IMPACT OF HIGH-COMPLEX-CARBOHYDRATE AND LOW-CARBOHYDRATE DIETS AND RESULTING BODY MASS LOSS ON CIRCULATING ACYLCARNITINE CONCENTRATIONS IN HEALTHY ADULTS WITH OVERWEIGHT AND OBESITY

By

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CERTIFICATE OF APPROVAL

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LIST OF ABBREVIATIONS

ACP …..... Acylcarnitine Profile

C0 ……Free Carnitine

C2 ……..Acetylcarnitine

C3 ……..Propionylcarnitine

C3-DC ……..Malonylcarnitine

C4……..Butyrylcarnitine

C4-OH ……3-Hydroxy-butyrylcarnitine

C4-DC ……Methylmalonyl-/succinylcarnitine

C5 ……Isovalerylcarnitine

C5-OH ……3-Hydroxy-isovalerylcarnitine

C5:1 ……Tiglylcarnitine

C5-DC ……..Glutarylcarnitine

C6 ……..Hexanoylcarnitine

C6-DC ……Adipylcarnitine

C6-OH ……3-OH-hexanoylcarnitine

C8 …… Octanoylcarnitine

C8-DC …… Suberylcarnitine

C8:1 …….. Octenoylcarnitine

C10 …….. Decanoylcarnitine

C10:1 …….. Decenoylcarnitine

C10:2 ……Decadienoylcarnitine

C10:3 ……Decatrienoylcarnitine

C12 ……..Dodecanoylcarnitine

C12-DC ……Dodecadiolylcarnitine

C12-OH ……3-Hydroxy-dodecanoylcarnitine

C12:1 ……Dodecenoylcarnitine

C14 ……Myristoylcarnitine

C14-OH ……3-Hydroxy-tetradecanoylcarnitine

C14:1 ……Tetradecenoylcarnitine

C14:1-OH ……3-Hyderoxy-tetradecenoylcarnitine

C14:2 ……Tetradecadienoylcarnitine

C16 ……Palmitoylcarnitine

C16-OH ……3-Hydroxy-hexadecanoylcarnitine

C16:1 ……Hexadecenoylcarnitine

C16:1-OH ……3-Hydroxy-hexadecenoylcarnitine

C16:2 ……Hexadecadienoylcarnitine

C18 ……Stearoylcarnitine

C18:1 …… Oleylcarnitine

C18:2 ……Linoleylcarnitine

ANOVA …… Analysis of Variance

ATP …… Adenosine Triphosphate

BBM …… Brush Border Membrane

BCAA …… Branched-Chain Amino Acid

BMI …… Body Mass Index

CAC …… Citric Acid Cycle

CACT …… Carnitine Acylcarnitine Translocase

CI …… Confidence Interval

CoA ..... Coenzyme A

CPT1 ..... Carnitine Palmitoyltransferase 1

CPT2 ..... Carnitine Palmitoyltransferase 2

DASH ..... Dietary Approaches to Stop Hypertension

DBP ..... Diastolic Blood Pressure

DEXA ..... Dual-Energy X-Ray Absorptiometry

EPIC ..... European Prospective Investigation into Cancer and Nutrition

ER ..... Endoplasmic Reticulum

ETC ..... Electron Transport Chain

FADH2 ..... Flavin Adenine Dinucleotide (Reduced)

FAO .....Fatty Acid Beta-Oxidation

FATP ..... Fatty Acid Transport Protein

FFA .....Free Fatty Acid

GCRC ..... General Clinical Research Center

HCC ..... High-Complex-Carbohydrate

HCl ..... Hydrochloric Acid

HPLC/MS/MS .....High Performance Liquid Chromatography-Tandem Mass Spectrometry

LC ..... Low-Carbohydrate

LCFA ..... Long-Chain Fatty Acid

LC/MS/MS ..... Liquid-Chromatography- Tandem Mass Spectrometry

LDL-CH ..... Low-Density Lipoprotein Cholesterol

MAD ..... Modified Atkins Diet

MRM ..... Multiple Reactions Monitoring

MS/MS ..... Tandem-Mass Spectrometry

NADH …….. Nicotinamide Adenine Dinucleotide (Reduced)

NEFA …….. Non-Esterified Fatty Acid

NHLBI …….. National Heart, Lung, and Blood Institute

OCTRI …….. Oregon Clinical Translational Research Institute

OCTN2 ……..Organic Cation Transporter Novel 2

OHSU …….. Oregon Health & Science University

SBP …….. Systolic Blood Pressure

T2DM ……..Type 2 Diabetes Mellitus

UFA …….. Un-Esterified Fatty Acid

VIP ……..The Variable Importance in the Projection

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ABSTRACT

Obesity is a public health concern with rising incidence and prevalence rates. Research exploring the metabolomic biomarkers for obesity suggests a potential association between elevated acylcarnitine concentrations in individuals with obesity compared to lean controls. Low-carbohydrate (LC) and high-complex-carbohydrate (HCC) dietary patterns are commonly followed by individuals with overweight and obesity seeking to lose weight. Yet, little is known about how these diets affect circulating acylcarnitine concentrations in combination with changes in body mass, lean mass, and fat mass.

The goal of this secondary analysis of data obtained from the Comparison of Health Benefits and Risks of High-Carbohydrate, Low-Fat or Very-Low-Carbohydrate Diets for Weight Loss Study was to determine the effect of LC and HCC dietary interventions on circulating acylcarnitine concentrations among healthy adults with overweight and obesity. The first aim was to describe the impact of consuming a LC or HCC diet on fasting acylcarnitine concentrations at baseline and 2, 4, 6, and 18-weeks after dietary intervention. The second aim was to define the relationships between changes in lean mass, fat mass, and fasting acylcarnitine concentrations within and between the ad libitum LC diet group and the energy-matched HCC diet group.

Twenty-five individuals with overweight and obesity, but otherwise healthy, participated in the Comparison of Health Benefits and Risks of High-Carbohydrate, Low-Fat or Very-Low-Carbohydrate Diets for Weight Loss Study. Data from 23 of the 25 participants who consumed either an ad libitum LC diet (n=11) or an energy-matched HCC diet (n=12) for 6-weeks of controlled dietary intervention and an additional 12-weeks of home dietary intervention were included in this analysis. Fasting whole blood samples collected at baseline and weeks 2, 4, 6, and 18 were analyzed for 21 individual acylcarnitine concentrations. Body mass, fat mass, and

fat-free mass were assessed using whole-body dual-energy x-ray absorptiometry (DEXA) at baseline and weeks 6 and 18. Lean mass was calculated by subtracting each participant's bone mineral density (BMD) from fat-free mass in kilograms.

Mean C4, C5, C5-DC, and C18 acylcarnitine concentrations were significantly affected by dietary intervention, time, and their interaction. C4, C5, C5-DC, and C18 acylcarnitine concentrations tended to increase among participants consuming a LC diet, whereas C4, C5, and C5-DC acylcarnitine concentrations tended to decrease among participants consuming a HCC diet. The majority of within and between group variations in individual acylcarnitine concentrations were observed during the 6-week controlled dietary intervention. Participants in both dietary groups lost significant mean body mass by weeks 6 and 18 ($p<0.001$). However, there were no significant differences in mean change in body mass, fat mass, lean mass, percent body fat, or percent lean mass between dietary groups at any time point ($p>0.20$). Participants, regardless of dietary group, lost an average of 5.17 ± 2.28 (95% CI: -6.33, -3.92; $p<0.001$) kg of body mass by week 6 and 6.88 ± 3.72 (95% CI: -8.04, -5.63; $p<0.001$) kg of body mass by week 18. There were no significant indications that mean change in C4 ($p=0.300$), C5 ($p=0.997$), or C18 ($p=0.258$) acylcarnitine concentrations were influenced by mean change in lean mass, diet, or their interaction from baseline to week 6. Participants who consumed a LC diet experienced a significant average 0.07 (95% CI: 0.02, 0.13; $p=0.015$) μmol/L increase in mean C5-DC acylcarnitine concentration from baseline to week 6. However, mean change in C5-DC acylcarnitine concentration from baseline to week 6 was not significantly influenced by either mean change in lean mass ($p=0.468$) or fat mass ($p=0.467$). Furthermore, there was no significant indication that mean change in C5 ($p=0.518$) acylcarnitine concentration was influenced by mean change in fat mass, diet, or their interaction from baseline to week 6. Yet, each 1 kg loss of fat mass was associated with a 0.16 (95% CI: -0.02, -0.30; $p=0.030$) μmol/L

decrease in C4 acylcarnitine concentration among participants consuming a LC diet but not a HCC diet (p=0.528). While, each 1 kg loss of fat mass was associated with a 0.20 (95% CI: -0.04, -0.36; p=0.016) μmol/L decrease in C18 acylcarnitine concentration, regardless of dietary intervention.

During active body mass loss, participants consuming the LC or HCC dietary interventions displayed significant within and between dietary group changes in mean C4, C5, C5-DC, and C18 acylcarnitine concentrations. Only changes in C4 and C18 acylcarnitine concentrations were associated with change in fat mass from baseline to week 6. However, the association between change in fat mass and change in C4 acylcarnitine concentration was exclusive to participants consuming a LC diet. These results are different from what we anticipated, as we hypothesized that change in fasting acylcarnitine concentrations would be positively correlated with loss of lean mass, and the correlation would be stronger among participants consuming the LC diet than the HCC diet. The findings from this secondary analysis provide preliminary and descriptive evidence of an effect of dietary intervention with varying macronutrient content on individal acylcarnitine concentrations, as well as an association between change in fat mass and change in C4 and C18 acylcarnitine concentrations. Our findings highlight the important role that dietary intervention may have on changes in individual acylcarnitine species among individuals with overweight and obesity. Yet, additional controlled dietary intervention studies with larger sample sizes that are statistically powered to detect differences in body composition and individual acylcarnitine concentrations after LC and HCC dietary intervention are needed to confirm these findings.

CHAPTER 1: SPECIFIC AIMS

Metabolomics is the comprehensive study and measurement of small-molecule metabolites involved in metabolism.[1] Metabolomics provides insight into changes in the chemical "signature" that results from specific cellular processes and environmental exposures.[1] Acylcarnitine blood profiling has been used to identify dysregulation of fatty acid beta-oxidation (FAO) and is recognized as important in the pathophysiology of obesity and insulin resistance.[2] A growing body of evidence from metabolomic studies describes differences in free- and acyl-carnitine concentrations among individuals with obesity compared to lean controls.[2-4] Some evidence suggests that individuals with obesity may share similar defects in the early phases of lipid metabolism as individuals with obesity and type 2 diabetes mellitus (T2DM).[2,3] The available research investigating the impact of low-and high-complex-carbohydrate dietary patterns and weight loss on circulating fasting acylcarnitine concentrations is sparse. Low-carbohydrate, high-fat, high-protein diets— such as the traditional Atkins or ketogenic diet, as well as high-complex-carbohydrate diets like the Dietary Approaches to Stop Hypertension (DASH) dietary pattern, are common dietary patterns followed by individuals with overweight or obesity seeking to lose weight. Yet, few studies exist analyzing how these dietary approaches affect circulating metabolic intermediates in combination with changes in body weight, lean mass, and fat mass.

Optimal weight loss is aimed at maximizing reductions in fat mass while limiting catabolism of skeletal muscle, where roughly 97% of carnitine is stored.[5] Dietary interventions that yield a negative energy balance may result in rapid and dramatic changes in body weight that may reflect significant loses of both fat and skeletal muscle mass. Any substantial amount of intentional weight loss in an individual with overweight or obesity may result in undesirable effects since muscle mass is responsible for the majority of resting metabolic rate, regulation of

core body temperature, preservation of skeletal integrity, and maintenance of function and quality of life as the body ages.[6]

The goal of this secondary analysis was to describe the effect of consuming energy-matched low-or high-complex-carbohydrate diets on fasting acylcarnitine concentrations, body mass, fat mass, and lean mass and to examine the relationships among these variables over time. To accomplish this goal, we conducted a secondary analysis of data collected during the Comparison of Health Benefits and Risks of High-Carbohydrate, Low-Fat or Very-Low-Carbohydrate Diets for Weight Loss Study that enrolled 25 healthy adults with overweight and obesity. Individuals were allocated to consume an ad libitum low-carbohydrate diet or an energy-matched high-complex-carbohydrate diet for 6-weeks followed by a 12-week home dietary intervention phase. Fasting whole blood samples were obtained at baseline and after 2, 4, 6, and 18-weeks. Body mass, fat mass and fat-free mass were measured at baseline, after the 6-week dietary intervention phase, and after the 12-week home dietary intervention phase at week 18. Lean mass was calculated by subtracting each participant's BMD from fat-free mass in kilograms. Data from 23 of the 25 participants were included in this analysis.

The specific aims of this secondary analysis were to:

1. Describe the impact of consuming an energy-matched low-or high-complex-carbohydrate diet on fasting acylcarnitine concentrations at baseline and 2, 4, and 6-weeks during the controlled dietary intervention phase, and at week 18 after the 12-week home dietary intervention phase.
2. Define the relationships between changes in lean mass, fat mass, and fasting acylcarnitine concentrations within and between the ad libitum low-carbohydrate diet group and the energy-matched high-complex-carbohydrate diet group.

Hypothesis: We hypothesized that the change in fasting acylcarnitine concentrations would be positively correlated with loss of lean mass, and the correlation would be stronger among participants consuming the ad libitum low-carbohydrate diet than the energy-matched high-complex-carbohydrate diet.

CHAPTER 2: BACKGROUND & REVIEW OF LITERATURE

Carnitine

Overview of Carnitine

The name "carnitine" is derived from the Latin word "carnus" or flesh, as the compound was first isolated in meat.[7] Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is a conditionally essential amino acid derivative that exists in two isomer forms, L-carnitine and D-carnitine. The L-carnitine isomer is the biologically active form that plays a vital role in fatty acid metabolism and energy production.[8,9] Carnitine is not an essential nutrient for adults or children and there is currently no recommended dietary allowance for this nutrient.[9] Carnitine homeostasis is maintained by a balance among absorption from dietary sources, endogenous biosynthesis from the essential amino acids lysine and methionine by the liver and kidneys, [8,9] maintenance of concentration gradients across cell membranes, and efficient renal reabsorption and excretion of carnitine.[9,10] Among healthy individuals, even with low carnitine intake, there is no indication of clinically relevant carnitine deficiency.[11] Humans who consume an omnivorous diet obtain and maintain ~75% of their carnitine stores from diet, primarily from animal sources such as red meat, fish, poultry, and milk products, [7,8] with roughly 25% synthesized endogenously.[10] Table 1 displays the relative amount of carnitine in various types of food.[12]

Table 1. Food sources of L-carnitine.
Table adapted from Demarquoy et al. (12)

Food Source (100 gram serving)	Amount (mg)
Raw ground beef	87.5
Raw beef steak	65
Raw lamb chop	40.5
Raw pork (ham)	33.5
Raw turkey breast	21.2
Raw chicken breast	10.4
Munster cheese	19.8
Goat cheese	15.3
0% fat yogurt	12.3
Avocado (no skin)	8.1
Cooked salmon	5.8
2% Milk	2.9
Lentils	2.1
Eggs	1.1
Mango (no skin)	0.8
Apple (no skin)	0.2
Banana (no skin)	0.2
Carrot	0.3
Tomato	0

Carnitine was independently discovered to be a quantitatively important nutrient in muscle tissue in 1905 by Gulewitsch and Krimberg,[13] and by Kutscher.[14] However, the chemical

structure was not confirmed until 1927 by Tomita and Sendju.[15] In humans, carnitine is primarily stored in skeletal and cardiac muscle tissue. Roughly 97% of carnitine is stored in skeletal muscle, although it is also present in most tissues in the body. In humans, the intracellular concentrations of carnitine in the liver and skeletal muscle are approximately 50 and 76 times higher than the concentrations in extracellular fluid.[5]

Absorption, Reabsorption, and Excretion of Carnitine

L-carnitine is absorbed from foods via both active and passive transport mechanisms across enterocyte membranes. The efficiency of L-carnitine absorption depends on an individual's carnitine status and their usual dietary composition. Individuals who consume a low carnitine diet, like vegetarians and vegans, are able to maintain normal carnitine concentrations through upregulated intestinal absorption, endogenous synthesis, and efficient renal tubular reabsorption of carnitine.[16] Intestinal absorption of carnitine among vegetarians and vegans is 66% to 86%, compared to an absorption rate of 54% to 72% in individuals who consume a carnitine-rich diet.[8,9]

Dietary free carnitine is absorbed through a high affinity carnitine transporter called the organic cation transporter novel 2 (OCTN2), which is expressed on the apical membrane of enterocytes.[17] As shown in Figure 1, the same OCTN2 is also expressed on the renal brush border membrane (BBM) and actively transports free carnitine across the BBM at normal circulating blood concentrations (20-50 µmol/L).[17] Carnitine reabsorption is highly efficient with approximately 95% of filtered carnitine reabsorbed by the kidneys in healthy humans. Gamma-butyrobetaine and short-chain acylcarnitine esters are also efficiently reabsorbed at the BBM and it is hypothesized that this reabsorption occurs through OCTN2.[9]

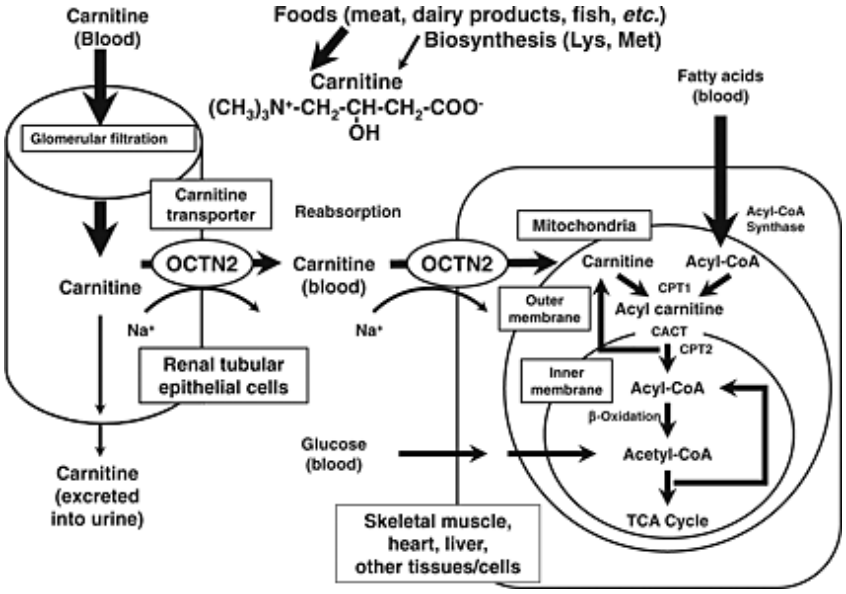


Figure 1. Involvement of the organic cation transporter novel 2 (OCTN2) in the reabsorption and disposition of carnitine within tissues.

Figure originally published by Tamai et al. (17)

Figure 2 illustrates a summary of carnitine homeostasis between dietary intake, endogenous synthesis from protein, and the amount of carnitine within tissues and in circulation.[18] As dietary carnitine intake decreases, the efficiency of carnitine reabsorption increases. In conjunction, as plasma carnitine concentrations increase, the rate of carnitine reabsorption decreases and the rate of carnitine excretion increases.[10] The remaining carnitine that is not reabsorbed by the kidneys is excreted in the urine as free carnitine, acylcarnitine esters, or trimethylamine oxide.[9,19] Additionally, dietary carnitine that is not absorbed in the small intestine is almost

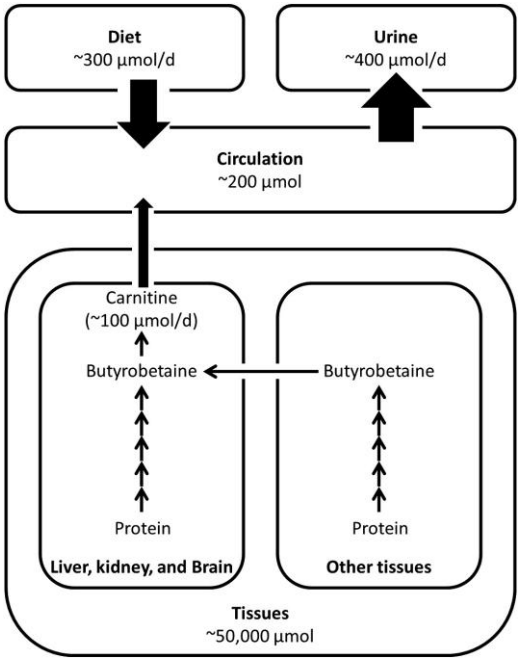


Figure 2. Carnitine homeostasis.

Figure originally published by El-Hattab et al. (18)

entirely degraded by bacteria within the large intestine and excreted as γ-butyrobetaine in the feces.[20]

Role of Carnitine in Fatty Acid Metabolism and Formation of Acylcarnitines

L-carnitine is present in biological systems as free carnitine, non-esterified, and esterified forms.[15] The primary role of carnitine is to transport long-chain fatty acids (LCFAs) through the inner mitochondrial membrane into the mitochondrial matrix for β-oxidation because the mitochondrial membrane is impermeable to LCFAs.[10,21] Carnitine's involvement in long-chain fatty acid oxidation in mitochondria was independently demonstrated between 1962 and 1963 by Fritz and Yue, and by Bremer.[22] During the fasting state, fatty acids are mobilized from adipose tissue to provide the predominant substrate for β-oxidation in the liver, cardiac muscle, and skeletal muscle.[21] Mitochondria, as well as peroxisomes, contain all the enzymes necessary for FAO although the mitochondria is the primary site for the oxidation of plasma free fatty acids (FFAs) and lipoprotein-associated triglycerides.[21]

In mammals, all known functions of carnitine involve the reversible esterification of the 3-hydroxyl group on carnitine[7] and the transfer of free fatty acids to and from coenzyme A (CoA), which is shown in Figure 3.[9] FFAs – also called un-esterified (UFA) or non-esterified fatty acids (NEFAs) – must first be converted into an active intermediate in the cytosol of the cell before being catabolized to produce energy within mitochondria.[9] Fatty acid transport proteins (FATPs) are integral transmembrane proteins that enhance the uptake of long-chain and very-long-chain fatty acids into cells.[21] FATPs contain the enzyme acyl-CoA synthetase

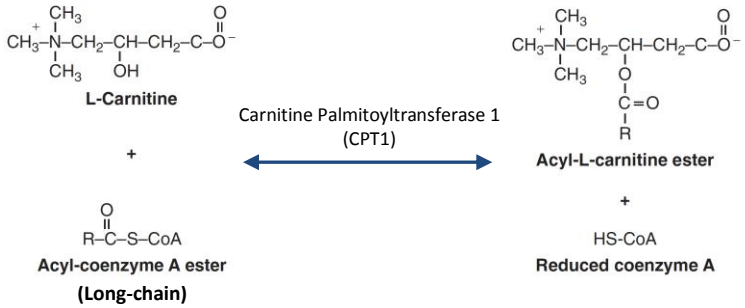


Figure 3. Reversible esterification of L-carnitine to an acyl-L-carnitine ester.

Figure originally published by Rebouche (9).

