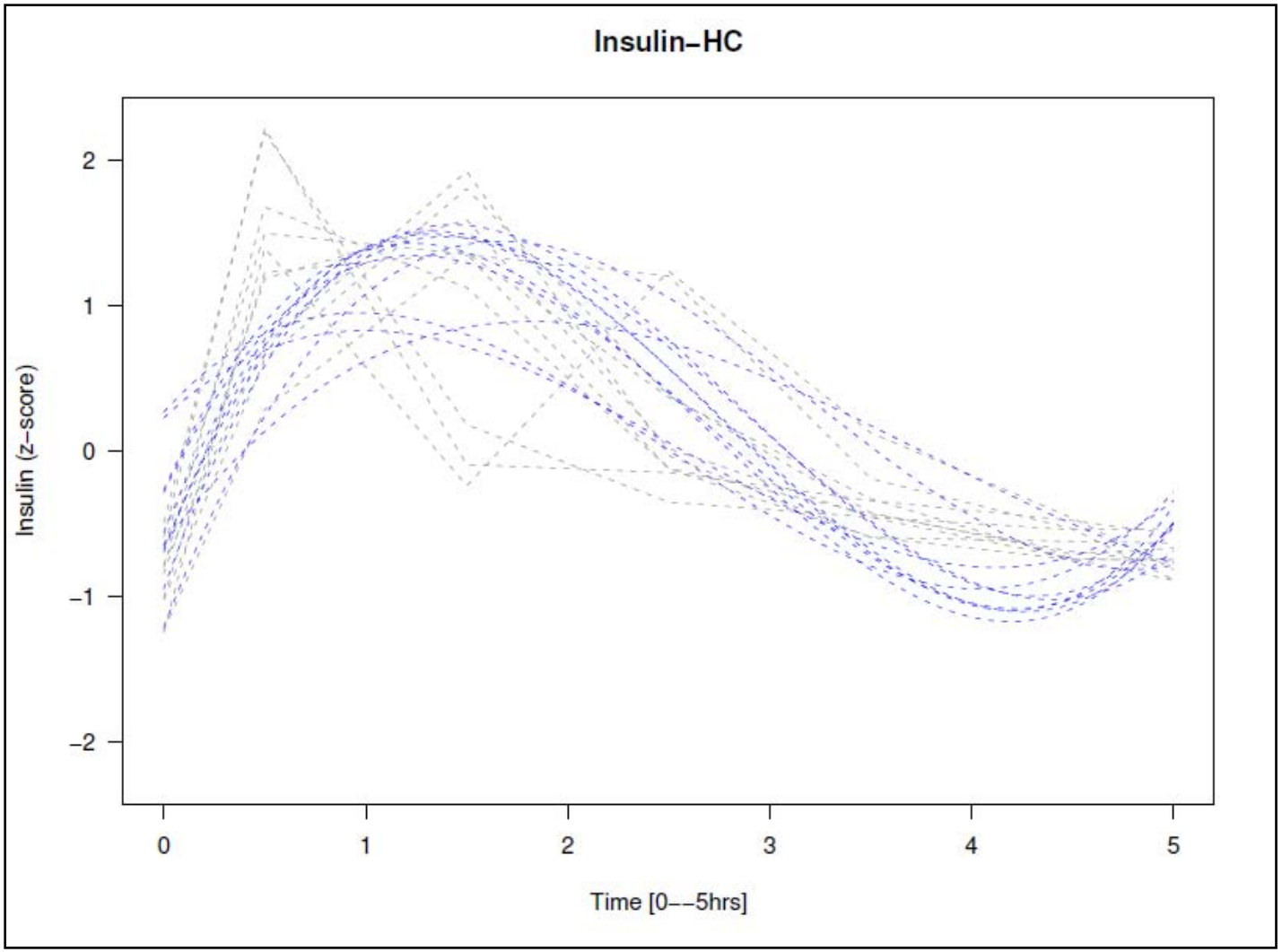
APPENDIX F: Graphs of Variability for NEFA and insulin



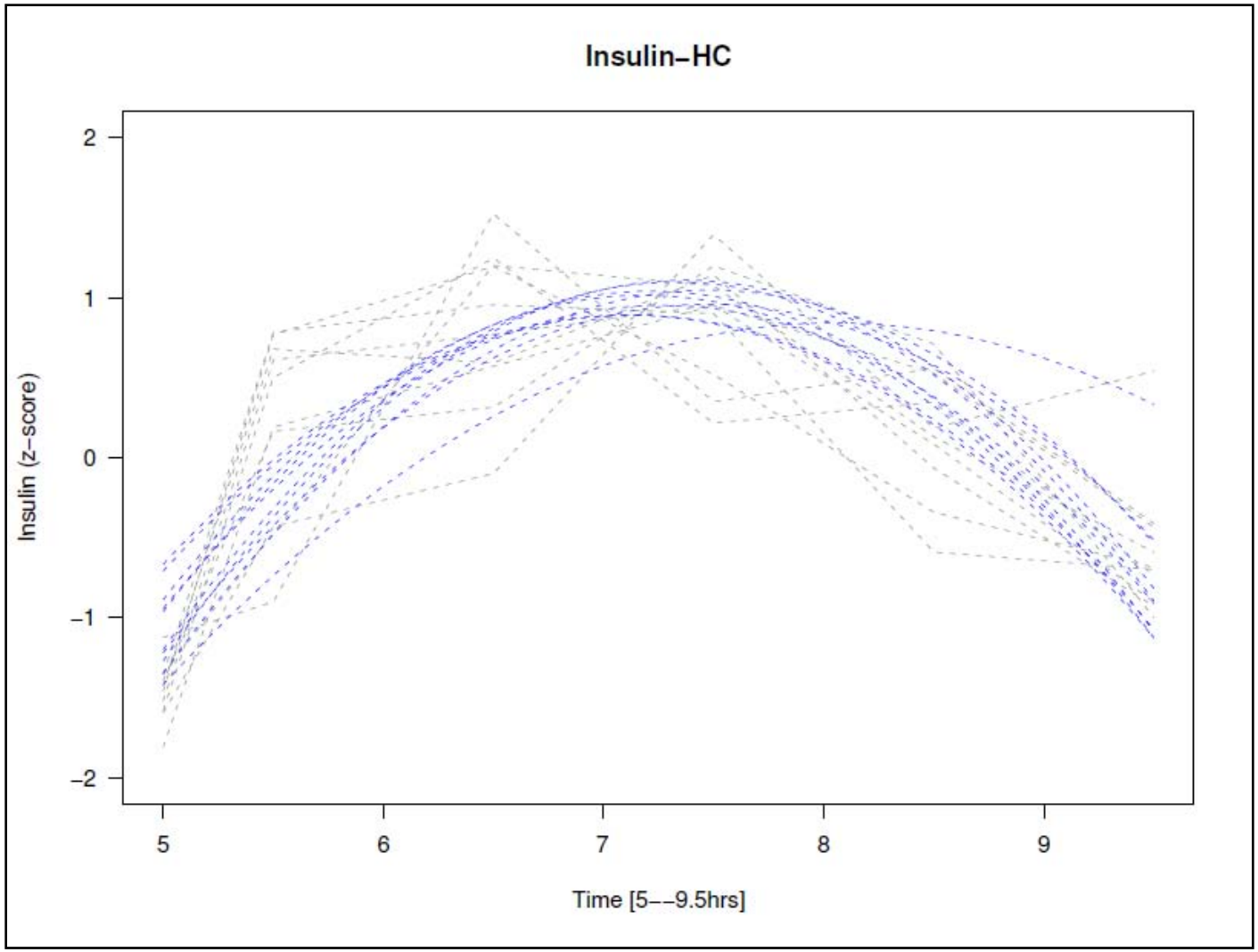
NEFA values for postprandial period after consumption of HC breakfast. Grey lines are actual values; Blue