(thiokinase), which in the presence of adenosine triphosphate (ATP) and CoA catalyzes the conversion of a FFA to an active fatty acid – also called an "acyl-CoA." [9,23] This conversion is shown in Figure 4.[23] Acyl-CoA synthetases are found in the endoplasmic reticulum (ER), in peroxisomes, and within the inner and outer mitochondrial membranes.[23]

Once inside the cell, carnitine is essential for the transport of long-chain acyl-CoAs through the inner mitochondrial membrane via the carnitine shuttle. To be transported across the inner mitochondrial membrane, the long-chain acyl group is transferred from the CoA molecule to carnitine by carnitine palmitoyltransferase I (CPT1), an enzyme embedded within the outer mitochondrial membrane.[24] Figures 4 [23] and 5 [25] display the transfer of carnitine to the long-chain acyl molecule to form an "acylcarnitine." An acylcarnitine is the broad name for any type of acyl functional group (R group–C=O) that is esterified to a carnitine molecule. Once the acylcarnitine molecule is formed, it can be transported through the inner mitochondrial membrane into the mitochondrial matrix by carnitine acylcarnitine translocase [(CACT), another enzyme embedded within the inner mitochondrial membrane] in exchange for a free carnitine molecule. The acyl group is then transferred back to a CoA molecule by carnitine palmitoyltransferase II (CPT2), reforming an acyl-CoA molecule, which can then enter into the β-oxidation cascade. The free carnitine in the mitochondrial matrix is then transported back into the intermembrane space via the CACT enzyme.[9,23] Each cycle of β-oxidation results in the cleavage of two carbons from the fatty acyl-CoA molecule (beginning at the carboxylic end). Each cycle generates large quantities of flavin adenine dinucleotide ($FADH_2$) and nicotinamide adenine dinucleotide (NADH), which enter the electron transport chain (ETC) to produce ATP (adenosine triphosphate). Moreover, the resulting acetyl-CoA molecules are integrated into other energy-producing pathways such as the citric acid cycle (CAC) or into ketogenesis.[23]

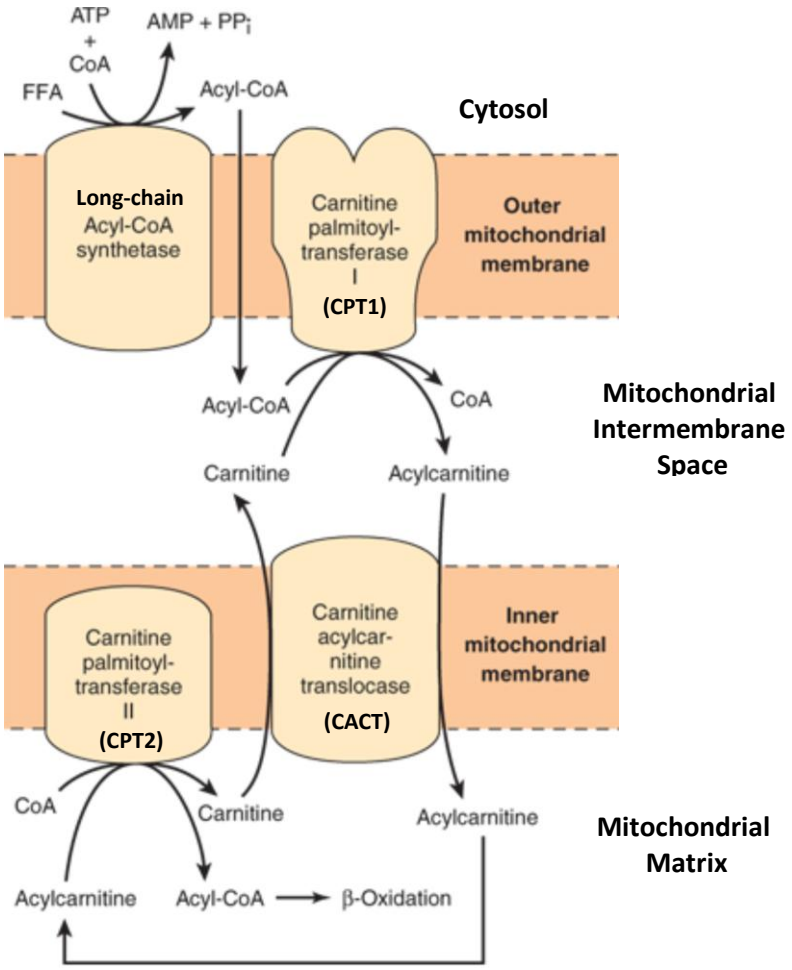


Figure 4. Carnitine in the transport of long-chain fatty acids.
Figure originally published by Botham et al. (23)

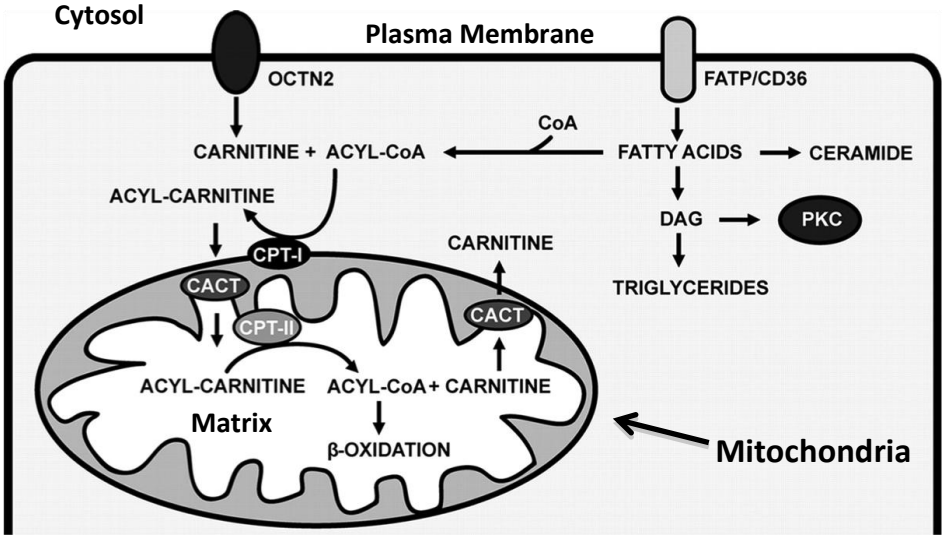


Figure 5. Lipid metabolism and the carnitine cycle.
Figure originally published by Glenn et al. (25)

Clinical Assessment of Free-and Acyl-Carnitine Concentrations

Free- and acyl-carnitines are intermediates of fatty acid and amino acid oxidation and are present in virtually all biological tissues and fluids including serum, plasma, dried blood spots, amniotic fluid, bile, dried bile spots, and urine.[26] The carnitine/acylcarnitine pool is reflective of metabolic homeostasis, therefore the quantification of total carnitine, free carnitine, and various acylcarnitines is of diagnostic value for the characterization of conditions related to altered carnitine metabolism, including FAO disorders.[27] Acylcarnitine profile analysis (ACP) is typically performed as part of the biochemical screening for inherited diseases of peroxisomal and mitochondrial oxidation processes (i.e. disorders of fatty acid oxidation and organic acid metabolism) and recently described as biomarkers of complex diseases like metabolic syndrome.[28]

In general, normal plasma free carnitine concentration is 20-50 μmol/L.[17] Any plasma free carnitine concentration of 20 μmol/L or less, or a total carnitine concentration of 30 μmol/L or less, is considered abnormally low. A ratio of esterified-to-free carnitine of 0.4 or greater in plasma or serum is considered indicative of abnormal carnitine metabolism.[9] The diagnoses of inherited diseases of peroxisomal and mitochondrial oxidation processes are almost exclusively a laboratory process of which acylcarnitine analysis is a key component.[26] High Performance Liquid Chromatography-tandem mass spectrometry (HPLC/MS/MS) is the most commonly used method to analyze and quantify carnitine and acylcarnitines species,[29] although other methods such as gas chromatography-mass spectrometry, tandem mass spectrometry, targeted liquid chromatography, flow-injection-tandem mass spectrometry, ultra-performance liquid chromatography, and capillary electrophoresis are also used.[26] Carnitine and acylcarnitines are most often analyzed as butyl-esters, although other methods do exist.[26] The HPLC/MS/MS method includes butylation of carnitine or acylcarnitines species using acidified butanol, HPLC

flow injection, and the measurement of acylcarnitines using precursor ion scan and multiple reactions monitoring (MRM).[29]

Free-and Acyl-Carnitine Concentrations in Adults with Obesity

Obesity is a major public health problem in both developed and developing countries worldwide with both incidence and prevalence continuing to rise.[30] The underlying biochemical and metabolic processes of obesity and its strong association with other affiliated comorbidities such as insulin resistance are still not fully understood.[31] However, recent research on metabolomic biomarkers of obesity have been conducted and a potential association may exist between alterations in cellular communication, certain metabolic pathways, and specific metabolites such as branch-chain amino acids (BCCAs), non-esterified fatty acids, organic acids, acylcarnitines, and phospholipids in the obese population that may impact an individual's susceptibility to other comorbidities.[4,31] Some evidence suggests that deregulation of β-oxidation may be associated with obesity and insulin resistance.[31]

Several cross-sectional analyses comparing the metabolomic profiles of individuals with obesity have observed varying degrees of alternations in free carnitine, short-chain (2-5 carbon chain), medium-chain (6-12 carbon chain), and long-chain (14-24 carbon chain) acylcarnitine concentrations compared to lean controls. The names for individual acylcarnitines are listed in the abbreviations list. Newgard et al.[4] performed a cross-sectional, metabolomic profiling study on serum samples from 73 “healthy” participants with obesity but without diabetes (median age 52 years old, BMI of 36.6 kg/m^2) and 67 lean controls (median age 50 years old, BMI of 23.2 kg/m^2) using tandem mass spectrometry (MS/MS) to describe the metabolomic profile of lean subjects and subjects with obesity. Among the 37 acylcarnitine species measured in serum, only mean C3, C5, C6, and C8:1 acylcarnitine concentrations were significantly higher in the

participants with obesity compared to the lean controls ($p \leq 0.009$), with the strongest differences seen in mean C3 and C5 acylcarnitine concentrations ($p < 0.001$).[4]

Another cross-sectional metabolomics study, Mihalik et al.[2] used MS/MS of dried bloodspots from fasting plasma samples to describe the free- and acyl-carnitine profiles of 14 "healthy" individuals with obesity but without diabetes (mean age 43 years old, BMI 34.3 kg/m^2) and 10 individuals with obesity and type 2 diabetes mellitus (T2DM) (mean age 45 years old, BMI 34.2 kg/m^2) compared to 12 lean sedentary controls (mean age 47 years old, BMI 23.9 kg/m^2). Similar to Newgard et al.,[4] the mean C5 acylcarnitine concentration was higher among the obese group compared to lean controls ($p < 0.05$) but was also higher in the T2DM group compared to the lean controls ($p < 0.05$). Free carnitine, C10:1, and individual long-chain acylcarnitine concentrations (C14:1, C14-OH, C16, C16-OH, C18, C18:1) were similarly higher in both the obese group and the T2DM group compared to the lean controls ($p < 0.05$). Yet, relative to the obese and lean groups, participants with T2DM had significantly higher mean concentrations of several short- and medium-chain acylcarnitine concentrations (C3, C4-OH, C4-DC, C5, C5-OH, C6-OH, C8; $P < 0.05$) with a larger contribution of elevated C3 and C5 concentrations coming from male participants with obesity and T2DM. Despite nearly identical mean concentrations of C4 acylcarnitine, participants in the T2DM group but not the obese group had significant elevations in C4, C4-DC, and C6 acylcarnitine concentrations compared to the lean participants ($p < 0.05$). However, the ratio of free carnitine-to-C16 acylcarnitine and total acylcarnitine-to-free carnitine was significantly lower in the obese group compared to the lean group ($p < 0.05$).[2]

Patel et al.[1] performed a cross-sectional metabolomics analysis of free carnitine and 45 acylcarnitines species in fasting plasma samples using MS/MS from 500 adults with overweight and obesity (mean age 55.9 years old, BMI 33.9 kg/m^2) but without diabetes mellitus who

participated in the Weight Loss Maintenance trial.[32] All blood samples were collected at baseline before any weight loss occurred to evaluate the differences across both race-(Caucasian versus African American) and sex-based subgroups (male versus female). Long-chain acylcarnitine concentrations (C14, C16, C18:1, C16:1-OH/C14:1-DC) were higher among Caucasians compared to African Americans ($p<0.0001$) and higher among males compared to females among all participants ($p<0.01$). Additionally, dicarboxylic acylcarnitine concentrations (C4-DC, C6-DC, C10-OH/C8-DC, C12:1, C12-OH/C10-DC, C14:1-OH/C12:1-DC, C14-OH/C12-DC, C8:1-DC) were also higher among males compared to females ($p<0.0001$).[1]

Floegal et al.[33] performed cross-sectional metabolomics analysis of free carnitine and 17 acylcarnitines in fasting serum samples using mass spectrometry from a subcohort of 100 individuals with overweight and obesity (mean age 49.8 years old, BMI 26.1 kg/m^2) randomly drawn from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Although statistical significance was not reported, partial correlation coefficient modeling displayed that acylcarnitine concentrations were positively associated with obesity. Mean free carnitine, C3, and C8:1 acylcarnitine concentrations were positively correlated with both BMI and waist circumference (0.3-0.4 multivariable adjusted partial correlation coefficient), whereas C16, and C18:1 acylcarnitine concentrations were positively correlated with only waist circumference (0.3-0.4 multivariable adjusted partial correlation coefficient). Mean free carnitine and C3 acylcarnitine concentrations were linked as a separate pair away from the acylcarnitine metabolite network and showed a partial correlation of 0.6-0.8.[33]

Low-Carbohydrate Diets

History

Modern day weight loss diets primarily differ by unique ratios of macronutrients and energy restriction.[34] Low-carbohydrate, high-protein, high-fat diets (typically referred to as low-

carbohydrate diets) have been popular since the 1860s, when William Banting – a carpenter in Victorian London struggling with obesity – published a pamphlet detailing the diet strategy that helped him lose 46 pounds in 12-months.[35] Many low-carbohydrate diet weight loss books have since been published, with the most familiar being *Dr. Atkins' New Diet Revolution,* first published in 1972 by Dr. Roberts C. Atkins.[36]

Diet Composition

There is no definitive definition of the exact composition of a low-carbohydrate diet since a variety of macronutrient distributions may be classified as "low-carbohydrate." However, the majority of energy in low-carbohydrate diets typically comes from fat (55-65% of total energy) and protein (25-40% of total energy).[37,38] Low-carbohydrate diets—such as the classic Atkins 20™ diet—are generally ad libitum for total energy, protein, and fat and provide roughly 5-20% of total energy from carbohydrate depending on the phase of the diet.[37] The Atkins 20™ diet aims to provide 15-20 grams of carbohydrates per day (primarily from non-starchy vegetable sources) during an induction phase (phase 1) that usually lasts around 14-days. Individuals who choose to remain in this phase for >14 -days to accelerate weight loss typically consume 5-10% of total energy from carbohydrate and the composition of the diet becomes more similar to a Modified Atkins Diet (MAD).[39] A MAD is a more liberal form of a very-low-carbohydrate ketogenic diet that is typically used clinically for the dietary management of drug-resistant childhood epilepsy.[40,41] A MAD focuses on encouraging unlimited protein and fat intake (~30-35% total energy from protein, ~65% total energy from fat) but limiting daily carbohydrate intake to 10 grams of carbohydrate in first month and extending it to 20 grams during the second month. The "classic" ketogenic diet is a highly restrictive very-low-carbohydrate diet that is primarily used to treat intractable epilepsy.[40,41] The "classic" ketogenic diet typically provides

90% of total energy from fat, 8% of total energy from protein, and 2% from carbohydrate while following either a 4:1 or 3:1 fat-to-combined protein and carbohydrate ratio.[42]

Over time, individuals on the induction phase of the Atkins 20™ diet may progress through the diet towards the maintenance phase by reintroducing more grams of carbohydrate into the diet during each of the four phases until s/he reaches and maintains the goal weight.[39,43] This type of low-carbohydrate diet is designed to facilitate quick weight-loss by encouraging participants to eat frequently and consume nutrient-dense foods, adequate protein, high-fiber vegetables, low glycemic fruits, and healthy fats.[39]

High-Complex-Carbohydrate Diets

Diet Composition

The Dietary Approaches to Stop Hypertension (DASH) is a high-complex-carbohydrate, low-fat dietary pattern that emphasizes eating nutrient-dense foods that are low in saturated fat, trans fat, and total fat. The typical distribution of energy in a high-complex-carbohydrate diet like the DASH diet is 55-60% of total energy from complex-carbohydrates, 15-20% of total energy from protein, and 20-30% of total energy from fat.[37] Other high-carbohydrate weight loss diets such as Weight Watchers, Nutrisystem, and Jenny Craig share similar diet compositions and are often used interchangeably in diet comparison studies.[37,44] The DASH dietary pattern is naturally high in potassium, calcium, magnesium, protein, and fiber, due to the high intake of fruits, vegetables, low-fat dairy products, whole grains, lean meats (e.g. poultry, fish), nuts, and legumes. The reduced intake of red meat, sweets, sugar-sweetened beverages, added salt, and sodium, aids in the prevention of heart disease by lowering blood pressure, and reducing low-density lipoprotein cholesterol (LDL-CH).[45] Thus, the DASH dietary pattern has been the foundation for weight reduction in those with pre-hypertension and stage I hypertension.[34,46]

History

The DASH dietary pattern is based on the findings from three major National Heart, Lung, and Blood Institute (NHLBI)-funded research trials [47] showing the health benefits associated with the DASH diet.[48-50] The results from these major trials and their follow-up analyses have helped establish the current DASH dietary plan recommendations.[51] The DASH diet is beneficial for reducing body weight, [48] LDL-CH,[45] and systolic (SBP) and diastolic blood pressure (DBP) [48-50]– with a greater reduction in blood pressure when the DASH diet is combined with a low sodium intake of 1,500 mg/day.[50]

Low-and High-Complex-Carbohydrate Diets and Changes in Body Composition with Weight Loss

Individuals with overweight and obesity from a variety of health backgrounds commonly follow high-complex-carbohydrate, low-fat diets or low-carbohydrate, high-fat, high-protein diets for the communal purpose of losing excess body weight and fat mass. However, randomized, controlled trials report conflicting findings on the superiority of low-and high-complex-carbohydrate diets for preservation of lean mass and loss of body weight and fat mass.

Shai et al.[43] and Hashimoto et al.[52] reported that adults with overweight and obesity lose more weight [43,52] and experience greater fat loss [52,53] when consuming low-carbohydrate diets. Shai et al.[43] conducted a 24-month randomized controlled trial looking at the effectiveness and safety of a low-carbohydrate, non-restricted energy diet with a low-fat, restricted-energy diet, and a Mediterranean, energy-restricted diet, among 322 adults with obesity (mean age 52 ± 7 years old, BMI 30.9 ± 3.6 kg/m^2) in a workplace setting. After 24-months, participants in the low-carbohydrate diet group lost on average 5.5 ± 7.0 kg compared to 3.3 ± 4.1 kg for the low-fat diet group and 4.6 ± 6.0 kg for the Mediterranean diet group (p=0.03 for comparison between the low-fat and the low-carbohydrate diet groups).[43] Maximum weight loss occurred

within the first 6-months in all three diet groups with a maintenance weight loss phase between 7- and 24-months in all diet groups.[43]

Additionally, a meta-analysis of 14 randomized controlled trials (study duration ranging from 2- to 24-months) conducted by Hashimoto et al.[52] reported that adults with overweight and obesity who consumed a low-carbohydrate diet (20 grams of carbohydrates per day to 45% of total energy from carbohydrates) experienced higher mean change in body weight (-0.70 kg; 95% CI: -1.07,-0.33 kg, $p<0.05$) and mean change in fat mass (-0.82 kg; 95% CI: -1.22, -0.42 kg, $p<0.05$) compared to a control diet. Data from 8 studies (666 participants) found that adults on a low-carbohydrate dietary intervention of <12-months lost on average 0.89 kg in body weight (95% CI: -1.43, 0.35 kg, $p<0.05$) and 0.98 kg in fat mass (95% CI: -1.60, -0.36 kg, $p<0.05$) compared to the control group. However, data from 6 studies (770 participants) found that a low-carbohydrate dietary intervention of >12-months was associated with a decrease in fat mass (-0.57 kg; 95% CI: -1.05, -0.09 kg, $p<0.05$) but not body weight when compared to the control group ($p>0.05$).[52]

Volek et al.[53] conducted a randomized controlled crossover trial studying the effects of a very-low-carbohydrate, ketogenic, energy-restricted diet and a low-fat, energy-restricted diet on 15 healthy, males with overweight and obesity (age 33.2 ± 2.9 years old, BMI 34.1 ± 1.1 kg/m^2) and 13 premenopausal females (age 34.0 ± 2.4 years old, BMI 29.6 ± 1.1 kg/m^2). Males consumed each diet for 50-days and females consumed each diet for 30-days. Participants on the free-living, very-low-carbohydrate, ketogenic, energy-restricted diet (-500 energy per day, <10% of total energy from carbohydrates) experienced greater weight loss and total fat loss, preferentially from the trunk region, compared to participants on the low-fat, energy-restricted diet (-500 energy per day, 60% energy from carbohydrate, <25% energy from fat, 15% energy from protein). Both men and women participants experienced a greater reduction in the ratio of

trunk fat/total fat when on the very-low-carbohydrate, ketogenic, energy-restricted diet compared to the low-fat, energy-restricted diet, although the superiority the very-low-carbohydrate, ketogenic, energy-restricted diet compared to the low-fat, energy-restricted diet was most dramatic for men in terms of weight loss, total fat loss, and trunk fat loss ($p<0.05$).[53]

Dansinger et al.[54] found that adults with overweight and obesity (mean age 49 years old, BMI 35 kg/m^2) consuming a low-carbohydrate Atkins diet for 12-months resulted in the lowest amount of weight loss (-2.1 ± 4.8 kg for Atkins diet, p=0.009, 53% of participants completed), compared to participants consuming a Zone diet (-3.2 ± 6.0 kg, p=0.002) 65% of participants completed), Weight Watchers diet (-3.0 ± 4.9 kg, p<0.001, 65% of participants completed), or Ornish diet (-3.3 ± 7.3 kg, p=0.007, 50% of participants completed). The participants in the Atkins group lost the greatest amount of body weight during the first 2-months of the dietary intervention (-3.6 ± 3.3 kg body weight). However, there was no statistically significant difference in weight loss observed among participants in any diet group at any time period during the study.[54]

High-complex-carbohydrate diets have been associated with greater weight loss compared to high-simple-carbohydrate diets.[55-57] A 6-month multi-center randomized controlled ad libitum feeding trial led by Saris et al.[57] reported greater mean weight loss (-1.8 ± 3.2 kg, p<0.001) and fat loss (-1.8 ± 3.9 kg, p<0.001) among participants with overweight and obesity (mean age 39 years old, BMI 30.4 kg/m^2) consuming a low-fat, high-complex-carbohydrate diet compared to a typical American diet (control group). In comparison, participants consuming the low-fat, high-simple-carbohydrate diet group lost on average 0.9 ± 3.6 kg of body weight ($p<0.05$) and 1.3 ± 3.6 kg of fat mass compared to the control diet ($p<0.01$).[57] Moreover, a 12-week randomized controlled ad libitum feeding trial by Hayes et al.[55] also concluded that participants with overweight and obesity (mean age 66 years old, BMI 30 kg/m^2) consuming a

high-complex-carbohydrate, low-fat diet without exercise lost on average 3.2 ± 1.2 kg body weight (p=0.02) and 2.2 ± 1.2% body fat compared to the control group (p=0.049). When combined with exercise, participants lost 4.8 ± 0.9 kg body weight (p=0.003) and 3.5 ± 0.7% body fat compared to the control group (p=0.01), who on average lost 0.1 ± 0.6 kg body weight and 0.2 ± 0.6% body fat.[55] Poppitt et al.[56] also found greater mean weight loss among overweight and obese participants (mean age 46 years old, BMI 32 kg/m^2) consuming a low-fat, high-complex-carbohydrate diet (-4.25 kg, p<0.01) after a 6-month dietary intervention. In fact, body weight loss was only statistically significant over time in participants consuming a low-fat, high-complex-carbohydrate diet when each diet was modeled separately (p<0.01).[56]

Sacks et al.[58] conducted a 6-month randomized controlled trial examining weight change after 24-months of follow-up in 811 participants with overweight and obesity on four diets with the same energy restriction that varied in content of fat (20%–40% of total energy), protein (15%–25% of total energy), and carbohydrate (35%–65% of total energy) content. The authors found that all energy-restricted diets were equally successful in promoting weight loss over 24-months. Between all diet groups, the most drastic weight loss occurred within the first 6-months and weight loss was not statistically different among participants assigned to either a high-fat or low-fat diet or based on carbohydrate level (35-65% of total energy). Twenty-three percent of all participants continued to lose weight between 6- to 24-months with no significant difference between diet groups, although weight regain occurred in all diet groups after 12-months.[58]

Two ad libitum randomized controlled trials comparing a traditional very-low-carbohydrate Atkins diet with a conventional high-carbohydrate, low-fat diet both found that participants in the low-carbohydrate diet group lost more weight after 6-months of dietary intervention [59,60] compared to the high-carbohydrate, low-fat diet group (mean percent weight change -7.0 ± 6.5% versus -3.2 ± 5.6%, p-value=0.02 between group difference;[59] mean weight

loss of -5.8 ± 8.6 kg versus -1.9 ± 4.2 kg, p=0.002).[60] However, results from a follow-up study of Samaha et al.[60,61] and additional results from Foster et al.[59] found that although not statistically significant, the mean percent weight change for the low-carbohydrate diet group after 12-months of dietary intervention still remained higher among participants in the low-carbohydrate diet group compared to the high-carbohydrate, low-fat diet group (-4.4 ± 6.7% versus -2.5 ± 6.3%, p=0.26 for between group differences at 12-months;[59] mean weight change for participants in the low-carbohydrate diet group was -5.1 ± 8.7 kg compared to -3.1 ± 8.4 kg for participants in the high-carbohydrate, low-fat diet group, p=0.02 for difference between groups).[61]

A 10-week randomized controlled trial investigating the effects of a low-fat, energy-restricted diet (1452 ± 61 kcals of energy/day) with a low-carbohydrate, energy-restricted diet (1534 ± 84 kcals of energy/day) on weight loss and body composition among 31 healthy adults with overweight and obesity (mean age 42 years old, mean BMI 32.2 kg/m^2) found that there was no difference in the pattern of weight loss over time between the two dietary interventions groups.[62] Participants consuming the low-fat, energy-restricted diet lost an average of 6.8 kg (no SD) of body weight compared to 7.0 kg (no SD) of body weight among participants consuming the low-carbohydrate, energy-restricted group ($p<0.05$ compared to baseline values). Significant losses of fat mass were observed in both diet groups [(low-fat, energy-restricted diet group: -5.4 kg (no SD); low-carbohydrate, energy-restricted diet group: -4.1 kg (no SD), $p<0.05$ compared to baseline)]. However, participants in the low-fat, energy-restricted diet group better preserved lean mass when compared to the low-carbohydrate, energy-restricted diet group. A significant decrease in lean mass was observed in the low-carbohydrate, energy-restricted diet group [(-1.9 kg (no SD), $p<0.05$ compared to baseline values)] but not in the low-fat, energy-restricted diet group [(-1.0 kg (no SD)]. Although, both groups experienced similar improvements in overall

body composition in terms of percentage of body fat and lean mass when controlling for total body weight changes.[62] Overall, many randomized clinical trials comparing the effects of low-carbohydrate, high-fat, high-protein diets (e.g. the Atkins diet) with high-complex-carbohydrate, low-fat diets (e.g. DASH diet) show varying effects on changes in fat mass and lean body mass with weight loss.