line is the calculated fitted line



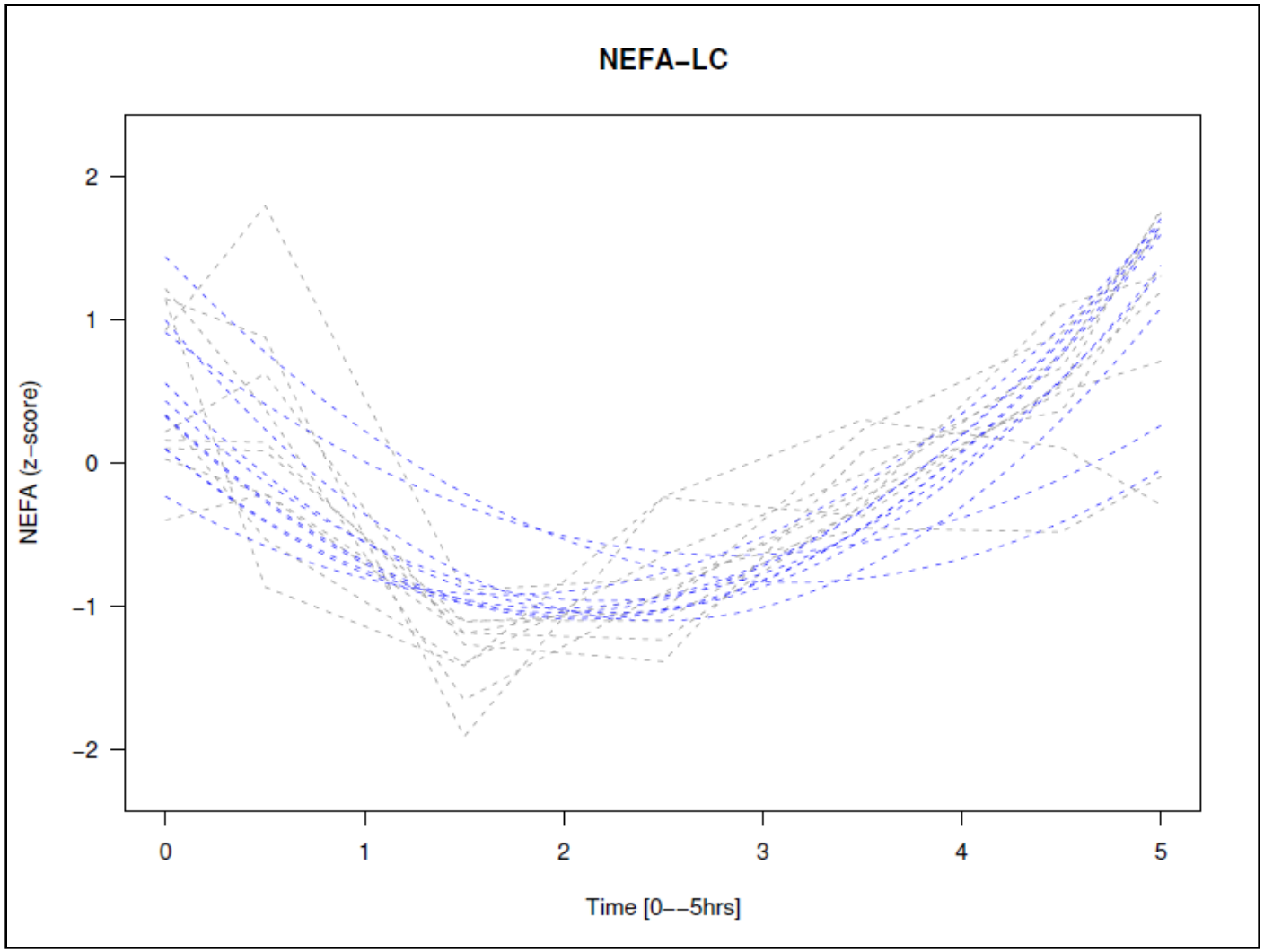
NEFA values for postprandial period after consumption of HC lunch. Grey lines are actual values; Blue line is the calculated fitted line



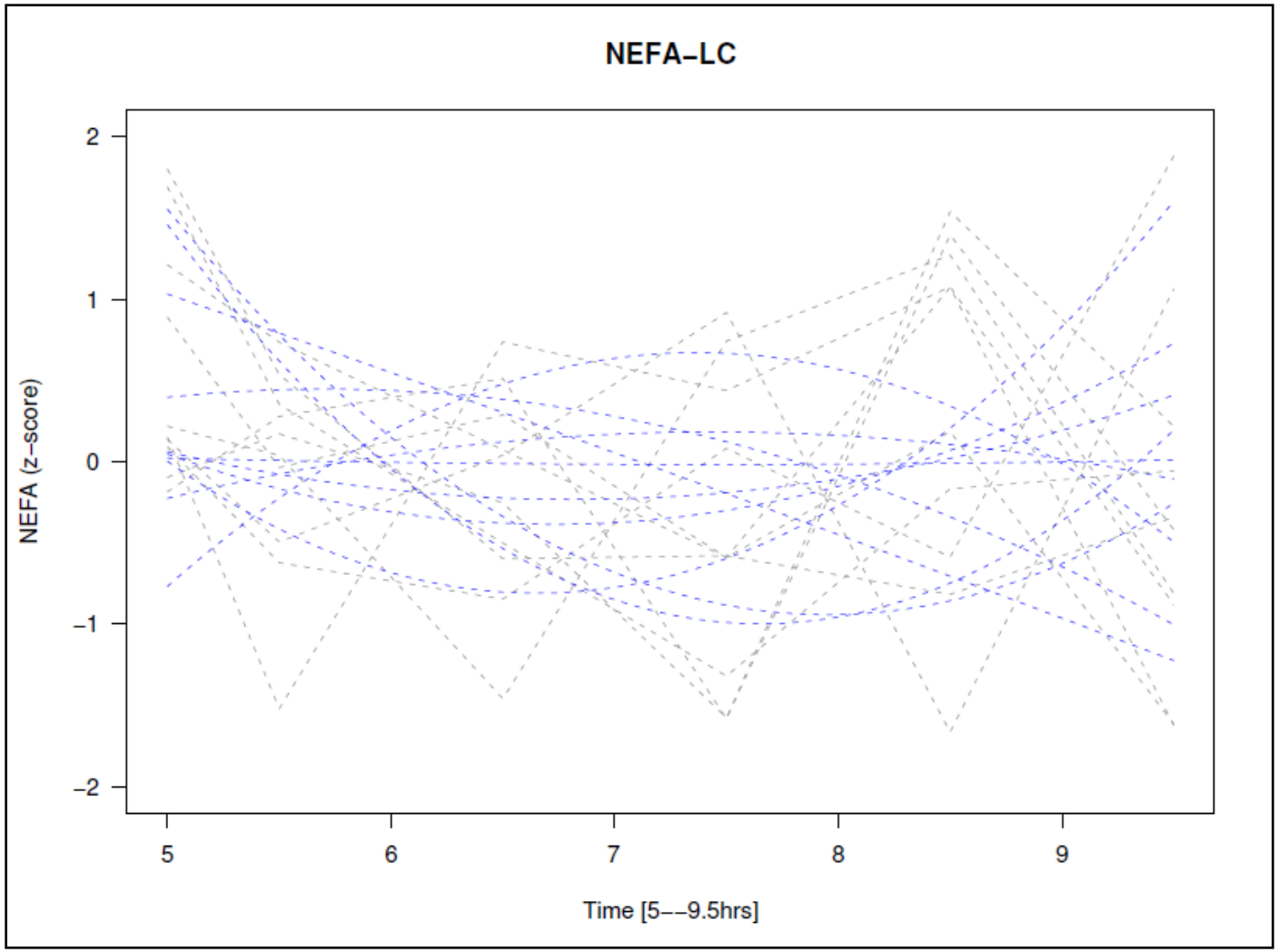
Insulin values for postprandial period after consumption of HC breakfast. Grey lines are actual values; Blue line is the calculated fitted line



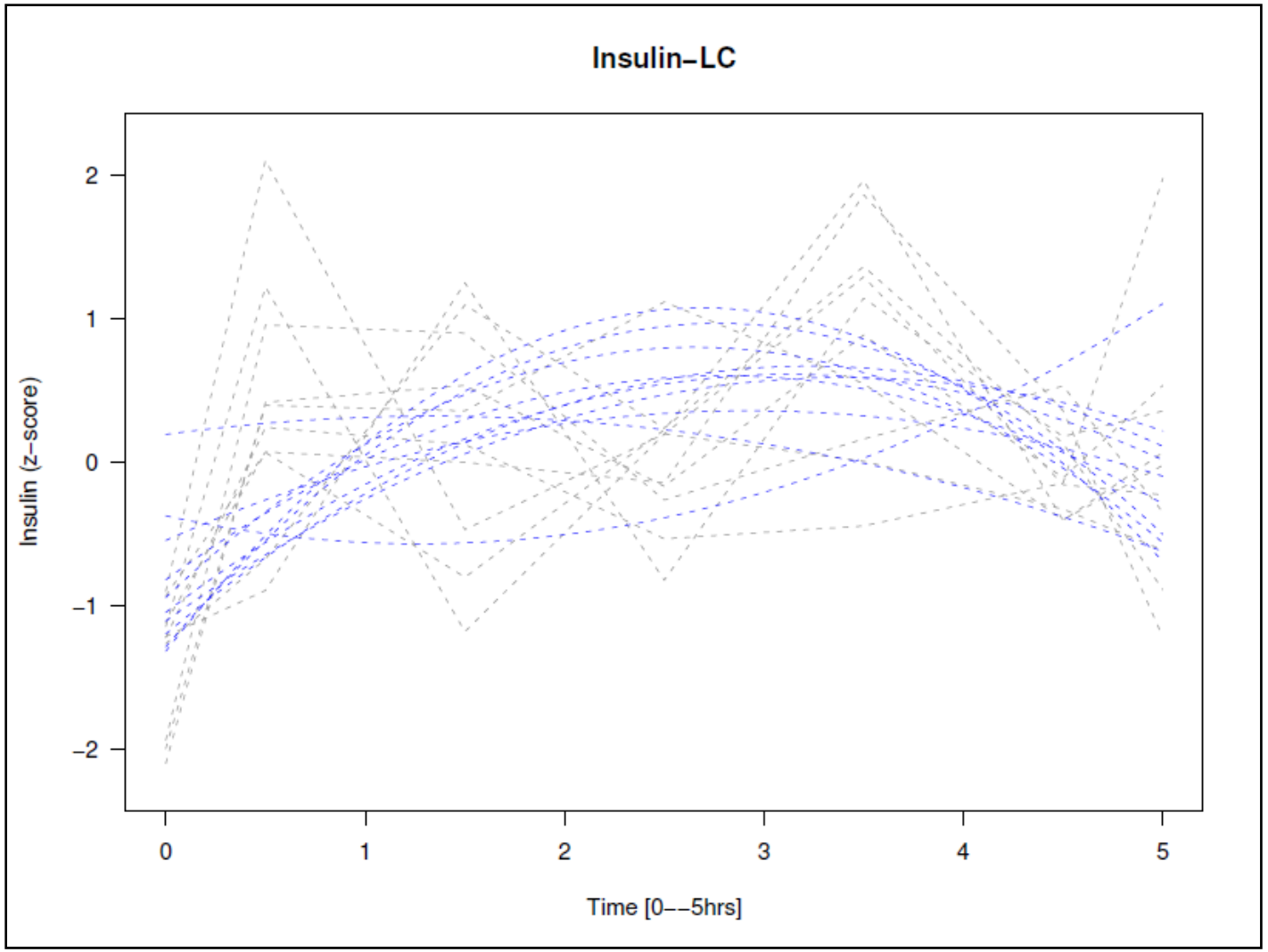