Results from various studies have suggested that very-low-carbohydrate diets accelerate the mobilization rate of fatty acids from fat tissue, increasing ketone production, and sparing muscle mass from degradation into amino acids to fuel gluconeogenesis during energy-restricted states; thus, preserving lean mass.[63-66] Noakes et al.[67] designed a 12-week randomized controlled trial comparing an isoenergetic, high-saturated fat, very-low-carbohydrate diet (Carbohydrate:Fat:Protein, percent saturated fat = 4:61:35, 20%), an isoenergetic, very-low-fat diet (70:10:20, 3%), and an isoenergetic high-unsaturated fat diet (50:30:20, 6%) among 83 participants with obesity (mean age 48 years old, BMI 33 kg/m^2). The authors concluded that a very-low-carbohydrate diet may not be more protective against loss of lean mass compared to the two other isoenergetic diets after 12-weeks of dietary intervention. The percent of fat mass loss was not statistically different among the three diet groups after 8-weeks of energy restriction (30% energy restriction) and 4-weeks of weight maintenance (very-low-carbohydrate diet group, -4.5 ± 0.5 kg; very-low-fat diet group, -4.0 ± 0.5; high-unsaturated fat diet group, –4.4 ± 0.6 kg, $p>0.05$). However, all dietary groups experienced a statistically significant loss of lean mass after 12-weeks (32% in the very-low-carbohydrate diet group, 31% in the very-low-fat diet group, and 21% in the high-unsaturated fat diet group, ($p<0.05$).[67]

<u>Low-and High-Complex-Carbohydrate Diets and Change in Free-and Acyl-Carnitine Concentrations</u>

Only a few studies have described the change in carnitine species before and after low-and high-carbohydrate dietary interventions. Cederblad et al.[68] conducted a randomized

controlled crossover feeding trial among 7 healthy male adults (median age 33 years old, BMI 20.4 kg/m^2) and assessed changes in fasting free plasma carnitine, total plasma carnitine, and urinary excretion of free- and acyl-carnitine concentrations at baseline and post-dietary intervention. Participants consumed either an isoenergetic, high-carbohydrate, low-fat diet (30% of total energy from fat, 51% of total energy from carbohydrate, 19% of total energy from protein) and a low-carbohydrate, high-fat diet (54% of total energy from fat, 29% of total energy from carbohydrate, 17% of total energy from protein) in a randomized order for 4-days on each diet. It is important to note that both dietary interventions contained the same amount of carnitine-rich foods.

Both diets significantly raised the fasting plasma acylcarnitine concentration and fasting plasma acylcarnitine-to-free carnitine concentration ratio by day 5 (day 5 versus day 1 within diet groups, $p<0.05$) but no differences were observed between diet groups by the end of each intervention period. Total plasma carnitine and free carnitine concentrations, as well as urinary excretion of both free-and acyl-carnitine concentrations rose significantly by day 3 after participants consumed the low-carbohydrate, high-fat diet but not after the high-carbohydrate, low-fat diet ($p<0.05$ for between group comparisons). There was also a progressive, statistically significant increase in urinary excretion of total-, free-, and acyl-carnitine concentrations between days 3-5 on the low-carbohydrate, high-fat diet compared to the high-carbohydrate, low-fat diet ($p<0.05$ for between group variation).[68]

Davis et al.[69] conducted a 2-month feeding trial among 10 female adults with obesity who were a part of an outpatient weight management clinic, assessing changes in fasting plasma and urinary excretion of total-, free-, short-chain, and long-chain acyl-carnitines concentrations at baseline and post-dietary intervention. Five participants consumed a very-low-carbohydrate liquid formula (420 kcals of energy/day, 30 grams carbohydrates, 70 gms protein, 4.4 μmol of

carnitine) and five participants consumed a very-low-carbohydrate diet high in meat, fish, and poultry (500-600 kcals of energy/day, 70% of total energy from protein, <10 gms carbohydrates, 30-40% of total energy from fat, ~375 μmol of carnitine). Plasma total carnitine concentration was higher among subjects receiving the meat/fish/poultry diet compared to the liquid diet over the intervention period ($p<0.05$). Plasma total carnitine concentration slightly increased in the meat/fish/poultry group but decreased by 21% in participants consuming the liquid formula diet. Plasma short-chain acylcarnitine concentration increased and free carnitine concentration decreased significantly ($p<0.05$) over the 2-month dietary intervention period in both very-low-carbohydrate diet groups. However, neither dietary intervention had any significant effect on plasma long-chain acylcarnitine concentration. At 2-months, participants in the liquid formula group (low dietary carnitine) group excreted significantly less urinary free-and acyl-carnitine than those receiving the meat/fish/poultry diet (high carnitine) ($p<0.05$).[69]

Bell et al.[70] and Seccombe et al.[71] both conducted low-and high-carbohydrate diet feeding trials using animal models to assess changes in fasting serum free-, total-, and acyl-carnitine concentrations after dietary intervention and prolonged fasting (24-96 hours). Bell et al.[70] conducted a randomized controlled crossover feeding trial on 21 male stumptail *Maca arctoides* monkeys (~6-10 years old) who were switched from a high-carbohydrate, low-fat diet (~10% of total energy from fat) to a low-carbohydrate, high-fat diet (~45% of total energy from fat) for 90-days and then returned to the original high-carbohydrate, low-fat diet for a subsequent 76-days. The ratio of free carnitine-to-acylcarnitine concentration rose significantly within 62-days of initiating the high-fat diet (0.22 ± 0.03 nmol/ml at baseline to 0.35 ± 0.02 nmol/ml, $p<0.05$) and remained significantly higher compared to baseline until the low-fat diet was reinitiated. The ratio of free carnitine-to-acylcarnitine concentration fell significantly to 0.20 ± 0.03 nmol/ml ($p<0.05$) within 3-days of starting the low-fat diet and then stabilized to 0.24

nmol/ml from day 93 to day 166. A subcohort of 8 monkeys maintained on a high-carbohydrate, low-fat diet for 6-months and fasted for 48-hours displayed a 65% increase in total carnitine after 24-hours and a 75% increase after 48-hours. This increase is largely attributable to the 270% increase in mean acylcarnitine concentration after 24-hours and a 410% increase in acylcarnitine concentration after 48-hours compared to baseline. Free carnitine concentration increased to a lesser degree (45% increase at 24-hours and 31% at 48-hours, no p-values reported).[70]

Seccombe et al.[71] conducted a short-term, 2-day feeding trial among 18 male white Wistar rats and assessed fasting serum total-, free-, and acyl-carnitine concentrations before and after dietary intervention, and after a 96-hour starvation period. Rats were fed either a "high-fat" long-chain triglyceride diet (n=8), a medium-chain triglyceride diet (n=10), or a high-carbohydrate diet (n=8) with all diets supplying the same amount of dietary carnitine. Mean serum free carnitine was lower in rats on the long-chain and medium-chain triglyceride diets compared to the high-carbohydrate diet ($p<0.0001$ when each group was compared to the high-carbohydrate group). Mean serum acylcarnitine concentration were significantly higher in rats on the medium-chain triglyceride diet compared to both the high-carbohydrate diet ($p<0.0001$) and the long-chain triglyceride diet ($p<0.0001$). Mean total serum carnitine concentration was highest in the high-carbohydrate group, lowest in the long-chain triglyceride diet group ($p<0.0001$ compared to the high-carbohydrate group) and higher in the medium-triglyceride diet group compared to the long-chain triglyceride group ($p<0.0001$). Serum acyl-to-free-carnitine concentration ratio was significantly higher in rats fed both high-fat diets compared to the high-carbohydrate group. After a 96-hour starvation period, mean free carnitine concentration significantly decreased after 24-hours of fasting ($p<0.01$ compared to baseline) but gradually increased to maximum concentration by 96-hours ($p<0.05$ compared to baseline). Mean

acylcarnitine concentrations significantly increase by 24-hours of fasting ($p<0.05$ compared to baseline) and reached its maximum concentration by 48-hours ($p<0.05$ compared to baseline and $p<0.01$ compared to 24-hours). Mean total carnitine concentration significantly decreased after 24-hours of fasting ($p<0.01$ compared to baseline) but gradually increased to maximum concentration by 96-hours ($p<0.01$ compared to baseline). The ratio of acylcarnitine-to-free carnitine concentration increased by 24-hours ($p<0.01$ compared to baseline) and continued to increase until 48-hours ($p<0.05$ compared to baseline).[71]

Mathew et al.[72] performed a secondary analysis of changes in fasting serum total-, free-, and various acyl-carnitine concentrations using data collected during a controlled feeding trial. Thirteen hypertensive adults with obesity (mean age 72 years old, BMI 35.5 kg/m^2) with heart failure, preserved ejection fraction, and various comorbidities (e.g. systemic hypertension, coronary artery disease, type 2 diabetes mellitus, chronic kidney disease)[73] consumed a sodium-restricted DASH diet for 21-days. The majority of fasting serum acylcarnitine concentrations had a non-statistically significant increase during the dietary intervention period except for C8:1, C10:1, C12, C12:1, C14:2, C16, C16-OH, C18, C18:2, C20:2, C20:3, and C20:4 acylcarnitine concentrations, which all decreased. The only statistically significant change in carnitine species between baseline and post-dietary intervention was an increase in short-chain acylcarnitine concentrations (C2, C3, C4, $p<0.03$) and in one medium-chain acylcarnitine concentration (C10, $p=0.04$).[72]

Overall, there is variability in fluctuations in serum and plasma total-, free-, and acyl-carnitine concentrations at specific time points during dietary intervention and during prolonged periods of fasting. Total plasma and serum carnitine concentrations quickly increase after both low-and high-carbohydrate dietary intervention [68,69,71] with the largest increase seen after consuming a high-carbohydrate diet[71] and greater increases among participants consuming a

low-carbohydrate diet with higher dietary carnitine content.[69] Plasma and serum free carnitine concentrations also quickly change after dietary intervention. Cederblad et al.[68] reported a greater increase in plasma free carnitine among participants consuming a low-carbohydrate, high-fat diet, whereas Seccombe et al.[71] reported a higher serum free carnitine concentration among participants on a high-carbohydrate, low-fat diet. Plasma and serum acylcarnitine concentrations increased on both low-carbohydrate[68,69,71] and the high-carbohydrate diets. [68,72] However, increases in both total- and acyl-carnitine concentrations after consuming a high-carbohydrate diet may only result when dietary carnitine content is equal to the low-carbohydrate diet group.[68] Low-carbohydrate, high-fat, high-protein diets usually provide a larger amount of dietary carnitine as a result of higher protein intake coming from animal sources. Due to the higher carnitine content, urinary excretion of total-, free-, and acyl-carnitines tends to be higher during consumption of a low-carbohydrate, high-fat, high-protein diets and lower among high-carbohydrate, low-fat diets.[68,69] During periods of prolonged starvation, serum total-, free-, and acyl- carnitine concentrations tend to increase during the first 24-hours and continue to increase over time,[70,71] although Seccombe et al.[71] found free carnitine to decrease within the first 24-hours.

The Obesity Phenotype and Changes in Body Composition

The obesity phenotype is a metabolically active environment that is characterized by its own distinct layout of fat-mass, fat distribution, fatty infiltrations of individual organs, and lean muscle mass.[74] Body weight encompasses not only fat mass but also the sum of individual organs, tissues, bone, fluids, and muscle mass that together are referred to as fat-free mass.[74] Optimal weight loss is aimed at maximizing reductions in fat-mass while limiting catabolism of skeletal muscle tissue,[6] which is not shielded from wasting away with the simultaneous loss of fat-mass during intentional weight loss regimes.[74] A secondary analysis comparing pooled

baseline DEXA-derived body composition data (fat mass and fat-free mass) from 275 weight-stable adults with overweight and obesity (mean age 45.5 ± 11.6 years old, BMI 36.4 ± 7 kg/m^2) against calculated estimates of fat mass and fat-free mass using the Pennington Fat-Free Mass calculator reported that the expected loss of fat-free mass-to-total body weight loss ratios in adult Caucasian males is 35-40% and 30-35% in females.[75] This predicted percentage is higher than the initial assumption of the "Quarter Fat-Free Mass Rule" (Δ fat-free mass/Δ body weight= ~25%).[76]

Dietary Intervention and Change in Free-and Acyl-Carnitine Concentrations with Weight Loss

Any substantial amount of intentional weight loss in an individual may result in undesirable effects since the non-adipose tissues in fat-free mass, much of which is skeletal muscle mass, is responsible for the majority of resting metabolic rate, regulation of core body temperature, preservation of skeletal integrity, and maintenance of function and quality of life as the body ages.[6] Ramos-Roman et al.[77] analyzed fasting plasma samples from 16 adults with overweight and obesity (mean age 45.8 years old, BMI 35.4 kg/m^2) and without diabetes using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and found that lean mass was positively associated with fasting plasma C3, C3-DC, C4, C5, C5:1, and C10:1 acylcarnitine concentrations, whereas fat mass was not associated with any acylcarnitine species (all $p<0.05$).[77] With the multitude of weight loss diets available, data still remains inconclusive on the anticipated changes in lean mass and circulating fasting free- and acyl-carnitine concentrations in adults following various with dietary interventions.

Energy-Restricted Diets

Several randomized controlled trials and secondary analyses of previously collected data have reported differentiation in body composition (body weight, fat mass, lean mass) and carnitine species (free-, total-, and acylcarnitines) in individuals with overweight and obesity after following dietary intervention. Laferrere et al.,[78] Redman et al.,[79] Schooneman et al.,[80] and Lien et al.[81] studied the effect of energy-restriction on changes in body composition and carnitine species among middle-aged adults with overweight and obesity. Laferrere et al.[78] compared the effects of a 10 kg diet-induced weight loss and Roux-en-Y gastric bypass surgery on changes in body composition and 45 fasting serum acylcarnitine concentrations among participants from the New York Obesity Nutrition Research Center (NYONRC). Eleven participants consumed a 1000 kcals of energy/day meal replacement until they lost 10 kg of

body weight (mean age 47.9 years old, baseline BMI 42.8 kg/m^2) and ten participants underwent Roux-en-Y gastric bypass surgery (mean age 43.3 years old, baseline BMI 44.9 kg/m^2). Participants in the gastric bypass surgery group were evaluated 1-month post-surgery. Significant weight change occurred among participants in both groups compared to baseline with faster weight loss in the gastric bypass surgery group (2.7 kg/week) compared to the dietary intervention group (1.3 kg/week, p=0.003). However, mean weight change between each group was not significantly different by the end of the study (gastric bypass group: -11.8 ± 5.3 kg, versus diet group: -9.9 ± 2.3kg, p=0.303). Serum acylcarnitine concentrations were negatively correlated with BMI (r= -0.521, p=0.015) in both groups before weight loss. Although the sum of all acylcarnitine concentrations increased after both types of weight loss interventions (p=0.005), serum C3 (p=0.004), C4-DC (p=0.019), C5 (p=0.027) acylcarnitine concentrations, and the sum of C3 and C5 acylcarnitine concentrations decreased significantly after gastric bypass surgery (p=0.001) but not after dietary intervention (p=0.956).[78]

A randomized controlled feeding trial by Redman et al.[79] investigated the effects of 25% energy-restriction from diet alone (n=12), with an 12.5% energy-restricted diet + exercise (12.5% increase in energy expenditure from aerobic exercise, n=12), compared to a control diet (100% estimated energy needs, n=11) among 35 adults who were overweight. After controlling for sex and age at baseline, fasting serum medium-chain-(C6-DC, C8, C8:1, C10, C10:1, C10:2, C10:3, C10-OH/C8-DC, C12, C12:1, C12-OH/C10-DC) and long-chain acylcarnitine concentrations (C14:1, C14:1-OH, C14:2, C16:2, C20-OH/C18-DC) were positively associated with percent body fat (R2=0.75, p=0.0001). The 25% energy deficit by energy-restriction alone and by energy-restriction + exercise resulted in equivalent losses of body weight (CR: -10 ± 1%; CR + EX: -10 ± 1%), fat mass (CR: -24 ± 3%; CR + EX: -25 ± 3%), abdominal visceral (CR: -28 ± 4%; CR + EX: -27 ± 3%) and subcutaneous fat stores (CR: -26 ± 4%; CR + EX:- 28 ± 3%). Despite similar weight loss,

there was a significant increase in fasting serum C2 and several medium-and long-chain acylcarnitine concentrations (C6-DC, C8, C10, C10:1, C10:2, C10-OH/C8-DC, C12, C12:1, C12-OH/C10-DC, C14, C14:1, C14:2, C14:1-OH, C14-OH/C12-DC, C16, C16:1, C16:2, C18:1, C18:2) in the energy-restricted group that was not seen to the energy-restricted diet group + exercise ($p=0.01$). C2, C14:1, C16, and C18:1 acylcarnitine concentrations were all uniquely increased from baseline to 3-months and baseline to 6-months in participants on the energy-restricted diet but not on the energy-restricted diet + exercise ($p<0.000$, $p=0.001$, $p=0.032$, $p=0.03$).[79]

Another randomized controlled trial by Schooneman et al.[80] measured the mean difference in fasting plasma free-and acyl-carnitine concentrations among 60 non-diabetic participants with obesity recruited for an outpatient study on weight loss prediction[82] (mean age 40 years old, BMI 34.8 kg/m^2) before and after intervention. Participants were randomized to one of three 12-week interventions, diet (–600 energy restriction/day) alone, diet with exercise, and diet with sibutramine weight loss drug. Mean whole-group weight loss between baseline and day 84 among all participants was -4.5 kg and mean lean mass decreased only until day 28 (-0.6 kg). The diet alone and diet with exercise groups exhibited weight loss only up to day 28 with weight remaining stable thereafter, whereas the sibutramine group displayed continued weight loss up to day 84. All weight loss interventions experienced an increase in plasma acylcarnitine concentrations after 84-days, with the greatest increase in the sibutramine group. Mean whole-group change in body weight was negatively correlated with C2 ($p=0.01$), C4-OH ($p<0.001$), C14:1 ($p=0.01$), C16 ($p=0.01$), and C18:1 acylcarnitine concentrations ($p<0.001$), with C2 and C4-OH acylcarnitine concentrations significantly higher after 28 days ($p<0.05$) and by day 84 ($p<0.05$ compared to baseline). Mean plasma C4-OH, C16, and C18:1 acylcarnitine concentrations increased over time and was significantly correlated with a reduction in both total and lean mass over time. Mean whole group free carnitine significantly increased between day 28 and day 84

compared to baseline ($p<0.05$). Mean whole group C10, C14:1, C16, and C18:1 acylcarnitine concentrations significantly increased after 28-days ($p<0.05$) followed by a significant decrease between day 28 & day 84 ($p<0.05$) that still remained higher than baseline.[80]

High-Complex-Carbohydrate Diets

A prospective analysis of pooled data collected from The Study of Effects of Diet on Metabolism and Nutrition (STEDMAN) Project examined the effects of a high-carbohydrate, low-fat DASH weight loss intervention on the metabolic profile of 27 adults with obesity (mean age 51 years old, BMI 32.6 kg/m^2) before and after a 6-month intensive behavioral intervention for achievement of weight loss.[81,83] All participants were part of the blinded Weight Loss Maintenance Study at Duke University that randomized participants during the phase 2 part of the study to one of three behavioral strategies for maintaining weight loss (monthly personal counseling by phone, an interactive website, or no-intervention control group).[32,81] Since this study used data collected during phase 2 of the blinded Weight Loss Maintenance Study that was still in progress at this time, the randomization assignments were unknown to Lien et al. and data from all treatment arms were pooled for this analysis.[81]

The mean weight change from baseline to 6-months was -13.90 (95% CI: -18.65 to -8.00, $p<0.0001$) lbs, mean fat mass change was -3.78 kg (95% CI: -5.51 to -1.38 kg, $p=0.0001$), and mean lean mass change was -1.43 kg (95% CI: -2.53 to 0.27 kg, $p=0.0121$), however, weight reverted towards baseline after 12-months of follow-up. Mean plasma C8:1 and C10:3 acylcarnitine concentrations displayed small increasing trends over the 6-month period but the only statistically significant change from baseline was seen at week 4, when C8:1 acylcarnitine concentration displayed a 0.03 uM increase (95% CI: 0.00 to 0.06 uM, $p=0.02$) and C10:3 acylcarnitine concentration displayed a 0.02 uM increase (95% CI: -0.01 to 0.06, $p=0.0102$).

C10:3 acylcarnitine concentration displayed a statistically significant increase of 0.02 uM (p=0.0242) at 12-months compared to baseline.[81]

A secondary retrospective analysis of data collected during a randomized controlled trial examined the impact of a high-carbohydrate, low-fat diet on changes in carnitine species among 80 Caucasian children with obesity compared to 80 controls with obesity who did not lose significant weight during the intervention (mean age 11 years old, BMI 29.9 kg/m^2). All participants had followed an outpatient lifestyle intervention called "Obeldicks," which included diet, exercise, and behavior therapy and adoption of a mixed diet (30% of total energy from fat, 15% of total energy from protein, 55% of total energy from carbohydrates) using a "traffic-light" system to encourage healthy food choices.[84] The analysis only included changes in 14 serum metabolites that were previously observed to be altered in children with obesity. Among these metabolites were C12:1 and C16:1 acylcarnitine concentrations. However, fasting serum C12:1 and C16:1 acylcarnitine concentrations were not significantly different after 1-year compared to baseline in either the weight loss group (p=0.967, p=0.604) or the weight stable group (p=0.906, p=0.700). This suggests that these acylcarnitines may not be affected by weight loss.[84]

Low-Carbohydrate, High-Fat, High-Protein Diets

Two randomized controlled feeding trials evaluated the impact of an energy-restricted low-carbohydrate, high-fat, high-protein diet on fasting serum carnitine species. Gu et al.[85] conducted an 8-week energy-restricted, low-carbohydrate, high-fat diet on fasting serum free carnitine and C2 acylcarnitine concentrations in 45 healthy adults with obesity (mean age 31.8 years old, BMI 32.58 kg/m^2) compared to 30 healthy controls (mean age 28.2 years old, BMI 21.9 kg/m^2). Free carnitine concentration was significantly higher at baseline in participants with obesity compared to healthy controls (the variable importance in the projection (VIP)=1.14, FC=1.25, p=2.26 x 10-4). No significant change in body weight was reported at week 4 or 8

within the obesity group compared to baseline. However, mean BMI was significantly reduced from 32.59 kg/m^2 to 30.59 kg/m^2 ($p<0.05$) after week 4 and further reduced to 29.88 kg/m^2 ($p<0.01$) by week 8. However, after the very-low-carbohydrate dietary intervention, carnitine was altered to a less significant degree, suggesting that a very-low-carbohydrate diet may attenuate the metabolic alteration of obesity. However, it did not reach a level of significance after 4-weeks (VIP=0.57, FC-=1.12, p=3.4 x 10-2) or 8-weeks of low-carbohydrate dietary intervention compared to healthy controls (VIP=0.90, FC=1.16, p=4.45 x 10-3). After 4-weeks of dietary intervention, mean C2 acylcarnitine concentration was higher compared to baseline (VIP=2.21, FC=1.47, p=6.89 x 10-6).[85]

Smith et al.[86] conducted another randomized controlled feeding trial among 27 postmenopausal females with obesity (mean age ~59 years old, BMI 35.5 kg/m^2) randomized to a regular protein, 30% energy-restricted weight loss diet (0.8 gm/kg body weight/day), a high-protein, 30% energy-restricted weight loss diet (1.2 gm/kg body weight/day), or a weight maintenance control diet. All participants attended weekly counseling sessions with a registered dietitian and both weight loss diet groups followed their assigned diet until they lost 8-10% of their initial body weight and were weight stable (<2% change in body weight) for 3-4 weeks. The weight maintenance group was studied after a time matched period of 27.4 ± 1.2 weeks. Participants in the two weight loss diet groups lost ~10% of their initial body weight compared to the weight maintenance group ($p<0.05$). The contribution of fat-free mass to total weight loss was ~45% less in the high-protein weight loss group than the normal protein weight loss group alone ($p=0.03$). However, the absolute loss of fat-free mass was small so that only ~ 700 grams or 1.5% of total fat-free mass was preserved by the high-protein weight loss diet compared to the normal protein weight loss diet. Mean plasma C3 and C5 acylcarnitine concentrations did

not change in the weight maintenance group and tended to decrease after weight loss in both weight loss groups, although not significantly.[86]

CHAPTER 3: MATERIALS & METHODS

General Experimental Design

A secondary analysis of data collected as part of the Comparison of Health Benefits and Risks of High-Carbohydrate, Low-Fat or Very-Low-Carbohydrate Diets for Weight Loss Study was performed to determine the impact of a low-or high-complex-carbohydrate diet, and the resulting body mass loss, on change in circulating acylcarnitine concentrations in free-living adults with overweight and obesity. Participants were allocated to consume either an ad libitum low-carbohydrate diet similar to the induction phase of the Atkins dietary pattern developed by Dr. Robert Atkins or to an energy-matched high-complex-carbohydrate, low-fat diet similar to DASH dietary pattern. The dietary interventions were administered using a parallel group design to adult participants with overweight and obesity but otherwise healthy. Blood samples were obtained before and 2, 4, and 6-weeks after initiating the controlled dietary intervention phase and after the 12-week home dietary intervention phase at week 18. Body composition (body mass, fat mass, and fat-free mass) were assessed at baseline and weeks 6 and 18. The study design is illustrated in Figure 6.

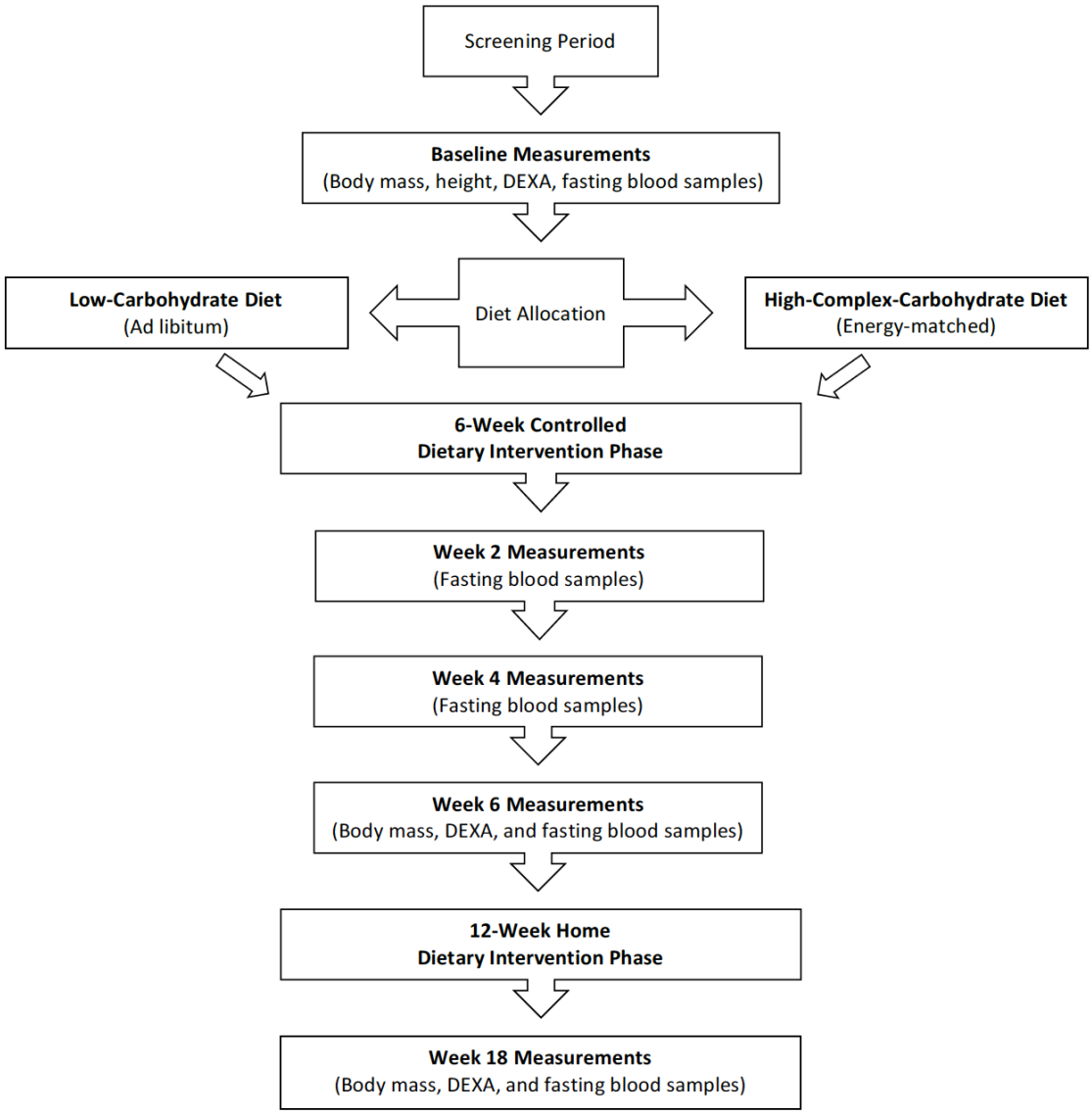


Figure 6. Comparison of Health Benefits and Risks of High-Carbohydrate, Low-Fat or Very-Low-Carbohydrate Diets for Weight Loss Study design.

Human Participants

Recruitment and Consent

Twenty-five healthy adults with overweight or obesity were recruited from the Portland, Oregon area through advertisements posted on the Oregon Health & Science University (OHSU) campus, on the OHSU Study Participation web page, and in local newspapers. Individuals who participated in the study successfully completed a medical history review, physical exam, health screenings, and a pre-intervention meal trial week. Table 2 summarizes the participant inclusion and exclusion criteria and Table 3 displays the study's work plan.

Table 2. Participant inclusion and exclusion criteria.

Inclusion Criteria	**Exclusion Criteria**
• BMI: 28-50 kg/m^2 • Age: 21-65 years old • Relative good health • Approved by primary care provider	• Major debilitating mental or physical illness that would interfere with participation • Diabetes, renal, or hepatic disease • Pregnancy or lactation within past 6 months • History of gallbladder disease • Hyperthyroidism or untreated hypothyroidism • Poorly controlled hypertension • Current excessive use of alcohol • Current/recent chronic use of tobacco or recreational drugs • Plans to leave area in next year

Table 3. Comparison of Health Benefits and Risks of High-Carbohydrate, Low-Fat or Very-Low-Carbohydrate Diets for Weight Loss Study work plan.

	Year 01				Year 02			
Study Quarters	1	2	3	4	1	2	3	4
Planning Phase								
Recruitment/Screening (Cohorts 1-6)	←	—	—	—	—	—	→	
Menu Planning/ Diet Formulation	←	→						
Intervention & Follow-Up								
Cohorts:								
1 Low-carbohydrate diet (n=4)		←	→					
2 High-complex-carbohydrate diet (n=4)			←	→				
3 Low-carbohydrate diet (n=4)				←	→			
4 High-complex-carbohydrate diet (n=4)					←	→		
5 Low-carbohydrate diet (n=4)						←	→	
6 High-complex-carbohydrate diet (n=4)							←	→
Data Analysis								
Data Collection & Analysis		←	—	—	—	—	—	→

Dietary Intervention Protocols

Group Allocation

Participants were allocated to consume either a low-or high-complex-carbohydrate diet using a covariate-adaptive balancing allocation procedure known as the minimization method.[87] This design-adaptive allocation method assigned each participant on a trial basis to each group and then a model was fit to predict the treatment in terms of prognostic factors (age, sex, BMI, and total fasting plasma cholesterol, and triglyceride concentrations). Each participant was assigned to the group for which the prediction was worst. This way, the two dietary intervention groups achieved balance at baseline for age, sex, BMI, and total fasting plasma cholesterol and triglycerides concentrations.

Participants in each dietary intervention group only ate foods prepared and provided to them by the bionutrition staff of the General Clinical Research Center (GCRC) at OHSU, now known as the Oregon Clinical & Translational Research Institute (OCTRI), during the 6-week controlled feeding phase of the study. Detailed information about the two dietary intervention

groups is provided in the following section. Each participant followed a 6-day repeating menu that provided a variety of foods and food combinations. Participants visited the GCRC every weekday morning (Monday through Friday) to be weighed, to complete other scheduled measurements, to eat one meal on-site, and to collect food for the remainder of the day. Weekend meals and snacks were packaged and provided to participants on Fridays. Participants were instructed to return any uneaten portions, which were weighed, recorded, and used to calculate daily energy, macro- and micronutrient consumption by each participant. Those taking vitamin, mineral, and other dietary supplements were asked to discontinue this practice for the duration of the study. All participants regardless of diet group were provided and consumed a standard adult-strength multivitamin/mineral supplement each day and were asked to maintain their typical physical activity level throughout the 6-week dietary intervention. After completing the 6-week controlled dietary phase, all participants continued to follow their assigned dietary plan for an additional 12-week home intervention phase. Each participant was provided with a copy of the Dr. Atkins New Diet Revolution or The DASH Dietary Pattern book, as well as recipes provided by the GCRC staff to encourage adherance to their asigned dietary plan.

Low-Carbohydrate Dietary Intervention

The low-carbohydrate dietary intervention was modeled after the induction phase of the Atkins dietary pattern such that carbohydrate intake was limited to no more than 28 grams per day. All moderate-to-high-carbohydrate containing foods were excluded including fruits, fruit juices, starchy vegetables, grains, and dairy products (except for cheese and cream). Food choices included meat, fish, poultry, eggs, cheese, whipping cream, oils, butter, and small amounts of non-starchy vegetables. Participants assigned to this group were offered pre-weighed meals that provided 120% of their estimated energy requirement (EER) for weight maintenance and were allowed to eat as much or as little of the food provided each day to

satisfy their hunger. Estimated energy requirements were calculated using the Harrison-Benedict equation, the Mifflin St. Jeor equation, and a nomogram developed by Walter Boothby and Joseph Berkson.[88]

High-Complex-Carbohydrate Dietary Intervention

The high-complex-carbohydrate dietary intervention was modeled after the Dietary Approaches to Stop Hypertension (DASH) dietary pattern,[46] which is the prototypical high-complex-carbohydrate dietary pattern that emphasizes consumption of whole fruits, vegetables, low-fat dairy products, whole grains, poultry, fish and nuts, and is reduced in fats, red meat, sweets, and sugar-containing beverages. Due to the structure of the dietary plan, the diet is naturally high in calcium, magnesium, potassium, and fiber, and lower in total fat, particularly saturated fat and cholesterol. The diet was designed to provide around 54% of energy from carbohydrate, 18% of energy from protein, and 28% energy from fat. Participants assigned to this group were provided with pre-weighed meals and were instructed to consume all food. Total daily energy intake from food was energy-matched to the low-carbohydrate dietary group, providing the cummulative average percent reduction of energy consumed by the previous low-carbohydrate dietary participants, as a ratio of their EER for weight maintenance. Estimated energy requirements were determined by multiplying the participant's EER by the average cummulative percent of EER for weight maintenance, consumed by the previous low-carbohydrate dietary participants, as described below in the "Energy Matching Protocol" section.

Nutrient Analyses

ProNutra (Viocare, Princeton, NJ) nutrient analysis software program was used by GCRC bionutritionists to develop the 6-day cycle menus for both dietary intervention groups, to

calculate the amount of foods needed to meet the EER for each participant, and to calculate individual daily intakes of energy, macro-and micronutrients.

Energy-Matching Protocol

Participants were enrolled into 1 of 6 successive cohorts of 4 individuals (see Table 3). To ensure similar relative energy intake between the participants in the low-and high-complex-carbohydrate dietary groups, participants within odd-numbered cohorts were allocated to receive the low-carbohydrate dietary intervention. Participants within even-numbered cohorts received the high-complex-carbohydrate dietary intervention. Participants in the low-carbohydrate dietary arm (cohorts 1, 3, 5) were provided with pre-weighed meals containing recommended foods meeting 120% of their EER for weight maintenance. Participants were allowed to eat as much or as little of the food provided each day to satisfy their hunger and were asked to return any uneaten food. The returned food and food containers were weighed, recorded, and used to calculate each participant's total daily energy consumption in the low-carbohydrate dietary group. In addition, each participant's average total daily energy consumption relative to their EER was reported and calculated as a percentage of their EER.

Participants in the successive high-complex-carbohydrate dietary cohorts (cohorts 2, 4, 6) were provided with pre-weighed meals containing recommended foods and were instructed to eat all of the food provided each day. The energy content of the foods provided were matched to the relative energy intake consumed by the previous low-carbohydrate dietary participants. Energy-matching was done by determining the actual daily energy intake of each low-carbohydrate dietary participant and dividing the amount by that individual's EER for weight maintenance. This value was then multiplied by 100 to calculate the "percent estimated energy requirement" consumed. The average percent EER was calculated for each individual, the cohort, and for all low-carbohydrate dietary participants as a group. The cumulative average

percent EER was then used to calculate the daily total energy content of the foods provided to the high-complex-carbohydrate dietary participants. This was done by multiplying the high-complex-carbohydrate dietary participant's EER for weight maintenance by the average percent of EER consumed by the low-carbohydrate dietary participants. In this way, the energy intakes for the two dietary groups were energy-matched. For instance, cohort 1 randomized to the low-carbohydrate dietary intervention comsumed an average of 75% of their EER to maintain weight then cohort 2 was provided with 75% of their estimated enery needs. Then, if cohort 3 consumed an average of 85% of their EER, then cohort 4 randomized to the high-complex-carbohydrate dietary intervention was given food providing 80% (the average of 75% and 85%) of their EER for weight maintenance.

Data Collection & Analysis

Body Composition Measurements

Body mass, fat mass, and fat-free mass were measured using whole-body dual-energy x-ray absorptiometry (DEXA) at baseline, after the 6-week controlled dietary intervention phase, and after the 12-week home dietary intervention phase at week 18. DEXA scans were performed by trained and licensed technicians in the Bone Mineral Research Lab at OHSU using a Hologic Discovery Series Densitometer (Hologic, Inc., Bedford, MA). Lean mass was calculated by subtracting each participant's bone mineral density (BMD) from fat-free mass in kilograms.

Height Measurements and BMI Calculation

Height was measured first thing in the morning without shoes after an overnight fast using a wall-mounted stadiometer (Harpenden Stadiometer, Holtain Ltd, Crymych, UK). Measurements were taken by the GCRC nursing staff and recorded to the nearest 0.1 cm at baseline. BMI was calculated by dividing each participant's body mass in kilograms by height in meters squared.

Blood Sample Collection and Analysis

Fasting whole blood samples were collected by the GCRC nursing staff after a 10-hour fast by venipuncture of an arm vein using a sterile technique at baseline and after 2, 4, 6, and 18-weeks of the dietary intervention. Single drops of whole blood were placed on standard newborn screening filter papers and allowed to air dry in a horizontal position for at least 3-hours at an ambient temperature (+18 –+25°C) and away from direct light. Samples were stored at room temperature (+20–+25°C) until analysis. A small disk around 3.2mm (1/8 inch) in diameter was punched out of each dried blood spot and the acylcarnitines were extracted by the addition of methanol. Known concentrations of isotopically-labeled acylcarnitines were added to each sample, which functioned as internal standards. Using a stream of nitrogen, the extract was dried and derivatized by the addition of 3.0 N HCl in n-butanol.[89] Samples were analyzed by the research staff at the Oregon State Public Health Laboratory for free- and acyl-carnitine concentrations using a 1445 tandem mass spectrometer (Wallac Oy, Turku, Finland; MDS Sciex, Inc., Framingham, MA) using a PerkinElmer NeoGram AAAC newborn screening kit (PerkinElmer, Inc., Walthan, MA). The acylcarnitines were measured as their butyl esters and the list of acylcarnitines measured by this method is provided in Table 4. Although measured, free carnitine was not included in analyses because the method used is an unrealible method to quantify free carnitine concentration.

Table 4. Acylcarnitine species.

Acylcarnitine Species	Name
Short-Chain Acylcarnitines	
C2	Acetylcarnitine
C3	Propionylcarnitine
C3-DC	Malonylcarnitine
C4	Butyrylcarnitine
C4-DC	Methylmalonylcarnitine
C5	Isovalerylcarnitine
C5-OH	3-Hydroxy-isovalerylcarnitine
C5-DC	Glutarylcarnitine
C5:1	Tiglylcarnitine
Medium-Chain Acylcarnitines	
C6	Hexanoylcarnitine
C6-DC	Adipylcarnitine
C8	Octanoylcarnitine
C10	Decanoylcarnitine
C10:1	Decenoylcarnitine
C12	Dodecanoylcarnitine
Long-Chain Acylcarnitines	
C14	Myristoylcarnitine
C14:1	Tetradecenoylcarnitine
C16	Palmitoylcarnitine
C16-OH	3-Hydroxy-hexadecanoylcarnitine
C18	Stearoylcarnitine
C18:1	Oleylcarnitine

Sample Size Consideration

Twenty-five participants were enrolled in the Comparison of Health Benefits and Risks of High-Carbohydrate, Low-Fat or Very-Low-Carbohydrate Diets for Weight Loss Study. This sample size was statistically powered to see changes in body mass and BMI over time. Since carnitine data was only available from 23 of the initial 25 participants, only data from these 23 participants were included in this secondary analysis. This secondary analysis was designed to provide preliminary and descriptive data for future statistically powered, controlled dietary intervention studies evaluating the impact of low- and high-complex-carbohydrate diets on body composition and acylcarnitine concentrations.

Statistical Analysis Plan

The primary specific aim of this secondary analysis was to examine data obtained from 23 of the initial 25 participants who consumed either a low-carbohydrate diet (n=11) or a high-complex-carbohydrate diet (n=12). Initial baseline characteristics of study participants, including age, sex, BMI (kg/m^2), body mass (kg), lean mass (kg), fat mass (kg), and lean and fat mass as percentages of total body mass, were summarized according to dietary group (Table 5). Means, standard deviations, and ranges were used to describe continuous variables while frequencies and percentages were used to describe categorical variables. The minimization method was used to balance the two dietary intervention groups at baseline for age, sex, BMI, and total fasting plasma cholesterol and triglycerides concentrations. Balance of baseline characteristics with respect to dietary group was checked by computing the difference between means, expressed as a percentage of the average standard deviation of the characteristic.

Linear mixed-effect models were fit to each of 21 fasting acylcarnitine concentrations to identify whether the mean concentration (1) varied over time within dietary groups (baseline, 2, 4, 6, and 18-weeks post-baseline), (2) differed between dietary groups (low- versus high-

complex-carbohydrate diet group), or (3) whether any changes over time, relative to baseline, were different between the two dietary groups (time:diet interaction). Models were fit in a hierarchical manner, starting with the most complex, and simplifying or removing terms when no significant ($p>0.10$) effect was noted. This strategy is outlined in Figure 7.

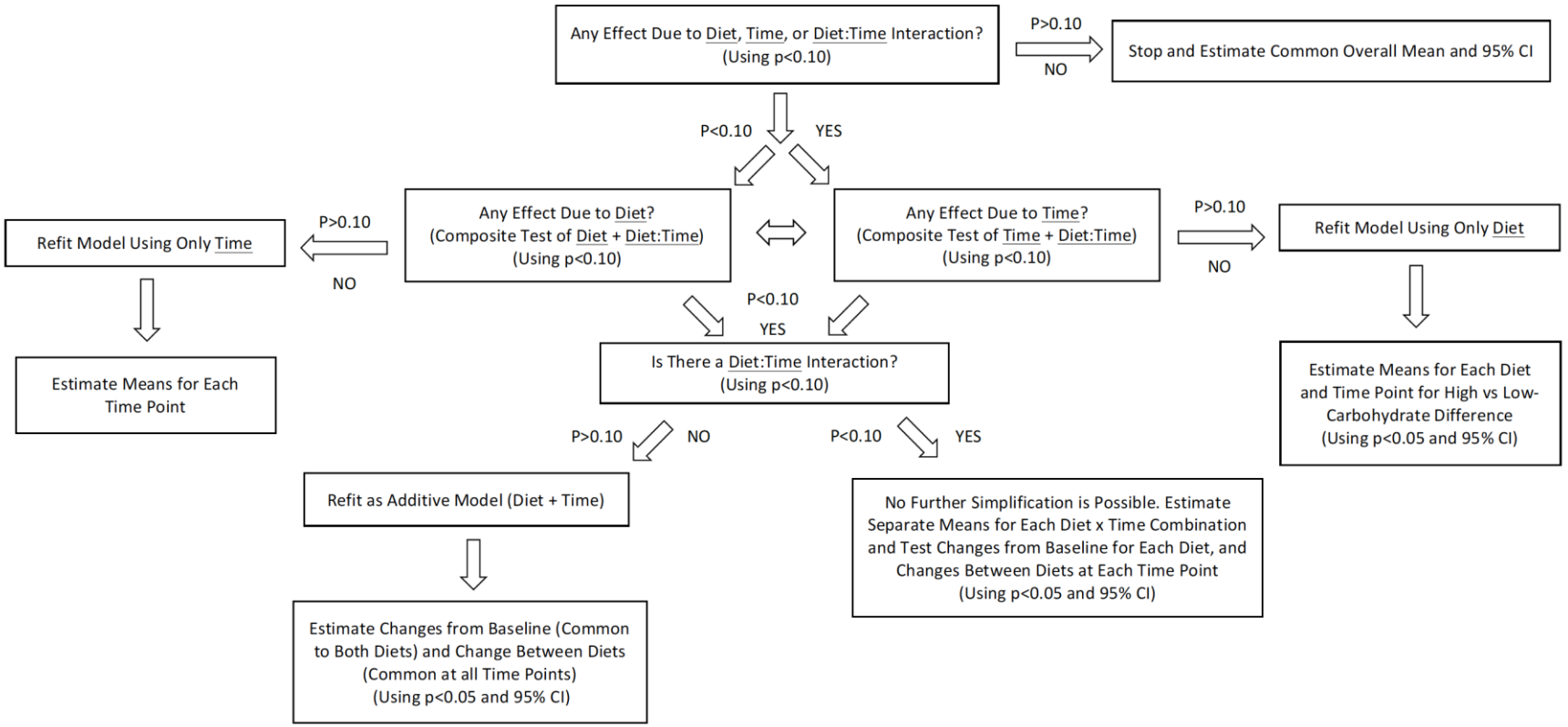


Figure 7. Hierarchical testing procedure for mixed models fit to each acylcarnitine concentration.

In an effort to distinguish significant effects for specific acylcarnitines, a single omnibus "alerting" model was conducted to test whether any effect of diet, time, or the interaction between diet and time (diet:time) was significant at a 0.10 level of significance. If $p>0.10$, then the model was refit to estimate a single common overall mean (and 95% CI) for each individual acylcarnitine that represented the same mean acylcarnitine concentration regardless of diet group or time point.

Upon passing the "alerting" model ($p<0.10$), two separate models were performed to determine whether diet was an important factor (combined diet + diet:time) or whether time was an important factor (combined time + diet:time). If neither were significant ($p>0.10$), the combined non-significant factor (diet or time) was removed and the model was refit to only include the significant factor. For example, if diet + diet:time was not significant, the model was refit to estimate and test changes over time, whereas if time + diet:time was not significant, the model was refit to estimate and test differences between diets. If both diet + diet:time and time + diet:time were significant ($p<0.10$), a specific test of the diet:time interaction was conducted.

If the diet:time interaction was significant ($p<0.10$), then separate means for each diet × time combination were estimated and changes over time were estimated/tested for each diet as well as differences between diets at each time point. If the interaction was not significant ($p>0.10$), then the model was refit as an additive model for both diet and time, where the effect of one factor was not modified by the other. The difference between low- and high-complex-carbohydrate diets (the same at each time point) was estimated/tested and differences at each time point compared to baseline were estimated/tested (the same for both diets). A similar strategy was used to assess any changes in body composition over time (baseline, week 6 and 18) or between diets. All screening tests of overall effects were conducted at the 0.10 level of

significance, while later tests and confidence intervals for the appropriately reduced models were held to a 0.05 level of significance and 95% confidence.

To address the second specific aim, linear regression analysis was used to evaluate if changes in lean mass (kg) or fat mass (kg) were associated with change in individual acylcarnitine concentrations. For the purposes of this thesis, only the individual acylcarnitines that displayed a significant overall effect of time and diet were tested in the linear regression analyses. All analyses were performed using STATA/IC™ (version 15; StataCorp LLC, College Station, TX). All figures were created using STATA/IC™ or Microsoft® Excel (Version 15.38; Redmond, WA).

CHAPTER 4: RESULTS

This secondary analysis is of samples obtained as part of the Comparison of Health Benefits and Risks of High-Carbohydrate, Low-Fat or Very-Low-Carbohydrate Diets for Weight Loss Study. This study was performed to determine the impact of a low-or high-complex-carbohydrate diet, and the resulting body mass loss, on change in circulating acylcarnitine concentrations in free-living adults with overweight and obesity. The association between change in lean mass (kg) and fat mass (kg) and individual acylcarnitine concentrations were assessed between dietary groups at week 6 compared to baseline.

Participant Baseline Characteristics

Demographic Characteristics

Twenty-five participants completed the 6-week controlled dietary intervention phase and the 12-week home dietary intervention phase. However, only 23 participants had complete carnitine concentration data. All participants (n=23) met the inclusion and exclusion criteria described in Table 2. For this analysis, a total of 12 participants were enrolled in the high-complex-carbohydrate dietary group and 11 participants were enrolled in the low-carbohydrate dietary group. Summary data regarding participant baseline characteristics are outlined in Table 5. The majority of participants in this analysis were female (n=15), characterizing 67% of participants in the high-complex-carbohydrate dietary group (n=8) and 64% of participants in the low-carbohydrate dietary group (n=7).

All participants included in this analysis were between 25 – 55 years old. The mean age $\pm$ standard deviation of participants within the high-complex-carbohydrate dietary group was 40 $\pm$ 7.4 years and 42 $\pm$ 8.5 years for the low-carbohydrate dietary group. Participants who identified themselves as Caucasian represented 75% of participants in the high-complex-

carbohydrate dietary group (n=9) and 64% of participants in the low-carbohydrate dietary group (n=7). The remaining 36% of participants in the low-carbohydrate dietary group (n=4) and 8% of participants in the high-complex-carbohydrate dietary group (n=1) identified themselves as black. Two participants (17% of participants) within the high-complex-carbohydrate dietary group identified themselves as Hispanic.