Insulin values for postprandial period after consumption of HC lunch. Grey lines are actual values; Blue line is the calculated fitted line



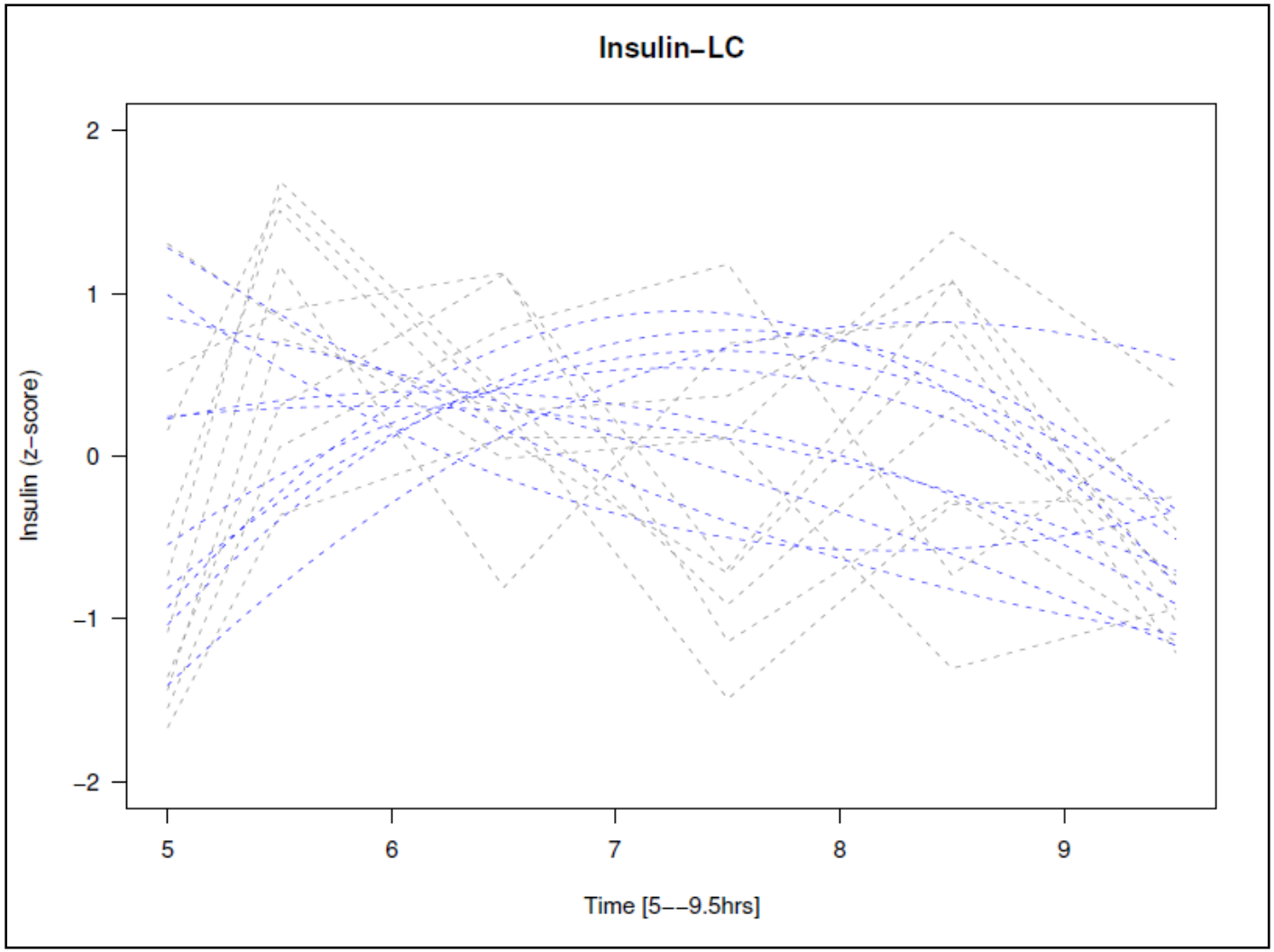
NEFA values for postprandial period after consumption of LC breakfast. Grey lines are actual values; Blue line is the calculated fitted line



NEFA values for postprandial period after consumption of LC lunch. Grey lines are actual values; Blue line is the calculated fitted line



Insulin values for postprandial period after consumption of LC breakfast. Grey lines are actual values; Blue line is the calculated fitted line



Insulin values for postprandial period after consumption of LC lunch. Grey lines are actual values; Blue line is the calculated fitted line