Physical Characteristics

A summary of body composition characteristics for each dietary group at baseline is also summarized in Table 5. All participants, regardless of dietary group, were classified as either "overweight" (BMI of 25 – 29.9 kg/m^2) or "obese" (BMI of 30 – 50 kg/m^2) at baseline. The mean BMI for the high-complex-carbohydrate dietary group was 34 $\pm$ 3.3 kg/m^2 compared to the low-carbohydrate dietary group, which was 35 $\pm$ 4.1 kg/m^2. The mean body mass of the high-complex-carbohydrate dietary group was 98 $\pm$ 11.9 kg at baseline compared to 103 $\pm$ 18.9 kg in the low-carbohydrate dietary group. Yet, overall body composition was similar between dietary groups. The low-carbohydrate dietary group tended to have slightly higher lean mass (61 $\pm$ 16.4 kg) than the high-complex-carbohydrate dietary group (58 $\pm$ 12.0 kg). However, as a percentage of total body mass, percent lean mass was nearly identical between dietary groups. The average percent lean mass was 59 $\pm$ 7.4% for the high-complex-carbohydrate dietary group and 59 $\pm$ 7.7% for the low-carbohydrate dietary group. Likewise, the same was true regarding fat mass at baseline. Mean fat mass was 37 $\pm$ 7.3 kg for participants in the high-complex-carbohydrate dietary group (38 $\pm$ 7.6% of total body mass as body fat) and 39 $\pm$ 9.7 kg for participants in the low-carbohydrate dietary group (39 $\pm$ 7.8% of total body mass as body fat).

Table 5. Participant baseline characteristics.

Characteristic	Dietary Group		Difference in Means[a]	Difference in Means as % of Average SDs[b]
	High-Complex-Carbohydrate n=12	Low-Carbohydrate n=11		
Group Allocation Balanced On:				
Age (years)	40 ± 7.4	42 ± 8.5	-2.1	26.7%
[Min, Max]	[30, 55]	[25, 55]		
Sex, count (%)				
Male	4 (33)	4 (36)	n/a	n/a
Female	8 (67)	7 (64)		
BMI (kg/m^2)	34 ± 3.3	35 ± 4.1	-1.2	33.4%
[Min, Max]	[29.2, 39.8]	[30.1, 41.8]		
Group Allocation Not Balanced On:				
Body Composition:				
Body mass, kg	98 ± 11.9	103 ± 18.9	-5.3	33.6%
[Min, Max]	[77.2, 120.8]	[69.8, 130.2]		
Lean mass, kg	58 ± 12.0	61 ± 16.4	-3.2	22.4%
[Min, Max]	[42.5, 82.2]	[36.9, 92.3]		
Fat mass, kg	37 ± 7.3	39 ± 9.7	-2.0	23.3%
[Min, Max]	[22.9, 48.2]	[31.2, 63.4]		
Lean mass, %	59 ± 7.4	59 ± 7.7	0.1	1.59%
[Min, Max]	[49.1, 71.3]	[46.9, 70.9]		
Fat mass, %	38 ± 7.6	39 ± 7.8	-0.2	2.55%
[Min, Max]	[25.2, 48.2]	[26.2, 50.8]		

Abbreviations: BMI, body mass index; Max, maximum value; Min, minimum value; n/a, not applicable; SD, standard deviation.
Values represent observed mean ± standard deviation, count, (percent), or [Min, Max]. [a] High-complex-carbohydrate mean minus low-carbohydrate mean. [b] Difference in means between diet groups are presented as a percentage of the average SDs: $\frac{|\bar{x}_1 - \bar{x}_2| \times 100}{\sqrt{(s_1^2 + s_2^2)/2}}$

Low-and High-Complex-Carbohydrate Diets and Changes in Body Composition

Change in Body Mass

The observed effect of a low- and high-complex-carbohydrate diet on changes in body composition is summarized in Table 6A. Both dietary interventions resulted in significant within dietary group loss of body mass by weeks 6 and 18 compared to baseline ($p<0.001$). However, there was no indication that diet, when relative energy intake was the same, played a significant role in mean change in body mass ($p=0.601$). In fact, no significant difference in mean body mass loss was observed between dietary groups at any time point during the study (data not shown). The average body mass loss when all participants were considered together, regardless of dietary group, was 5.17 ± 2.28 kg (95% CI: -6.33, -3.92; $p<0.001$) of body mass after the 6-week controlled dietary intervention phase and continued to lose an average 1.67 (95% CI: -2.88, -0.46; $p=0.008$) kg of body mass during the 12-week home dietary intervention phase; leading to an average body mass loss of 6.88 ± 3.72 (95% CI: -8.04, -5.63; $p<0.001$) kg by week 18.

Change in Fat Mass

As shown in Tables 6B and 6C, both dietary interventions resulted in significant within dietary group loss of fat mass (kg) by weeks 6 and 18 ($p\leq0.001$), and lower percent body fat by week 18 ($p<0.001$) compared to baseline. There was no indication that diet, when relative energy intake was the same, played a significant role in change in fat mass ($p=0.866$) or percent body fat ($p=0.855$), with no significant differences in mean change of fat mass or percent body fat between dietary groups at any time point during the study (data not shown). Regardless of dietary group, the 6-week controlled dietary intervention phase resulted in a 2.34 ± 1.04 (95% CI: -3.13, -1.46; $p<0.001$) kg reduction in fat mass and an additional 1.98 (95% CI: -2.81, -1.15; $p<0.001$) kg loss of fat mass during the 12-week home dietary intervention phase. By week 18, the average fat mass loss when all participants were considered together, regardless of dietary

group, was 4.38 ± 2.45 kg (95% CI: -5.16, -3.50; p<0.001) of fat mass. Yet, fat mass as a percentage of total body mass only differed significantly from baseline at week 18, where average percent body fat was 1.92 ± 1.39% (95% CI:-2.47,-1.32; p<0.001) lower among all participants. Percent body fat was lower at week 6 compared to baseline [-0.45 ± 1.17% (95% CI: -1.00, 0.16; p=0.149)] but this difference was not significant. However, percent body fat was significantly lower [1.45% (95% CI:-2.03, -0.87; p<0.001)] at week 18 compared to week 6.

Change in Lean Mass

As shown in Tables 6B and 6C, both dietary interventions resulted in significant within dietary group change in lean mass (kg) by weeks 6 and 18 (p<0.001), and change in percent lean mass by week 18 (p<0.001) compared to baseline. There was no indication that diet, when relative energy intake was the same, played a significant role in change in lean mass (p=0.416) or percent lean mass (p=0.813), with no significant differences in mean change of lean mass or percent lean mass between dietary groups at any time point during the study (data not shown). Regardless of dietary group, the 6-week controlled dietary intervention phase resulted in significantly lower lean mass at week 6 [-2.83 ± 2.30 kg (95% CI: -3.65, -1.96; p<0.001)] and week 18 [-2.54 ± 2.29 kg (95% CI: -3.35, -1.66; p<0.001) compared to baseline. However, no significant change in lean mass was observed between weeks 6 and 18 (p=0.434). Percent lean mass was higher at week 6 [0.28 ± 1.23% (95% CI: -0.28, 0.88; p=0.300)] and week 18 [1.68 ± 1.36% (95% CI: 1.12, 2.27; p<0.001) than baseline, although this difference was only significant at week 18. In fact, percent lean mass was 1.41% (95% CI: 0.83, 1.99; p<0.001) higher at week 18 than week 6.

Table 6A. Within and between dietary group differences in observed mean body mass, lean mass, and fat mass before and after 6-week controlled dietary intervention and 12-week home dietary intervention.

		Baseline	Controlled Dietary Intervention	Home Dietary Intervention	Difference from Baseline[a]		P-value	
			Week 6	Week 18	Δ Week 6	Δ Week 18	Time[b]	Diet[b]
Body mass, kg								
HC	Mean ± SD	97.88 ± 11.86	93.33 ± 10.55	91.11 ± 11.13	**-4.56 ± 1.84**	**-6.77 ± 2.85**		
	95% CI	(89.54, 106.23)	(84.98, 101.67)	(82.77, 99.46)	**(-6.24, -2.87)**	**(-8.46, -5.09)**		
	P-value				**< 0.001**	**< 0.001**		
LC	Mean ± SD	103.18 ± 18.86	97.35 ± 17.43	96.19 ± 17.25	**-5.83 ± 2.61**	**-6.99 ± 4.64**	**< 0.001**	0.601
	95% CI	(94.47, 111.90)	(88.64, 106.07)	(87.48, 104.91)	**(-7.59, -4.07)**	**(-8.75, -5.23)**		
	P-value				**<0.001**	**< 0.001**		
Overall[c]	Mean ± SD	100.42 ± 15.47	95.25 ± 14.07	93.54 ± 14.28	**-5.17 ± 2.28**	**-6.88 ± 3.72**		
	95% CI	(94.10, 106.73)	(88.94, 101.57)	(87.23, 99.86)	**(-6.33, -3.92)**	**(-8.04, -5.63)**	n/a	n/a
	P-value				**<0.001**	**<0.001**		

Abbreviations: CI, confidence interval; HC, high-complex-carbohydrate diet; LC, low-carbohydrate diet; n/a, not applicable; Δ, change.
All effects were tested using mixed models. Values represent observed mean, standard deviation, 95% confidence interval, and p-value. Each mean and standard deviation is based on n=12 (HC) or n=11 (LC). [a] Values for each diet group represent the average difference ± standard deviation of the difference (e.g., week 6 minus baseline). [b] P-values for each term indicate its overall effect (e.g., time + time:diet for overall effect of time; diet + time:diet for overall effect of diet). [c] Includes all participant data (n=23).
Bolded values are significant at p<0.05.

Table 6B. Within and between dietary group differences in observed mean body mass, lean mass, and fat mass before and after 6-week controlled dietary intervention and 12-week home dietary intervention.

		Baseline	Controlled Dietary Intervention	Home Dietary Intervention	Difference from Baseline[a]		P-value	
			Week 6	Week 18	Δ Week 6	Δ Week 18	Time[b]	Diet[b]
Fat mass, kg								
HC	Mean ± SD	37.40 ± 7.34	35.22 ± 7.39	33.30 ± 7.53	**-2.18 ± 0.86**	**-4.10 ± 1.86**	**< 0.001**	0.866
	95% CI	(32.74, 42.06)	(30.55, 39.88)	(28.64, 37.96)	**(-3.36, -1.01)**	**(-5.27, -2.93)**		
	P-value				**0.001**	**< 0.001**		
LC	Mean ± SD	39.41 ± 9.73	36.89 ± 9.07	34.73 ± 8.33	**-2.52 ± 1.23**	**-4.68 ± 3.04**		
	95% CI	(34.54, 44.28)	(32.02, 41.76)	(29.86, 39.60)	**(-3.74, -1.29)**	**(-5.91, -3.45)**		
	P-value				**< 0.001**	**< 0.001**		
Overall[c]	Mean ± SD	38.36 ± 8.43	36.02 ± 8.09	33.98 ± 7.77	**-2.34 ± 1.04**	**-4.38 ± 2.45**	n/a	n/a
	95% CI	(34.87, 41.86)	(32.52, 39.51)	(30.49, 37.48)	**(-3.13, -1.46)**	**(-5.16, -3.50)**		
	P-value				**<0.001**	**<0.001**		
Lean mass, kg								
HC	Mean ± SD	57.75 ± 11.99	55.38 ± 10.36	55.05 ± 10.21	**-2.37 ± 2.12**	**-2.70 ± 2.16**	**< 0.001**	0.416
	95% CI	(50.14, 65.36)	(47.77, 62.98)	(47.44, 62.65)	**(-3.54, -1.21)**	**(-3.86, -1.54)**		
	P-value				**< 0.001**	**< 0.001**		
LC	Mean ± SD	60.96 ± 16.36	57.63 ± 15.40	58.61 ± 15.59	**-3.33 ± 2.48**	**-2.36 ± 2.52**		
	95% CI	(53.02, 68.91)	(49.69, 65.57)	(50.66, 66.55)	**(-4.55, -2.12)**	**(-3.57, -1.14)**		
	P-value				**< 0.001**	**< 0.001**		
Overall[c]	Mean ± SD	59.29 ± 14.01	56.45 ± 12.76	56.75 ± 12.88	**-2.83 ± 2.30**	**-2.54 ± 2.29**	n/a	n/a
	95% CI	(53.57, 65.00)	(50.74, 62.17)	(51.04, 62.47)	**(-3.65, -1.96)**	**(-3.35, -1.66)**		
	P-value				**<0.001**	**<0.001**		

Abbreviations: CI, confidence interval; HC, high-complex-carbohydrate diet; LC, low-carbohydrate diet; n/a, not applicable; Δ, change.
All effects were tested using mixed models. Values represent observed mean, standard deviation, 95% confidence interval, and p-value. Each mean and standard deviation is based on n=12 (HC) or n=11 (LC). [a] Values for each diet group represent the average difference ± standard deviation of the difference (e.g., week 6 minus baseline). [b] P-values for each term indicate its overall effect (e.g., time + time:diet for overall effect of time; diet + time:diet for overall effect of diet). [c] Includes all participant data (n=23).
Bolded values are significant at p<0.05.

Table 6C. Within and between dietary group differences in observed mean body mass, lean mass, and fat mass before and after 6-week controlled dietary intervention and 12-week home dietary intervention.

		Baseline	Controlled Dietary Intervention	Home Dietary Intervention	Difference from Baseline[a]		P-value	
			Week 6	Week 18	Δ Week 6	Δ Week 18	Time[b]	Diet[b]
Fat mass, %								
HC	Mean ± SD	38.49 ± 7.56	37.88 ± 7.52	36.65 ± 7.45	-0.60 ± 1.06	**-1.83 ± 1.35**	**< 0.001**	0.855
	95% CI	(34.12, 42.85)	(33.52, 42.25)	(32.29, 41.02)	(-1.42, 0.21)	**(-2.65, -1.02)**		
	P-value				0.143	**< 0.001**		
LC	Mean ± SD	38.68 ± 7.84	38.41 ± 7.99	36.67 ± 7.97	-0.27 ± 1.32	**-2.01 ± 1.50**		
	95% CI	(34.12, 43.24)	(33.85, 42.97)	(32.11, 41.23)	(-1.12, 0.57)	**(-2.86, -1.16)**		
	P-value				0.517	**< 0.001**		
Overall[c]	Mean ± SD	38.58 ± 7.52	38.13 ± 7.58	36.66 ± 7.52	-0.45 ± 1.17	**-1.92 ± 1.39**	n/a	n/a
	95% CI	(35.32, 41.84)	(34.88, 41.39)	(33.41, 39.92)	(-1.00, 0.16)	**(-2.47, -1.32)**		
	P-value				0.149	**<0.001**		
Lean mass, %								
HC	Mean ± SD	58.72 ± 7.36	59.18 ± 7.29	60.31 ± 7.19	0.46 ± 1.08	**1.59 ± 1.33**	**< 0.001**	0.813
	95% CI	(54.47, 62.97)	(54.93, 63.43)	(56.06, 64.56)	(-0.35, 1.27)	**(0.78, 2.40)**		
	P-value				0.254	**< 0.001**		
LC	Mean ± SD	58.60 ± 7.67	58.69 ± 7.82	60.37 ± 7.77	0.09 ± 1.40	**1.77 ± 1.46**		
	95% CI	(54.16, 63.04)	(54.25, 63.12)	(55.93, 64.81)	(-0.76, 0.93)	**(0.92, 2.61)**		
	P-value				0.834	**< 0.001**		
Overall[c]	Mean ± SD	58.66 ± 7.34	58.94 ± 7.38	60.34 ± 7.30	0.28 ± 1.23	**1.68 ± 1.36**	n/a	n/a
	95% CI	(55.49, 61.83)	(55.78, 62.11)	(57.17, 63.51)	(-0.28, 0.88)	**(1.12, 2.27)**		
	P-value				0.300	**<0.001**		

Abbreviations: CI, confidence interval; HC, high-complex-carbohydrate diet; LC, low-carbohydrate diet; n/a, not applicable; Δ, change.
All effects were tested using mixed models. Values represent observed mean, standard deviation, 95% confidence interval, and p-value. Each mean and standard deviation is based on n=12 (HC) or n=11 (LC). [a] Values for each diet group represent the average difference ± standard deviation of the difference (e.g., week 6 minus baseline). [b] P-values for each term indicate its overall effect (e.g., time + time:diet for overall effect of time; diet + time:diet for overall effect of diet). [c] Includes all participant data (n=23).
Bolded values are significant at p<0.05.

Low-and High-Complex-Carbohydrate Diets and Change in Acylcarnitine Concentrations

Effect of Diet, Time, or their Interaction on Acylcarnitine Concentrations

A mixed model was run on the individual acylcarnitines listed in Table 4 to determine the overall effect of diet, time, or the interaction between diet and time (diet:time). Results from the fitting strategy are shown in Figure 8, together with the number of acylcarnitines that "passed" each screening step and the appropriate table references for the specific models fit. Tables 7A and 7B summarize the presence or absence of an overall effect on each individual acylcarnitine species, in addition to the observed mean and standard deviation for each individual acylcarnitine concentration (μmol/L) by dietary group at baseline and weeks 2, 4, 6, and 18.

In summary, only C2 (p=0.002), C3 (p=0.001), C4 (p=0.023), C5 (p=0.061), C5-DC (p=0.040), C10 (p=0.049), C16 (p=0.092), C18 (p=0.034), and C18:1 (p=0.039) acylcarnitine concentrations exhibited an overall effect of diet, time, or their interaction. Since there was no evidence of any effect due to diet, time, or their interaction on the remaining 12 acylcarnitines at a 0.10 level of significance, the data for each non-significant acylcarnitine species was summarized by a single estimated mean and 95% confidence interval. This estimated mean represents the average concentration for each non-significant acylcarnitine species (C3-DC, C4-DC, C5-OH, C5:1, C6, C6-DC, C8, C10:1, C12, C14, C14:1, and C16-OH acylcarnitines) regardless of dietary group or time.

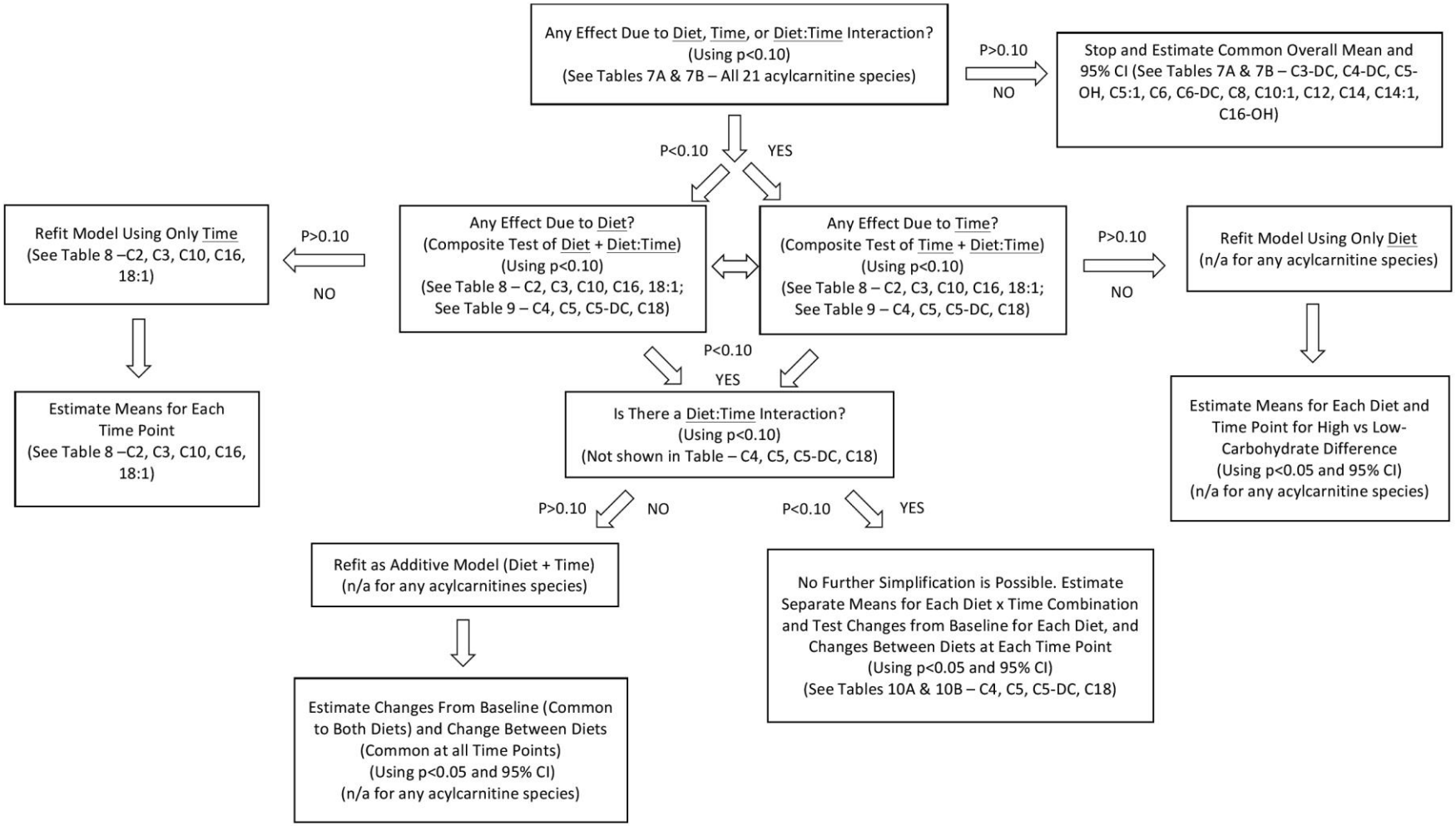


Figure 8. Hierarchical testing procedure and results for mixed models fit to each acylcarnitine concentration.

Table 7A. Effect of diet, time, or their interaction on observed and estimated mean acylcarnitine concentrations (μmol/L) at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.

	Baseline		Controlled Dietary Intervention						Home Dietary Intervention		Estimated Mean (95% CI)[b]	P-value[c]
			Week 2		Week 4		Week 6		Week 18			
Acylcarnitine, μmol/L	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**		
C2[a]	13.02 ± 1.73	11.42 ± 2.26	14.47 ± 3.41	14.37 ± 2.83	13.57 ± 1.84	13.54 ± 2.67	14.66 ± 2.60	13.93 ± 2.50	14.02 ± 1.63	13.69 ± 1.80	n/a	**0.002**
C3[a]	2.22 ± 1.06	1.73 ± 0.59	2.86 ± 1.08	2.43 ± 0.93	2.34 ± 0.92	2.16 ± 0.89	2.46 ± 1.13	1.72 ± 0.76	2.15 ± 0.97	2.05 ± 0.78	n/a	**0.001**
C3-DC[a]	0.17 ± 0.10	0.13 ± 0.06	0.10 ± 0.03	0.12 ± 0.06	0.13 ± 0.07	0.12 ± 0.05	0.11 ± 0.04	0.10 ± 0.03	0.15 ± 0.10	0.12 ± 0.06	0.13 (0.11, 0.14)	0.351
C4[a]	0.56 ± 0.27	0.32 ± 0.23	0.37 ± 0.15	0.43 ± 0.27	0.34 ± 0.16	0.51 ± 0.36	0.46 ± 0.24	0.40 ± 0.27	0.36 ± 0.18	0.24 ± 0.11	n/a	**0.023**
C4-DC[a]	0.83 ± 0.14	0.61 ± 0.25	0.73 ± 0.26	0.58 ± 0.22	0.66 ± 0.28	0.63 ± 0.21	0.67 ± 0.31	0.54 ± 0.18	0.67 ± 0.26	0.76 ± 0.38	0.67 (0.59, 0.75)	0.141
C5[a]	0.31 ± 0.14	0.29 ± 0.14	0.23 ± 0.12	0.27 ± 0.14	0.21 ± 0.07	0.40 ± 0.22	0.33 ± 0.14	0.29 ± 0.18	0.22 ± 0.17	0.31 ± 0.14	n/a	**0.061**
C5-DC[a]	0.17 ± 0.08	0.09 ± 0.03	0.11 ± 0.05	0.10 ± 0.07	0.12 ± 0.06	0.11 ± 0.05	0.13 ± 0.07	0.16 ± 0.08	0.12 ± 0.07	0.14 ± 0.07	n/a	**0.040**
C5-OH[a]	0.47 ± 0.42	0.33 ± 0.18	0.36 ± 0.16	0.43 ± 0.22	0.39 ± 0.17	0.45 ± 0.19	0.36 ± 0.19	0.49 ± 0.22	0.34 ± 0.22	0.42 ± 0.28	0.40 (0.33, 0.47)	0.541
C5:1[a]	0.13 ± 0.06	0.09 ± 0.04	0.09 ± 0.02	0.09 ± 0.08	0.11 ± 0.05	0.11 ± 0.05	0.10 ± 0.05	0.10 ± 0.03	0.10 ± 0.05	0.11 ± 0.05	0.10 (0.09, 0.12)	0.530
C6[a]	0.22 ± 0.20	0.12 ± 0.04	0.14 ± 0.07	0.17 ± 0.10	0.18 ± 0.09	0.17 ± 0.08	0.12 ± 0.06	0.12 ± 0.03	0.21 ± 0.15	0.20 ± 0.10	0.17 (0.15, 0.19)	0.137
C6-DC[a]	0.20 ± 0.11	0.23 ± 0.23	0.17 ± 0.09	0.14 ± 0.07	0.24 ± 0.14	0.20 ± 0.14	0.12 ± 0.06	0.14 ± 0.08	0.19 ± 0.11	0.21 ± 0.13	0.18 (0.16, 0.21)	0.383

Abbreviations: CI, confidence interval; DC, dicarboxylic acid in the acyl group; HC, high-complex-carbohydrate diet; LC, low-carbohydrate diet; n/a, not applicable; OH, hydroxyl group attached to the acyl group.

[a] Values represent observed mean ± standard deviation. [b] Values represent estimated overall mean and 95% confidence interval for each acylcarnitine regardless of diet group or time point. [c] All effects were tested using mixed models. P-values reflect overall significiance (e.g., any of diet, time, or their interaction). **Bolded** acylcarnitine species and corresponding p-values are significant at p<0.10.

Table 7B. Effect of diet, time, or their interaction on observed and estimated mean acylcarnitine concentrations (μmol/L) at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.

	Baseline		Controlled Dietary Intervention						Home Dietary Intervention		Estimated Mean (95% CI)[b]	P-value[c]
			Week 2		Week 4		Week 6		Week 18			
Acylcarnitine, μmol/L	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**		
C8[a]	0.17 ± 0.09	0.15 ± 0.14	0.15 ± 0.06	0.23 ± 0.09	0.19 ± 0.16	0.25 ± 0.16	0.18 ± 0.09	0.14 ± 0.07	0.17 ± 0.08	0.14 ± 0.08	0.18 (0.15, 0.20)	0.216
C10[a]	0.13 ± 0.06	0.11 ± 0.06	0.16 ± 0.07	0.22 ± 0.14	0.12 ± 0.06	0.24 ± 0.12	0.19 ± 0.19	0.18 ± 0.13	0.16 ± 0.11	0.21 ± 0.12	n/a	**0.049**
C10:1[a]	0.10 ± 0.06	0.12 ± 0.06	0.12 ± 0.05	0.15 ± 0.08	0.16 ± 0.12	0.18 ± 0.10	0.15 ± 0.07	0.14 ± 0.04	0.18 ± 0.10	0.16 ± 0.10	0.15 (0.13, 0.16)	0.290
C12[a]	0.22 ± 0.27	0.19 ± 0.13	0.25 ± 0.16	0.29 ± 0.13	0.20 ± 0.07	0.21 ± 0.13	0.19 ± 0.10	0.20 ± 0.06	0.25 ± 0.19	0.18 ± 0.08	0.22 (0.19, 0.24)	0.782
C14[a]	0.14 ± 0.08	0.11 ± 0.09	0.12 ± 0.08	0.13 ± 0.06	0.11 ± 0.07	0.14 ± 0.06	0.09 ± 0.05	0.13 ± 0.06	0.09 ± 0.04	0.11 ± 0.08	0.12 (0.10, 0.13)	0.490
C14:1[a]	0.09 ± 0.04	0.11 ± 0.09	0.13 ± 0.11	0.11 ± 0.05	0.12 ± 0.04	0.10 ± 0.05	0.10 ± 0.04	0.08 ± 0.04	0.09 ± 0.04	0.10 ± 0.04	0.10 (0.09, 0.12)	0.573
C16[a]	1.48 ± 0.91	1.25 ± 0.51	1.28 ± 0.58	1.47 ± 0.45	1.37 ± 0.63	1.37 ± 0.36	1.20 ± 0.30	1.19 ± 0.31	1.05 ± 0.32	0.99 ± 0.29	n/a	**0.092**
C16-OH[a]	0.12 ± 0.07	0.08 ± 0.03	0.12 ± 0.10	0.07 ± 0.01	0.08 ± 0.02	0.10 ± 0.05	0.12 ± 0.06	0.11 ± 0.06	0.09 ± 0.02	0.13 ± 0.08	0.10 (0.09, 0.12)	0.106
C18[a]	0.79 ± 0.47	0.68 ± 0.21	0.68 ± 0.29	1.22 ± 0.60	0.72 ± 0.27	0.98 ± 0.49	0.73 ± 0.27	1.00 ± 0.44	0.70 ± 0.30	0.81 ± 0.49	n/a	**0.034**
C18:1[a]	1.62 ± 0.49	1.43 ± 0.41	1.86 ± 0.49	1.76 ± 0.51	1.97 ± 0.90	1.74 ± 0.56	2.06 ± 0.70	1.55 ± 0.38	1.47 ± 0.42	1.65 ± 0.81	n/a	**0.039**

Abbreviations: CI, confidence interval; HC, high-complex-carbohydrate diet; LC, low-carbohydrate diet; n/a, not applicable; OH, hydroxyl group attached to the acyl group.

[a] Values represent observed mean ± standard deviation. [b] Values represent estimated overall mean and 95% confidence interval for each acylcarnitine regardless of diet group or time point. [c] All effects were tested using mixed models. P-values reflect overall significiance (e.g., any of diet, time, or their interaction). **Bolded** acylcarnitine species and corresponding p-values are significant at p<0.10.

Effect of Diet or Time and their Interaction on Acylcarnitine Concentrations

A different mixed model was run on the remaining 9 acylcarnitine species (C2, C3, C4, C5, C5-DC, C10, C16, C18, and C18:1 acylcarnitines) that previously exhibited some overall effect of diet, time, or their interaction. This second mixed model tested for an effect of either diet (as a main effect or an interaction with time) or time (as a main effect or an interaction with diet) on the remaining 9 acylcarnitine concentrations.

Overall, C2 ($p=0.002$), C3 ($p<0.001$), C10 ($p=0.051$), C16 ($p=0.059$), and C18:1 ($p=0.031$) acylcarnitine concentrations displayed a significant effect of time (either as a main effect or an interaction with diet) and no effect of diet (either as a main effect or an interaction with time). Table 8 summarizes these acylcarnitine species accompanied by p-values for these overall effects, in addition to the observed means and standard deviations for each individual acylcarnitine concentration (μmol/L) by diet group at baseline and weeks 2, 4, 6, and 18. Since there was no evidence at a 0.10 level of significance of any effect due to diet, a single estimated mean was reported for each non-significant acylcarnitine species (C2, C3, C10, C16, and C18:1 acylcarnitines), common to both diets, that best represents the typical mean response of each acylcarnitine concentration at each time point. This estimated mean represents the average acylcarnitine concentration for each non-significant acylcarnitine species at each time point regardless of dietary group.

Table 8. Mean acylcarnitine concentrations (µmol/L) affected by time and not diet at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.

	Baseline		Controlled Dietary Intervention						Home Dietary Intervention		P-value	
			Week 2		Week 4		Week 6		Week 18		Time[c]	Diet[c]
Acylcarnitine, µmol/L	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**		
C2[a]	13.02 ± 1.73	11.42 ± 2.26	14.47 ± 3.41	14.37 ± 2.83	13.57 ± 1.84	13.54 ± 2.67	14.66 ± 2.60	13.93 ± 2.50	14.02 ± 1.63	13.69 ± 1.80	**0.002**	0.569
Estimated Mean[b]	12.25		14.42		13.55		14.31		13.86			
C3[a]	2.22 ± 1.06	1.73 ± 0.59	2.86 ± 1.08	2.43 ± 0.93	2.34 ± 0.92	2.16 ± 0.89	2.46 ± 1.13	1.72 ± 0.76	2.15 ± 0.97	2.05 ± 0.78	**< 0.001**	0.174
Estimated Mean[b]	1.99		2.65		2.26		2.11		2.10			
C10[a]	0.13 ± 0.06	0.11 ± 0.06	0.16 ± 0.07	0.22 ± 0.14	0.12 ± 0.06	0.24 ± 0.12	0.19 ± 0.19	0.18 ± 0.13	0.16 ± 0.11	0.21 ± 0.12	**0.051**	0.103
Estimated Mean[b]	0.12		0.19		0.18		0.18		0.19			
C16[a]	1.48 ± 0.91	1.25 ± 0.51	1.28 ± 0.58	1.47 ± 0.45	1.37 ± 0.63	1.37 ± 0.36	1.20 ± 0.30	1.19 ± 0.31	1.05 ± 0.32	0.99 ± 0.29	**0.059**	0.678
Estimated Mean[b]	1.37		1.37		1.37		1.20		1.02			
C18:1[a]	1.62 ± 0.49	1.43 ± 0.41	1.86 ± 0.49	1.76 ± 0.51	1.97 ± 0.90	1.74 ± 0.56	2.06 ± 0.70	1.55 ± 0.38	1.47 ± 0.42	1.65 ± 0.81	**0.031**	0.187
Estimated Mean[b]	1.53		1.81		1.86		1.82		1.56			

Abbreviations: HC, high-complex-carbohydrate diet; LC, low-carbohydrate diet.

[a] Values represent observed mean ± standard deviation. [b] Values represent overall estimated mean for each acylcarnitine regardless of diet group. [c] All effects were tested using mixed models. P-values reflect each term's overall effect (e.g., time + time:diet for overall time effect; diet + time:diet for overall diet effect). **Bolded** acylcarnitine species and corresponding p-values are significant at p<0.10.

In contrast, C4 (p=0.014 for time; p=0.028 for diet), C5 (p=0.063 for time; p=0.033 for diet), C5-DC (p=0.025 for time; p=0.031 for diet), and C18 (p=0.065 for time; p=0.021 for diet) acylcarnitine concentrations displayed a significant effect of time and diet at a 0.10 level of significance. Subsequent models at a 0.10 level of significance revealed that C4 (p=0.014), C5 (p=0.029), C5-DC (p=0.015), and C18 (p=0.041) acylcarnitines concentrations were also significantly influenced by a time:diet interaction and all experienced changes over time that varied by dietary group (not shown in Table 9). Therefore, there were no simple effects of diet or time for these 4 acylcarnitines because both time and diet interacted to modify the behavior of each other. Table 9 summarizes the observed means, standard deviations, and effect of diet (as a main effect or interaction with time), time (as a main effect or interaction with diet), and interaction of time and diet alone for these acylcarnitine species (µmol/L) by diet group at baseline and weeks 2, 4, 6, and 18. The estimated mean for these 4 acylcarnitines at each time point within each dietary group could not be further simplified. As a result, the estimated mean is not listed within Table 9 because it matches the observed mean at each time point within each dietary group.

Table 9. Mean acylcarnitine concentrations (μmol/L) affected by time and diet at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.

	Baseline		Controlled Dietary Intervention						Home Dietary Intervention		P-value	
			Week 2		Week 4		Week 6		Week 18		Time[b]	Diet[b]
Acylcarnitine, μmol/L	HC	LC	HC	LC	HC	LC	HC	LC	HC	LC		
C4[a]	0.56 ± 0.27	0.32 ± 0.23	0.37 ± 0.15	0.43 ± 0.27	0.34 ± 0.16	0.51 ± 0.36	0.46 ± 0.24	0.40 ± 0.27	0.36 ± 0.18	0.24 ± 0.11	**0.014**	**0.028**
C5[a]	0.31 ± 0.14	0.29 ± 0.14	0.23 ± 0.12	0.27 ± 0.14	0.21 ± 0.07	0.40 ± 0.22	0.33 ± 0.14	0.29 ± 0.18	0.22 ± 0.17	0.31 ± 0.14	**0.063**	**0.033**
C5-DC[a]	0.17 ± 0.08	0.09 ± 0.03	0.11 ± 0.05	0.10 ± 0.07	0.12 ± 0.06	0.11 ± 0.05	0.13 ± 0.07	0.16 ± 0.08	0.12 ± 0.07	0.14 ± 0.07	**0.025**	**0.031**
C18[a]	0.79 ± 0.47	0.68 ± 0.21	0.68 ± 0.29	1.22 ± 0.60	0.72 ± 0.27	0.98 ± 0.49	0.73 ± 0.27	1.00 ± 0.44	0.70 ± 0.30	0.81 ± 0.49	**0.065**	**0.021**

Abbreviations: HC, high-complex-carbohydrate diet; LC, low-carbohydrate diet.

[a] Values represent observed mean ± standard deviation. [b] Values represent overall estimated mean for each acylcarnitine regardless of diet group. [c] All effects were tested using mixed models. P-values reflect each term's overall effect (e.g., time + time:diet for overall time effect; diet + time:diet for overall diet effect). **Bolded** acylcarnitine species and corresponding p-values are significant at p<0.10.

Effect of Time, Diet, and their Interaction on Acylcarnitine Concentrations

To describe the within and between dietary group differences at various time points, multiple mixed models were run on the remaining 4 acylcarnitine species (C4, C5, C5-DC, and C18 acylcarnitines) that were previously reported to have a significant effect of diet, time, and their interaction. Table 10 summarizes the observed means, 95% confidence intervals, and p-values representing the means at baseline and weeks 2, 4, 6, and 18 for C4, C5, C5-DC, and C18 acylcarnitines concentrations within and between dietary groups.

Table 10A. Within and between dietary group differences in observed mean acylcarnitine concentrations (μmol/L) affected by time, diet, and their interaction at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.

Acylcarnitine, μmol/L		Baseline	Controlled Dietary Intervention			Home Dietary Intervention	Difference from Baseline[a]			
			Week 2	Week 4	Week 6	Week 18	Δ Week 2	Δ Week 4	Δ Week 6	Δ Week 18
C4										
HC	Mean	0.56	0.37	0.34	0.46	0.36	**-0.19**	**-0.22**	-0.10	**-0.21**
	95% CI	(0.43, 0.69)	(0.24, 0.50)	(0.21, 0.48)	(0.33, 0.59)	(0.22, 0.49)	**(-0.36, -0.02)**	**(-0.38, -0.05)**	(-0.27, 0.07)	**(-0.37, -0.04)**
	P-value						**0.025**	**0.012**	0.233	**0.016**
LC	Mean	0.32	0.43	0.51	0.40	0.24	0.11	**0.19**	0.09	-0.08
	95% CI	(0.18, 0.46)	(0.30, 0.57)	(0.37, 0.65)	(0.27, 0.54)	(0.10, 0.38)	(-0.06, 0.29)	**(0.02, 0.36)**	(-0.09, 0.26)	(-0.25, 0.10)
	P-value						0.197	**0.034**	0.330	0.376
Δ HC – LC[b]	Mean	**0.24**	-0.06	-0.16	0.06	0.12	**-0.31**	**-0.41**	-0.19	-0.13
	95% CI	**(0.05, 0.44)**	(-0.26, 0.13)	(-0.36, 0.03)	(-0.14, 0.25)	(-0.08, 0.31)	**(-0.55, -0.06)**	**(-0.65, -0.17)**	(-0.43, 0.05)	(-0.37, 0.11)
	P-value	**0.014**	0.523	0.095	0.566	0.239	**0.014**	**0.001**	0.127	0.296
C5										
HC	Mean	0.31	0.23	0.21	0.33	0.22	-0.08	-0.10	0.02	-0.09
	95% CI	(0.22, 0.39)	(0.14, 0.31)	(0.12, 0.29)	(0.24, 0.42)	(0.14, 0.31)	(-0.19, 0.03)	(-0.21, 0.01)	(-0.08, 0.13)	(-0.19, 0.02)
	P-value						0.134	0.068	0.681	0.115
LC	Mean	0.29	0.27	0.40	0.29	0.31	-0.02	**0.11**	0.00	0.02
	95% CI	(0.20, 0.38)	(0.18, 0.36)	(0.31, 0.49)	(0.21, 0.38)	(0.22, 0.40)	(-0.13, 0.09)	**(0.00, 0.23)**	(-0.11, 0.12)	(-0.09, 0.13)
	P-value						0.693	**0.047**	0.932	0.712
Δ HC – LC[b]	Mean	0.02	-0.04	**-0.19**	0.04	-0.09	-0.06	**-0.21**	0.02	-0.11
	95% CI	(-0.11, 0.14)	(-0.17, 0.08)	**(-0.32, -0.07)**	(-0.09, 0.16)	(-0.21, 0.04)	(-0.21, 0.10)	**(-0.37, -0.06)**	(-0.14, 0.17)	(-0.26, 0.05)
	P-value	0.774	0.515	**0.003**	0.574	0.164	0.449	**0.008**	0.824	0.175

Abbreviations: CI, confidence interval; HC, high-complex-carbohydrate diet; LC, low-carbohydrate diet; n/a, not applicable; Δ, change.

All effects were tested using mixed models. Values represent observed mean, 95% confidence interval, and p-value. Each mean and 95% confidence interval is based on n=12 (HC) or n=11 (LC). [a] Values represent the mean difference from baseline within diet group. [b] Values represent the between diet group difference in means (high-complex-carbohydrate diet group mean minus low-carbohydrate diet group mean). **Bolded** values are significant at p<0.05.

Table 10B. Within and between dietary group differences in observed mean acylcarnitine concentrations (μmol/L) affected by time, diet, and their interaction at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.

Acylcarnitine, μmol/L		Baseline	Controlled Dietary Intervention			Home Dietary Intervention	Difference from Baseline[a]			
			Week 2	Week 4	Week 6	Week 18	Δ Week 2	Δ Week 4	Δ Week 6	Δ Week 18
C5-DC										
HC	Mean	0.17	0.11	0.12	0.13	0.12	**-0.06**	**-0.05**	-0.04	**-0.05**
	95% CI	(0.13, 0.21)	(0.07, 0.15)	(0.08, 0.15)	(0.09, 0.17)	(0.08, 0.16)	**(-0.11, -0.01)**	**(-0.10, -0.01)**	(-0.09, 0.00)	**(-0.10, 0.00)**
	P-value						**0.013**	**0.026**	0.072	**0.035**
LC	Mean	0.09	0.10	0.11	0.16	0.14	0.01	0.02	**0.07**	**0.05**
	95% CI	(0.05, 0.13)	(0.06, 0.14)	(0.07, 0.15)	(0.12, 0.20)	(0.10, 0.18)	(-0.04, 0.06)	(-0.03, 0.07)	**(0.02, 0.12)**	**(0.00, 0.10)**
	P-value						0.575	0.414	**0.006**	**0.049**
Δ HC – LC[b]	Mean	**0.08**	0.01	0.01	-0.03	-0.02	**-0.07**	**-0.07**	**-0.11**	**-0.10**
	95% CI	**(0.03, 0.14)**	(-0.05, 0.06)	(-0.05, 0.06)	(-0.09, 0.02)	(-0.07, 0.04)	**(-0.14, -0.01)**	**(-0.14, -0.01)**	**(-0.18, -0.05)**	**(-0.17, -0.03)**
	P-value	**0.003**	0.778	0.770	0.251	0.501	**0.033**	**0.034**	**0.001**	**0.004**
C18										
HC	Mean	0.79	0.68	0.72	0.73	0.70	-0.11	-0.07	-0.07	-0.09
	95% CI	(0.57, 1.02)	(0.46, 0.91)	(0.50, 0.95)	(0.50, 0.95)	(0.47, 0.92)	(-0.39, 0.18)	(-0.36, 0.21)	(-0.35, 0.22)	(-0.38, 0.19)
	P-value						0.446	0.615	0.647	0.515
LC	Mean	0.68	1.22	0.98	1.00	0.81	**0.54**	**0.30**	**0.32**	0.14
	95% CI	(0.44, 0.91)	(0.98, 1.45)	(0.74, 1.21)	(0.76, 1.23)	(0.58, 1.05)	**(0.24, 0.84)**	**(0.00, 0.60)**	**(0.02, 0.62)**	(-0.16, 0.43)
	P-value						**0.001**	**0.049**	**0.036**	0.360
Δ HC – LC[b]	Mean	0.12	**-0.53**	-0.26	-0.27	-0.12	**-0.65**	-0.37	-0.38	-0.23
	95% CI	(-0.21, 0.45)	**(-0.86, -0.20)**	(-0.59, 0.08)	(-0.60, 0.06)	(-0.45, 0.22)	**(-1.06, -0.24)**	(-0.78, 0.04)	(-0.80, 0.03)	(-0.64, 0.18)
	P-value	0.488	**0.002**	0.129	0.110	0.490	**0.002**	0.077	0.067	0.267

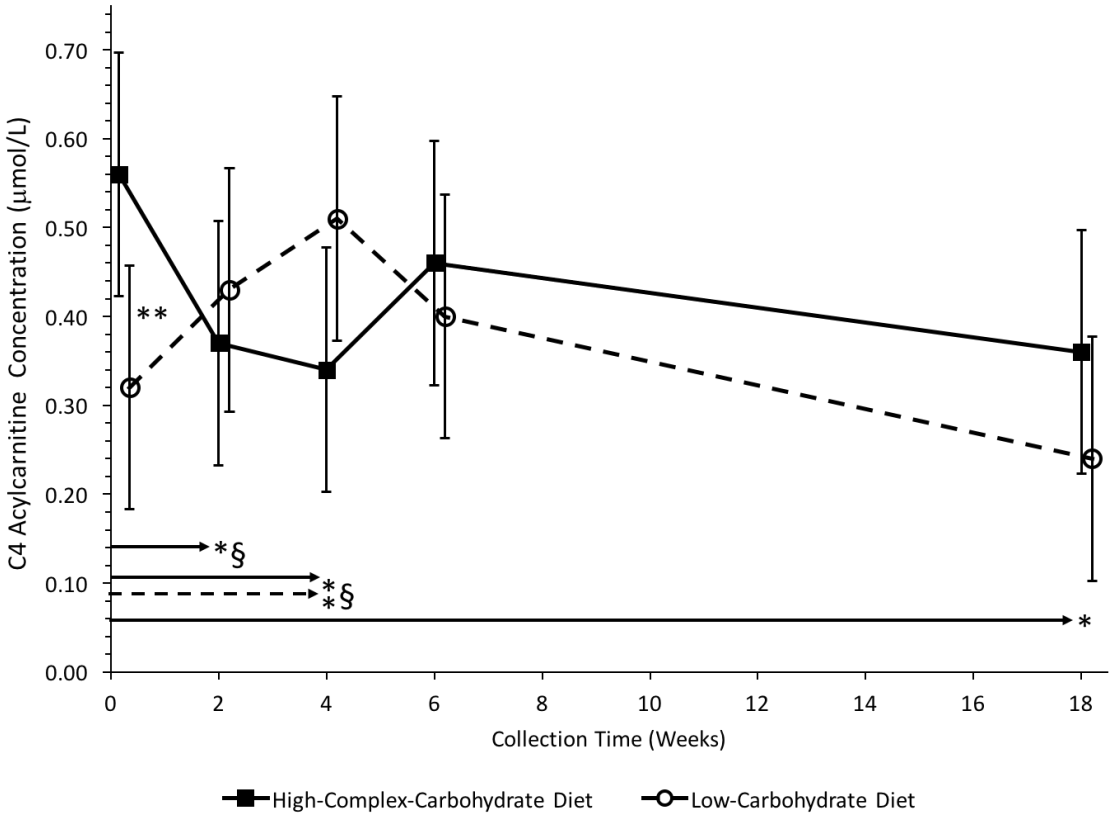
Abbreviations: CI, confidence interval; HC, high-complex-carbohydrate diet; LC, low-carbohydrate diet; n/a, not applicable; Δ, change.

All effects were tested using mixed models. Values represent observed mean, 95% confidence interval, and p-value. Each mean and 95% confidence interval is based on n=12 (HC) or n=11 (LC). [a] Values represent the mean difference from baseline within diet group. [b] Values represent the between diet group difference in means (high-complex-carbohydrate diet group mean minus low-carbohydrate diet group mean). **Bolded** values are significant at p<0.05.

Table 10A and Figure 9 display the within and between dietary group changes in C4 acylcarnitine concentration over time. The only significant difference in C4 acylcarnitine concentration at any time point between dietary groups during the study was at baseline, where the high-complex-carbohydrate dietary group had an average C4 acylcarnitine concentration that was 0.24 (95% CI: 0.05, 0.44; p=0.014) μmol/L higher than the low-carbohydrate dietary group (0.56 ± 0.27 μmol/L versus 0.32 ± 0.23 μmol/L at baseline). The high-complex-carbohydrate dietary group experienced significant changes in C4 acylcarnitine concentration at weeks 2, 4, and 18 compared to baseline but not at week 6 (p=0.233). While the low-carbohydrate dietary group experienced a significant change in C4 acylcarnitine concentration at week 4 compared to baseline but not at weeks 2 (p=0.197), 6 (p=0.330), or 18 (p=0.376). Participants consuming a high-complex-carbohydrate diet displayed an average 0.19 (95% CI: -0.36, -0.02; p=0.025) μmol/L reduction in mean C4 acylcarnitine concentration at week 2, an average 0.22 (95% CI: -0.38, -0.05; p=0.012) μmol/L reduction at week 4, and an average 0.21 (95% CI: -0.37, -0.04; p=0.016) μmol/L reduction at week 18 compared to baseline. In contrast, participants consuming a low-carbohydrate diet only experienced an average 0.19 (95% CI: 0.02, 0.36; p=0.034) μmol/L increase at week 4 compared to baseline.

The only significant differences between dietary groups in C4 acylcarnitine concentration at each time point compared to baseline were observed at weeks 2 and 4 but not at 6 (p=0.127) or 18 (p=0.296). The high-complex-carbohydrate dietary group displayed an average change in C4 acylcarnitine concentration from baseline to week 2 that was 0.31 (95% CI: -0.55, -0.06; p=0.014) μmol/L lower than the low-carbohydrate dietary group and average change in C4 acylcarnitine concentration from baseline to week 4 that was 0.41 (95% CI: -0.65, -0.17; p=0.001) μmol/L lower than the low-carbohydrate dietary group.

Figure 9. C4 acylcarnitine concentration (µmol/L) at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.



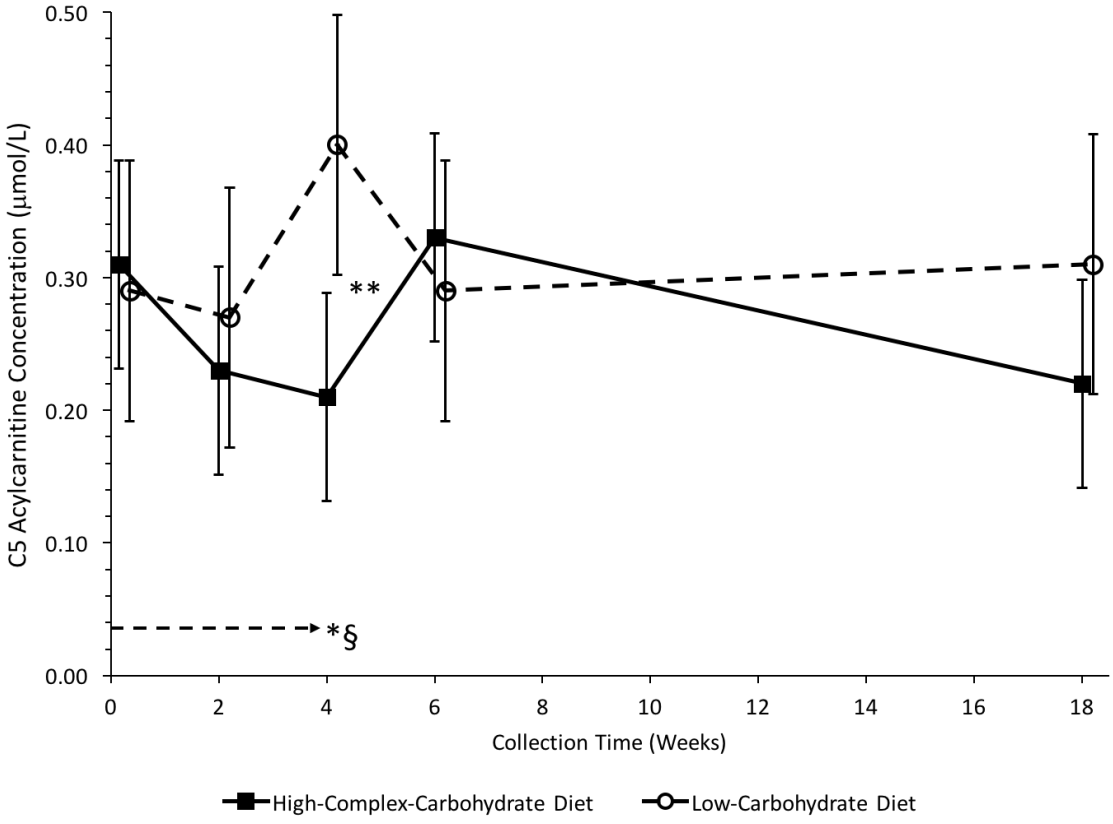
All effects were tested using mixed models. Values represent observed mean, 95% confidence interval, and p-value for low-carbohydrate (dotted grey) and high-complex-carbohydrate (dotted black) diet groups. Each mean is based on n=12 (High-complex-carbohydrate diet group) or n=11 (Low-carbohydrate diet group). All between group differences were computed by subtracting the low-carbohydrate diet group mean from the high-complex-carbohydrate diet group mean. All within group differences were computed by subtracting mean baseline values from the mean at each time point. (e.g. Week 6 – Baseline).

* C4 acylcarnitine concentration is significantly different from baseline within the specified diet group and at the specified time point at a 0.05 level of significance. ** C4 acylcarnitine concentration is significantly different between diet groups at the specified time point at a 0.05 level of significance. § The difference in C4 acylcarnitine concentration at the specified time point from baseline is significantly different between diet groups at a 0.05 level of significance.

Table 10A and Figure 10 display the within and between dietary group changes in C5 acylcarnitine concentration over time. The only significant difference in C5 acylcarnitine concentration between dietary groups at any time point during the study was at week 4, where the high-complex-carbohydrate dietary group had an average C5 acylcarnitine concentration that was 0.19 (95% CI: -0.32, -0.07; p=0.003) μmol/L lower than the low-carbohydrate dietary group (0.21 ± 0.07 μmol/L versus 0.40 ± 0.22 μmol/L at week 4). The high-complex-carbohydrate dietary group did not experience any significant changes in C5 acylcarnitine concentration at weeks 2 (p=0.134), 4 (p=0.068), 6 (p=0.681), or 18 (p=0.115). However, the low-carbohydrate dietary group experienced a significant change in C5 acylcarnitine concentration at week 4 compared to baseline but not at weeks 2 (p=0.693), 6 (p=0.932), or 18 (p=0.712). On average, participants consuming a low-carbohydrate diet experienced a 0.11 (95% CI: 0.00, 0.23 μmol/L; p=0.047) μmol/L increase in C5 acylcarnitine concentration at week 4 compared to baseline.

The only significant difference in C5 acylcarnitine concentration between dietary groups at each time point compared to baseline was at week 4, where the high-complex-carbohydrate dietary group displayed an average change in C5 acylcarnitine concentration from baseline to week 4 that was 0.21 (95% CI: -0.37, -0.06; p=0.008) μmol/L lower than the low-carbohydrate dietary group. No significant differences between dietary groups in C5 acylcarnitine concentration at each time point compared to baseline were observed at weeks 2 (p=0.449), 6 (p=0.824) or 18 (p=0.175).

Figure 10. C5 acylcarnitine concentration (µmol/L) at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.



All effects were tested using mixed models. Values represent observed mean, 95% confidence interval, and p-value for low-carbohydrate (dotted grey) and high-complex-carbohydrate (dotted black) diet groups. Each mean is based on n=12 (High-complex-carbohydrate diet group) or n=11 (Low-carbohydrate diet group). All between group differences were computed by subtracting the low-carbohydrate diet group mean from the high-complex-carbohydrate diet group mean. All within group differences were computed by subtracting mean baseline values from the mean at each time point. (e.g. Week 6 – Baseline).

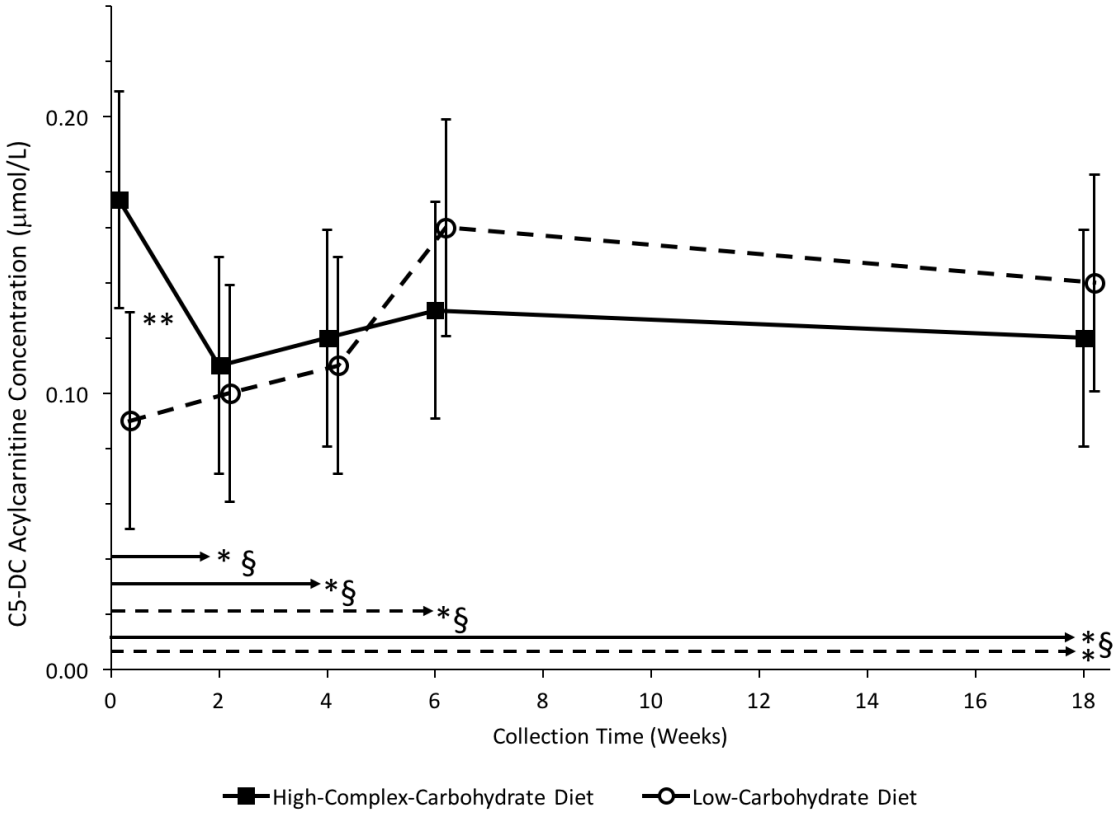
* C5 acylcarnitine concentration is significantly different from baseline within the specified diet group and at the specified time point at a 0.05 level of significance. ** C5 acylcarnitine concentration is significantly different between diet groups at the specified time point at a 0.05 level of significance. § The difference in C5 acylcarnitine concentration at the specified time point from baseline is significantly different between diet groups at a 0.05 level of significance.

Table 10B and Figure 11 display the within and between dietary group changes in C5-DC acylcarnitine concentrations over time. The only significant difference in C5-DC acylcarnitine concentration between dietary groups at any time point during the study was at baseline, where the high-complex-carbohydrate dietary group had an average C5-DC acylcarnitine concentration that was 0.08 (95% CI: 0.03, 0.14; p=0.003) μmol/L higher than the low-carbohydrate dietary group (0.17 ± 0.08 μmol/L versus 0.09 ± 0.03 μmol/L at baseline). The high-complex-carbohydrate dietary group experienced significant changes in C5-DC acylcarnitine concentration at weeks 2, 4, and 18 compared to baseline but not at week 6 (p=0.072). While the low-carbohydrate dietary group experienced a significant change in C5-DC acylcarnitine concentration at weeks 6 and 18 compared to baseline but not at weeks 2 (p=0.575) or 4 (p=0.414). Participants consuming a high-complex-carbohydrate diet displayed an average 0.06 (95% CI: -0.11, -0.01; p=0.013) μmol/L reduction in mean C5-DC acylcarnitine concentration at week 2, an average 0.05 (95% CI: -0.10, -0.01; p=0.026) μmol/L reduction at week 4, and an average 0.05 (95% CI: -0.10, 0.00; p=0.035) μmol/L reduction at week 18 compared to baseline. Whereas, participants consuming a low-carbohydrate diet experienced an average 0.07 (95% CI: 0.02, 0.12; p=0.006) μmol/L increase in mean C5-DC acylcarnitine concentration at week 6 and an average 0.05 (95% CI: 0.00, 0.10 μmol/L; p=0.049) μmol/L increase at week 18 compared to baseline.

There were significant differences between dietary groups in C5-DC acylcarnitine concentration at each time point compared to baseline at weeks 2, 4, 6, and 18. The high-complex-carbohydrate dietary group displayed an average reduction in C5-DC acylcarnitine concentration from baseline to week 2 that was 0.07 (95% CI: -0.14, -0.01; p=0.033) μmol/L lower than the low-carbohydrate dietary group and an average reduction from baseline to week 4 that was also 0.07 (95% CI: -0.14, -0.01; p=0.034) μmol/L lower than the low-carbohydrate

diet. Furthermore, the average reduction from baseline to week 6 was 0.11 (95% CI: -0.18, -0.05; p=0.001) µmol/L lower than the low-carbohydrate diet and an average reduction from baseline to week 18 that was 0.10 (95% CI: -0.17, -0.03; p=0.004) µmol/L lower than the low-carbohydrate dietary group.

Figure 11. C5-DC acylcarnitine concentration (µmol/L) at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.



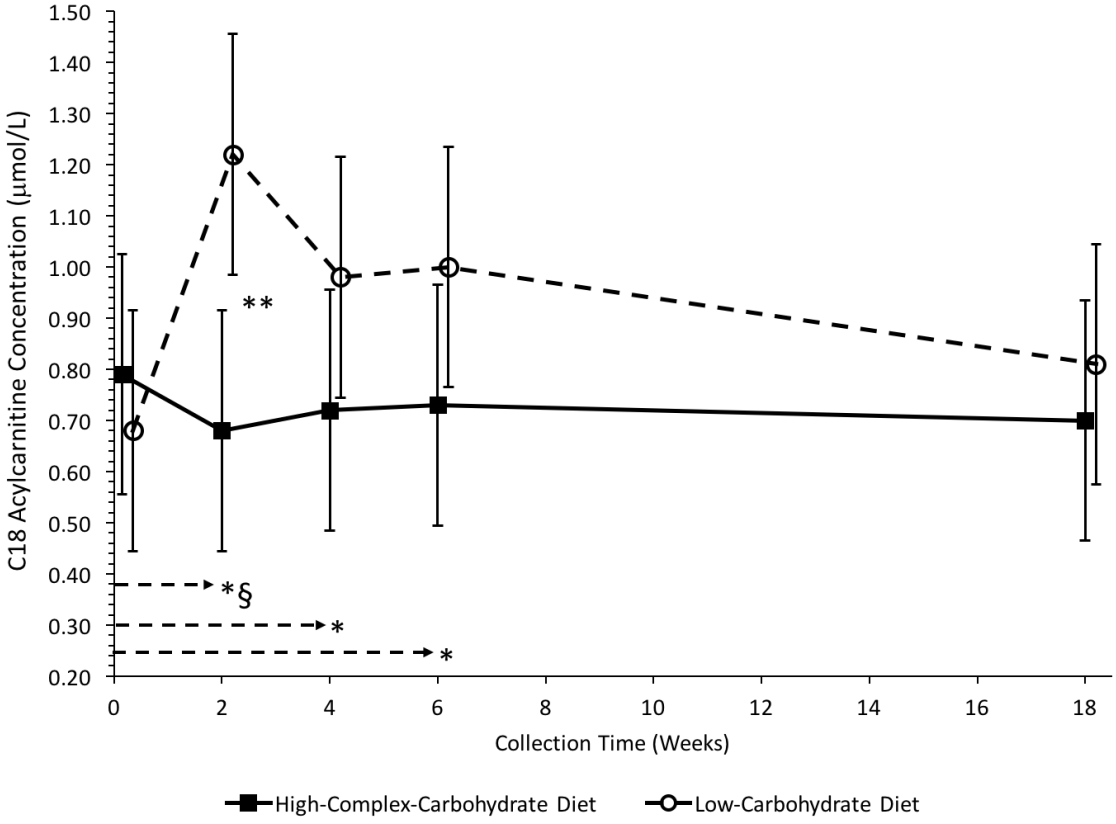
All effects were tested using mixed models. Values represent observed mean, 95% confidence interval, and p-value for low-carbohydrate (dotted grey) and high-complex-carbohydrate (dotted black) diet groups. Each mean is based on n=12 (High-complex-carbohydrate diet group) or n=11 (Low-carbohydrate diet group). All between group differences were computed by subtracting the low-carbohydrate diet group mean from the high-complex-carbohydrate diet group mean. All within group differences were computed by subtracting mean baseline values from the mean at each time point. (e.g. Week 6 – Baseline).

* C5-DC acylcarnitine concentration is significantly different from baseline within the specified diet group and at the specified time point at a 0.05 level of significance. ** C5-DC acylcarnitine concentration is significantly different between diet groups at the specified time point at a 0.05 level of significance. § The difference in C5-DC acylcarnitine concentration at the specified time point from baseline is significantly different between diet groups at a 0.05 level of significance.

Table 10B and Figure 12 display the within and between dietary group changes in C18 acylcarnitine concentrations over time. The only significant difference in C18 acylcarnitine concentration between dietary groups at any time point during the study was at week 2, where the high-complex-carbohydrate dietary group had an average C18 acylcarnitine concentration that was 0.53 (95% CI: -0.86, -0.20; p=0.002) µmol/L lower than the low-carbohydrate dietary group (0.68 ± 0.29 µmol/L versus 1.22 ± 0.60 µmol/L at week 2). The high-complex-carbohydrate dietary group did not experience any significant changes in C18 acylcarnitine concentration at weeks 2 (p=0.446), 4 (p=0.615), 6 (p=0.647), or 18 (p=0.515). Whereas, the low-carbohydrate dietary group experienced significant changes in C18 acylcarnitine concentration at weeks 2, 4, and 6 compared to baseline but not at week 18 (p=0.360). On average, participants consuming a low-carbohydrate diet experienced a 0.54 (95% CI: 0.24, 0.84; p=0.001) µmol/L increase in mean C18 acylcarnitine concentration at week 2, a 0.30 (95% CI: 0.00, 0.60; p=0.049) µmol/L increase at week 4, and an average 0.32 (95% CI: 0.02, 0.62; p=0.036) µmol/L increase at week 6 compared to baseline.

The only significant difference between dietary groups in C18 acylcarnitine concentration at each time point compared to baseline was at week 2, where the high-complex-carbohydrate dietary group displayed an average change in C18 acylcarnitine concentration from baseline to week 2 that was 0.65 (95% CI: -1.06, -0.24; p=0.002) µmol/L lower than the low-carbohydrate dietary group. No significant between dietary group differences in C18 acylcarnitine concentration at each time point compared to baseline were observed at weeks 4 (p=0.077), 6 (p=0.067) or 18 (p=0.267).

Figure 12. C18 acylcarnitine concentration (µmol/L) at baseline and after 2, 4, and 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention.



All effects were tested using mixed models. Values represent observed mean, 95% confidence interval, and p-value for low-carbohydrate (dotted grey) and high-complex-carbohydrate (dotted black) diet groups. Each mean is based on n=12 (High-complex-carbohydrate diet group) or n=11 (Low-carbohydrate diet group). All between group differences were computed by subtracting the low-carbohydrate diet group mean from the high-complex-carbohydrate diet group mean. All within group differences were computed by subtracting mean baseline values from the mean at each time point. (e.g. Week 6 – Baseline).

* C18 acylcarnitine concentration is significantly different from baseline within the specified diet group and at the specified time point at a 0.05 level of significance. ** C18 acylcarnitine concentration is significantly different between diet groups at the specified time point at a 0.05 level of significance. § The difference in C18 acylcarnitine concentration at the specified time point from baseline is significantly different between diet groups at a 0.05 level of significance.

Linear Regression Models

Association Between Change in Lean Mass (kg) and Acylcarnitine Concentrations

Linear regression analysis was performed to test whether an association exists between change in lean mass (kg) and change in the 4 acylcarnitine species (C4, C5, C5-DC, and C18 acylcarnitines) that displayed the interaction between diet and time. Models focused on change in lean mass (kg) and changes in acylcarnitine concentrations during the controlled feeding intervention phase of the study (baseline to week 6). Figure 13 represents the linear associations between change in lean mass and change in C4 (Panel A), C5 (Panel B), C5-DC (Panel C), and C18 (Panel D) acylcarnitine concentrations from baseline to week 6 according to dietary group.

There was no significant indication that change in C4 (p=0.300), C5 (p=0.997), or C18 (p=0.258) acylcarnitine concentrations were influenced by change in lean mass, diet, or the interaction between change in lean mass and diet from baseline to week 6. Since there was no influence due to diet, change in lean mass, or their interaction on C4, C5, or C18 acylcarnitine concentrations, the best estimate of the change in acylcarnitine concentration was represented by an overall average of all the data in both groups combined (n=23). The estimated mean change for C4 acylcarnitine concentration, regardless of loss of lean mass or dietary group, was -0.01 (95% CI: -0.14, 0.12) μmol/L, 0.01 (95% CI: -0.06, 0.09) μmol/L for C5 acylcarnitine concentration, and 0.12 (95% CI: -0.08, 0.32) μmol/L for C18 acylcarnitine concentration.

Only change in C5-DC acylcarnitine concentration displayed a significant influence of diet alone (p=0.010) and not change in lean mass (either as a simple effect or an interaction between diet and change in lean mass; p=0.468). Although not statistically significant, participants who consumed a high-complex-carbohydrate diet experienced an average 0.04 (95% CI: -0.10, 0.01; p=0.101) μmol/L reduction in mean C5-DC acylcarnitine concentration. Yet,

participants who consumed a low-carbohydrate diet experienced a significant average 0.07 (95% CI: 0.02, 0.13; p=0.015) μmol/L increase in mean C5-DC acylcarnitine concentration. This separation between dietary groups with respect to the average change in C5-DC acylcarnitine concentration (0.07 μmol/L versus -0.04 μmol/L) is significant, with the average change for the high-complex-carbohydrate dietary group estimated to be 0.11 (95% CI: 0.04, 0.19; p=0.005) μmol/L lower than the average change for the low-carbohydrate dietary group.

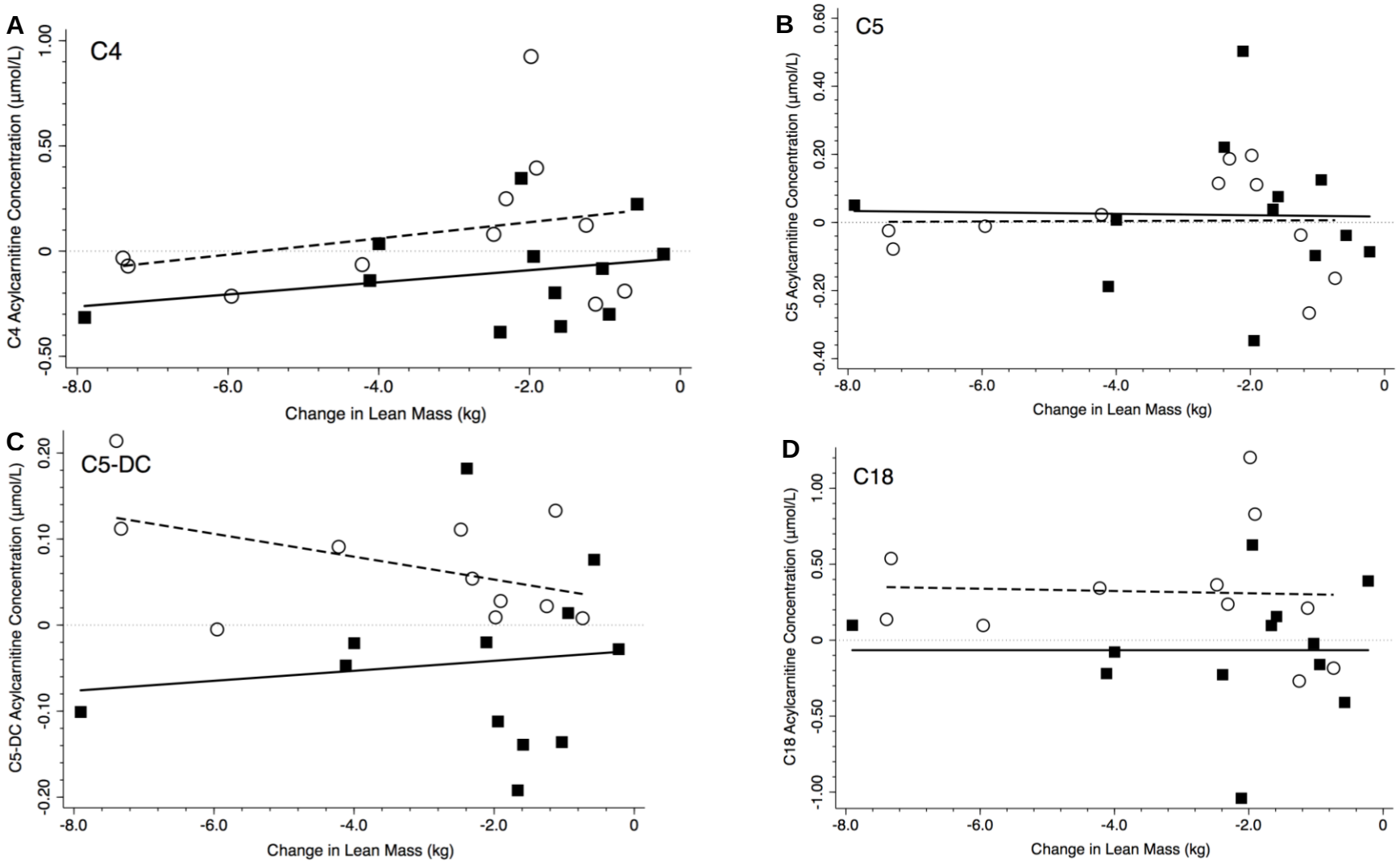


Figure 13. Association between change in lean mass (kg) and change in C4 (Panel A), C5 (Panel B), C5-DC (Panel C), and C18 (Panel D) acylcarnitine concentrations (µmol/L) after 6-weeks of controlled dietary intervention.

Participants consumed either a high-complex-carbohydrate diet (——■——) or a low-carbohydrate diet (– – ○ – –) for 6-weeks.

Association Between Change in Fat Mass (kg) and Acylcarnitine Concentrations

Linear regression analysis was also performed to test whether an association exists between change in fat mass (kg) and the 4 acylcarnitine species (C4, C5, C5-DC, and C18 acylcarnitines) that displayed an effect of diet, time, and their interaction. Similar to change in lean mass, linear regression analysis was only performed for changes in fat mass (kg) and changes in acylcarnitine concentrations during the controlled feeding intervention phase of the study (baseline to week 6). Figure 14 represents the linear associations between change in fat mass and change in C4 (Panel A), C5 (Panel B), C5-DC (Panel C), and C18 (Panel D) acylcarnitine concentrations from baseline to week 6 according to dietary group.

Change in fat mass was associated with change in C4 acylcarnitine concentration for the low-carbohydrate dietary group and not the high-complex-carbohydrate dietary group. There was a significant overall effect of change in fat mass, diet, or their interaction from baseline to week 6 (p=0.060), with a significant interaction indicating that change in fat mass is modified by diet (p=0.071). The average change in C4 acylcarnitine concentration for participants in the low-carbohydrate dietary group was estimated to decrease 0.16 (95% CI: -0.02, -0.30; p=0.030) μmol/L for each 1 kg loss of fat mass. There was no significant association between change in fat mass and change in C4 acylcarnitine concentration in the high-complex-carbohydrate dietary group (p=0.528).

Change in fat mass was associated with change in C18 acylcarnitine concentration in both dietary groups. Although there was a significant effect of diet (p=0.031) and change in fat mass (p=0.050), there was no significant interaction between dietary group and change in fat mass (p=0.542), which demonstrates that both the low-and high-complex-carbohydrate dietary groups exhibit a similar trend in change in C18 acylcarnitine concentration as a function of

change in fat mass. Regardless of dietary group, each 1 kg loss of fat mass was associated with a 0.20 (95% CI: -0.04, -0.36 μmol/L; p=0.016) μmol/L decrease in C18 acylcarnitine concentrations.

There was a significant overall effect of change in fat mass, diet, or their interaction on C5-DC acylcarnitine concentrations from baseline to week 6 (p=0.031). However, no significant effect of change in fat mass and diet was observed (p=0.651), thus indicating that change in fat mass is not modified by diet. Change in C5-DC acylcarnitine concentrations displayed a significant influence of diet alone (p=0.004) but not change in fat mass (either as a main effect or an interaction between diet and change in fat mass; p=0.467). The effect of consuming a high-complex-carbohydrate or low-carbohydrate diet on change in mean C5-DC acylcarnitine concentrations was described in the previous section.

There was no significant indication that change in C5 (p=0.518) acylcarnitine concentration was influenced by change in fat mass, diet, or the interaction between change in fat mass and diet from baseline to week 6. Since there was no influence due to diet, change in fat mass, or the interaction between change in fat mass and diet on C5 acylcarnitine concentration, the best estimate of the overall average change in C5 acylcarnitine concentration from all data (n=23) was 0.01 (95% CI: -0.06, 0.09) μmol/L.

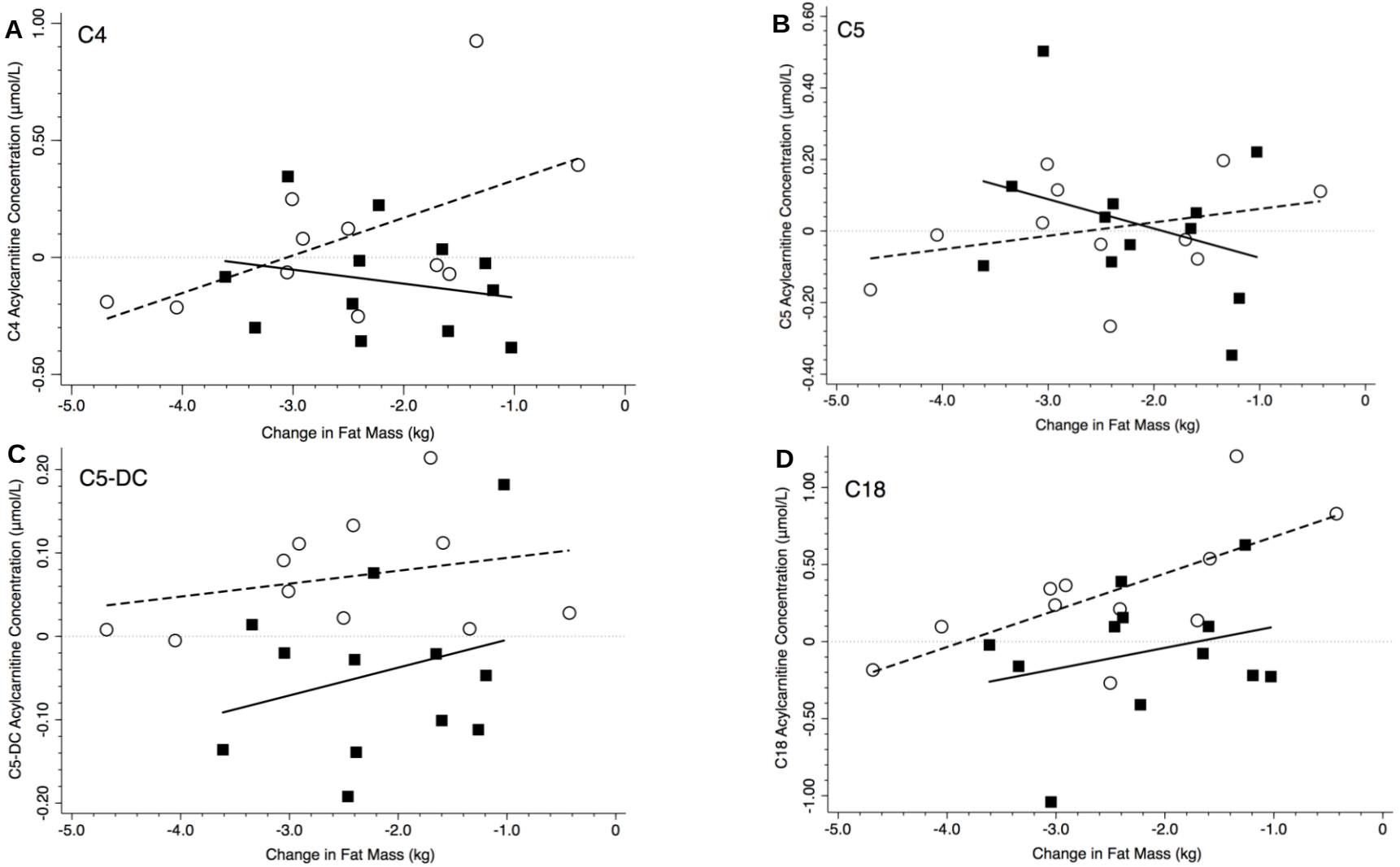


Figure 14. Association between change in fat mass (kg) and change in C4 (Panel A), C5 (Panel B), C5-DC (Panel C), and C18 (Panel D) acylcarnitine concentrations (µmol/L) after 6-weeks of controlled dietary intervention.

Participants consumed either a high-complex-carbohydrate diet (——■——) or a low-carbohydrate diet (– – ○ – –) for 6-weeks.

CHAPTER 5: DISCUSSION

Summary

This secondary analysis of healthy, free-living adults with overweight and obesity during a controlled metabolic feeding study was performed to determine the impact of a low-or high-complex-carbohydrate diet, and the resulting loss of body mass, on change in circulating acylcarnitine concentrations. Twenty-five participants completed the study that included a 6-week controlled dietary intervention and a 12-week home dietary intervention phase, of which 23 participants had complete sets of carnitine and body composition data. Eleven participants were allocated to an ad libitum low-carbohydrate diet similar to the induction phase of the Atkins dietary pattern developed by Dr. Robert Atkins. Twelve participants were allocated to an energy-matched high-complex-carbohydrate, low-fat diet similar to the DASH dietary pattern. Fasting blood samples were obtained before and 2, 4, and 6-weeks after initiating the controlled dietary intervention phase and after the 12-week home dietary intervention phase at week 18. Body composition (body mass, fat mass, and fat-free mass) was assessed at baseline and at weeks 6 and 18. The primary objective of this secondary analysis was to provide preliminary and descriptive data for future statistically powered, controlled dietary intervention studies evaluating the impact of low-and high-complex-carbohydrate diets on change in body mass, fat mass, lean mass, and individual acylcarnitine concentrations.

Low-and High-Complex-Carbohydrate Diets and Changes in Body Composition

Numerous studies have displayed the effectiveness of ad libitum and energy-restricted low-and high-complex-carbohydrate diets for loss of body weight and fat mass. High-complex-carbohydrate diets are associated with greater weight loss compared to both high-simple-carbohydrate[56,57] and control diets.[55-57] Low-carbohydrate diets have been found to induce greater initial weight loss compared to other dietary intervention.[43,52-54] Our analysis

demonstrated that adults with overweight and obesity who consumed the same relative energy intake as a proportion of their estimated energy requirement for weight maintenance, lost similar amounts of body mass, fat mass, and lean mass regardless of their assigned dietary intervention group (low-or high-complex-carbohydrate diets).

Meckling et al.[62] performed a randomized controlled trial among 31 healthy male and female participants with overweight and obesity (mean age 42 years old, mean BMI 32.3 kg/m^2), where participants either received an energy-restricted low-carbohydrate diet or an energy-matched low-fat diet for 10-weeks. Similar to our study, there was no difference in the pattern of weight or fat loss over time between the two dietary intervention groups ($p>0.05$ for between group differences). Participants in the low-fat dietary group lost an average 6.8 kg (no SD reported) of body weight and 5.4 kg (no SD reported) fat mass, compared to 7.0 kg body weight and 4.1 kg (no SD reported) fat mass among participants in the low-carbohydrate dietary group ($p<0.05$ compared to baseline within each dietary group).[62] In contrast to our study, the low-carbohydrate dietary group experienced a significant decrease [-1.9 kg (no SD reported), $p<0.05$ compared to baseline] in lean mass but not the low-fat dietary group [-1.0 kg (no SD), $p>0.05$ compared to baseline]. Although participants in the low-carbohydrate dietary group lost more lean mass than those in the high-complex-carbohydrate dietary group in our study, there was no significant difference between dietary groups. Our results were also similar to the findings of Sacks et al.,[58] Stern et al.,[61] and Noakes et al.,[67] which all found similar changes in body composition between participants within the dietary interventions that they studied.

Participants in our study lost more body mass after the 6-week controlled dietary intervention (mean body mass loss of 5.17 ± 2.28 kg among all participants pooled together compared to baseline) than after the 18-week combined dietary intervention (mean body mass loss of 6.88 ± 3.72 kg among all participants). These results are reasonable since food choice and

energy intake were not controlled during the home dietary intervention phase, thus leaving more opportunity for inconsistent dietary adherence among participants. Other studies have also reported greater weight loss during the initial phase of dietary intervention[58,59] of randomized controlled trials with weight regain during follow-up post-dietary intervention.[58]

Low-and High-Complex-Carbohydrate Diets and Change in Acylcarnitine Concentrations without Body Composition Changes

Few human or animal studies have tested the effect of a low-or-high-complex-carbohydrate diet on circulating acylcarnitine concentrations. The majority of these studies report change in free carnitine, total carnitine, or total acylcarnitine concentrations in serum[70,71] or plasma[68] but not the change in individual acylcarnitine concentrations. Our study did not analyze total-or free-carnitine concentrations but did analyze the concentrations of 21 individual acylcarnitines in whole blood samples measured using tandem mass spectrometry. The samples were collected at multiple time points from healthy male and female participants with overweight and obesity consuming energy-matched low-or-high-complex-carbohydrate diets. We observed that only 4 acylcarnitine species (C4, C5, C5-DC, and C18 acylcarnitines) were affected by dietary intervention, time, and the interaction between diet and time (diet:time). The C2, C3, C10, C16, and C18:1 acylcarnitine concentrations from participants in both dietary groups displayed an overall effect of time ($p<0.10$) that was seen by a fluctuation in individual acylcarnitine concentrations over time. However, each of these individual acylcarnitines' pattern of change during the study did not display an effect of diet; meaning that each acylcarnitine's concentration was not significantly different between diet groups ($p>0.10$). The other 12 acylcarnitines measured (C3-DC, C4-DC, C5-OH, C5:1, C6, C6-DC, C8, C10:1, C12, C14, C14:1, C16-OH) did not show an overall effect of time, diet, or their interaction and thus were not included in subsequent analyses.

There are no studies published to this date that we are aware of that describe the effect of energy-matched low-and high-complex-carbohydrate dietary interventions on individual short-, medium-, or long-chain acylcarnitine concentrations. In 1990, Davis et al.[69] published results from a controlled feeding study that described the effect of two hypoenergetic, very-low-carbohydrate diets with varying carnitine content on plasma total- and free-carnitine concentrations, as well as total-short-chain and total-long-chain acylcarnitine concentrations using a radioisotopic assay. This study sample included 10 female adults with obesity of whom half were allocated to consume a very-low-carbohydrate, low-fat liquid formula supplying 420 kcals of energy/day (70 gms of protein, 30 gms of carbohydrate, 4.4 μmol carnitine, negligible amount of fat). The other half consumed a very-low-carbohydrate diet supplying 500-600 kcals of energy/day from mainly lean meat, fish, or poultry (70% of total energy from protein, <10 gms carbohydrate, 30-40% of total energy from fat, ~375 μmol carnitine) for 2-months. Total plasma short-chain-acylcarnitine concentration significantly increased in both low-energy, low-carbohydrate dietary interventions ($p<0.05$), which could be possibly explained by the increased protein-content of both diets. C3, C5, and other short-chain acylcarnitines can be produced from the conversion of amino acids such as lysine, tryptophan, valine, leucine, and isoleucine.[24] Thus, short-chain acylcarnitine concentrations may be increased over time when the primary source of energy is protein, amino acids from endogenous protein metabolism, or from breakdown of lean tissue. Based on these findings by Davis et al.,[69] we would expect to see an increase in the concentration of short-chain acylcarnitines (C2, C3, C3-DC, C4, C4-DC, C5, C5-OH, C5-DC, C5:1) in the low-carbohydrate dietary group of our sample population, especially during the 6-week controlled dietary intervention phase. However, the only short-chain acylcarnitines that were affected by diet within both dietary interventions of our study were C4, C5, and C5-DC acylcarnitine concentrations. In the low-carbohydrate dietary group, both C4 and C5

acylcarnitine concentrations peaked at week 4 and exhibited significant differences in mean concentrations at week 4 compared to baseline. The only significant difference between dietary groups was observed for C5 acylcarnitine concentration at week 4. C5-DC acylcarnitine displayed a mean peak concentration at week 6 and demonstrated a significant difference in concentration at weeks 6 and 18 compared to baseline with no between dietary group differences at any time point.

Davis et al.[69] also found that neither low-carbohydrate dietary intervention resulted in a significant effect on total plasma long-chain acylcarnitine concentration, which is most likely due to the low intake of fat in both dietary groups (~17-27 gms/day). Our study only found the long-chain C18 acylcarnitine to be affected by diet. In the low-carbohydrate dietary group, the mean peak C18 acylcarnitine concentration occurred at week 2. However, there was a significant increase in C18 acylcarnitine concentration compared to baseline at weeks 2, 4, and 6 during the controlled dietary intervention phase. These findings are consistent with Kien et al.,[90] which demonstrated that even a 1-week alteration in dietary fatty acid composition produced corresponding changes in several long-chain acylcarnitine concentrations. C16 and C18:1 acylcarnitine concentrations are reported to increase with increased fatty acid loads coming from diet,[62] which can be explained by the higher dietary fat content in the low-carbohydrate dietary intervention. Our analyses also showed a significant difference in acylcarnitine concentrations between dietary groups at week 2, indicating that the higher fat intake during the low-carbohydrate dietary group may be responsible for the overall increase from baseline and the between group difference.

In 2015, Mathew et al.[72] published results from a secondary analysis of acylcarnitine concentrations collected during a controlled feeding study analyzing the effect of a sodium-restricted DASH diet on serum short-, medium-, and long-chain acylcarnitine concentrations

using targeted liquid chromatography-mass spectrometry. This study sample included 13 male and female older adults with obesity, treated hypertension, and stable heart failure with preserved ejection fraction who received a sodium-restricted DASH diet for a 3-week intervention. The majority of acylcarnitine concentrations increased non-significantly over the dietary intervention (C5, C5-DC, C6, C8, C12-OH, C14, 14:1, C14-OH, C16:1, C16:2, C18:1, C18:2-OH, C20, C20:1), except for C8:1, 10:1, C12, C12:1, C14:2, C16, C16-OH, C18, C18:2, C20:2, C20:3, and C20:4, which all decreased non-significantly. The only statistically significant change from baseline to week 3 was an increase in C2, C3, C4 ($p<0.03$ for each), and C10 acylcarnitine concentrations ($p=0.04$).[72] Our study did not find any effect of dietary intervention on C2, C3, or C10 acylcarnitines, although these acylcarnitines did significantly change over time regardless of dietary group and tended to display an upward trend during the study. Similar to Mathew et al.,[72] we also found a non-significant decrease in mean C16 acylcarnitine concentrations over time, but this trend was not significantly affected by a specific dietary intervention. Although our study did find C4 acylcarnitine concentration to be one of the 4 acylcarnitines affected by a high-complex-carbohydrate dietary intervention, we observed C4 acylcarnitine concentrations to decrease during the dietary intervention and remained below its initial baseline concentration throughout the study. In our study, C4 and C5 acylcarnitine concentrations both experienced their greatest decrease by week 4. However, significant within dietary group differences from baseline was only observed at weeks 2, 4, and 18 for C4 acylcarnitine concentration and not at any time point for C5 acylcarnitine concentration. Our study also found C5-DC acylcarnitine concentration to reach its lowest concentration at week 2, with significant within dietary group differences at weeks 2, 4, and 18 compared to baseline. Mathew et al.[72] reported a non-significant decrease in C18 acylcarnitine concentration by week 3. Our study also observed a non-significant decrease in C18 acylcarnitine concentration by week 2 that was

significantly different than the low-carbohydrate dietary group. However, C18 acylcarnitine in the high-complex-carbohydrate dietary group did not display any within dietary group differences at any time point during the study.

Low-and High-Complex-Carbohydrate Diets and Change in Acylcarnitine Concentrations with Body Composition Changes

Our secondary objective was to describe the relationship between changes in lean mass and fat mass with change in individual acylcarnitine concentrations during the 6-week controlled dietary intervention phase. Individuals lose varying proportions of both lean and fat mass during weight loss, and since 97% of carnitine stores are found in skeletal muscle mass,[5] we hypothesized that fasting acylcarnitine concentrations would be positively correlated with loss of lean mass. Furthermore, we predicted that that this association would be strongest among participants in the ad libitum low-carbohydrate dietary group compared to the energy-matched high-complex-carbohydrate dietary group since individuals on low-carbohydrate diets typically experience rapid weight loss within the first few months of dietary intervention.[54] This hypothesis was in part postulated from the descriptive findings of adults with overweight and obesity without diabetes by Ramos-Roman et al.,[77] who reported a positive association between lean mass and mean fasting plasma C3, C3-DC, C4, C5, C5:1, and C10:1 acylcarnitine concentrations and no association between fat mass and any acylcarnitine species at baseline.

Participants in both the low-and-high-complex-carbohydrate dietary intervention groups in our study sample lost similar amounts of body mass, fat mass, and lean mass. A study conducted by Schooneman et al.[80] found that a -600 kcals of energy restriction/day increased plasma carnitine concentrations after 12-weeks of intervention, with a significant correlation between a reduction in both total body mass and lean body mass, and increases in C4-OH, C16, and C18:1 acylcarnitine concentrations over time. In our study, we chose to only perform linear

regression analyses on the 4 acylcarnitines (C4, C5, C5-DC, C18 acylcarnitines) that displayed a significant effect of dietary intervention, time, and interaction between time:diet. Our previous analyses included C10, C14:1, C16, and C18:1 acylcarnitine concentrations, however, these acylcarnitines did not display any effect of dietary intervention so were not included in further modeling. In contrast to Schooneman et al.,[80] our linear regression analyses indicated that no association existed between change in lean mass and change in any of the 4 acylcarnitines (C4, C5, C5-DC, C18 acylcarnitines) that were affected by dietary intervention, time, and the interaction between time:diet. These results are different from what we anticipated, as we hypothesized that change in fasting acylcarnitine concentrations would be positively correlated with loss of lean mass, and the correlation would be stronger among participants in the low-carbohydrate dietary group than the high-complex-carbohydrate dietary group. Our linear regression analyses indicated that there was only an association between change in fat mass and change in C4 acylcarnitine concentration among participants on a low-carbohydrate diet, and an association between change in fat mass and change in C18 acylcarnitine concentration regardless of dietary intervention. Redman et al.[79] found a positive association between percent body fat and fasting serum medium-chain-(C6-DC, C8, C8:1, C10, C10:1, C10:2, C10:3, C12, C12:1) and long-chain-(C14:1,C14:1-OH, C14:2, C16:2) acylcarnitine concentrations at baseline (R^2=0.75, p=0.0001). However, using multiple linear regression analysis, Redman et al.[79] did not report any associations between change in percent fat mass and change in any acylcarnitines species, despite similar loss of body weight and fat mass in both intervention groups.

The majority of studies investigating the impact of body composition change on acylcarnitine concentrations involve interventions consisting of energy restriction,[78-81,86] gastric bypass-induced weight loss,[78] or medication-induced weight loss.[80] Only Reinehr et al.[84] and Lien et al.[81] have described changes in specific acylcarnitine concentrations in individuals with

obesity after a high-complex-carbohydrate dietary intervention resulting in weight loss. However, unlike our study, both Reinehr et al.[84] and Lien et al.[81] only reported change in specific acylcarnitine concentrations after weight loss but did not assess for an association between change in lean or fat mass and change in individual acylcarnitine concentrations. Similar to our study, Lien et al.[81] also conducted a prospective analysis of data collected from individuals with obesity consuming a high-complex-carbohydrate dietary intervention. Participants consumed a high-complex-carbohydrate diet for 12-months (~52-weeks), compared to 6-weeks of controlled dietary intervention and 12-weeks of home dietary intervention in our study. Lien et al.[81] measured plasma acylcarnitine concentrations at baseline and at weeks 2 and 4, and at 6 and 12-months, as well as change in body weight, fat mass, and lean mass at baseline, 6- and 12-months. However, only results for plasma C8:1 and C10:3 acylcarnitine concentrations were reported and Lien et al.[81] did not test whether an association existed with change in body composition and either acylcarnitine species. We measured C8, C10, and C10:1 acylcarnitine concentrations; however, we did not measure the C8:1 or C10:3 acylcarnitine concentrations included in the Lien et al.[81] analysis. Additionally, Reinehr et al.[84] only focused on change in 14 serum metabolites that were previously reported to be altered in children with obesity. Among these 14 metabolites, the only "altered" acylcarnitine concentrations assessed were C12:1 and C16:1 acylcarnitine concentrations, which we did not measure in our study. Although we did measure C12, C16, and C16-OH acylcarnitine concentrations, none of these acylcarnitines species were among the 4 acylcarnitine concentrations that were included in the linear regression analysis testing for an association between change in lean or fat mass and change in acylcarnitine concentrations.

To our knowledge, only Smith et al.[86] has described changes in specific acylcarnitine cocnentrations in individuals with obesity after a high-protein, lower-carbohydrate dietary

intervention resulting in weight loss. Yet, Smith et al.[86] only reported the sum of plasma C3 and C5 acylcarnitine concentrations and reported a non-significant ($p=0.11$) decrease of ~15% in their sum concentration after ~10% body weight loss in both energy-restricted dietary intervention groups (regular protein weight loss group: ~49% of energy from carbohydrate, ~29% of energy from fat, ~22% of energy from protein; high-protein weight loss group: ~43% of energy from carbohydrate, ~26% of energy from fat, ~31% of energy from protein). Both dietary interventions resulted in a significant decrease in body mass compared to the weight maintenance group ($p<0.05$). The contribution of fat-free mass to total body mass loss was ~45% less in the high-protein weight loss group compared to the weight loss group, although the amount preserved by the high-protein diet was only ~1.5% of total fat-free mass (~700 grams). In terms of fat mass, only intra-abdominal adipose tissue was reported. However, both dietary groups lost ~20% of their intra-abdominal adipose tissue post-dietary intervention, with no significant differences between dietary intervention groups.

Although body mass, fat-free mass, and percent body fat were assessed before and after the ~27-week dietary interventions, Smith et al.[86] did not test for an association between change in either fat mass or fat-free mass and change C3 and C5 acylcarnitine concentrations. In our study, C3 acylcarnitine concentration did show an overall effect of time, regardless of dietary intervention, but the concentration tended to fluctuate over time and was higher after dietary intervention compared to baseline. C5 acylcarnitine concentration displayed an overall effect of time and diet but only displayed a significant decrease in concentration at week 2 and remained greater than baseline thereafter. Gu et al.[85] also conducted a randomized controlled feeding trial evaluating the impact of an energy-restricted, low-carbohydrate, high-fat, high-protein diet on fasting carnitine species; however, only free carnitine and C2 acylcarnitine concentrations were described and no information regarding change in body composition was

reported. Overall, limited studies exist reporting change in body composition with low-and high-complex-carbohydrate dietary interventions, and fewer studies have tested for an association between change in body composition and change in individual acylcarnitine concentrations.

Strengths

The strengths of this secondary analysis include the use of data collected during a well-designed, tightly controlled feeding trial with a respectable sample size for this study design. A controlled feeding trial is considered to be the gold standard for producing high-quality evidence, as it maximizes participant adherence to dietary intervention and minimizes other confounding dietary habits.[91] Allocation of participants to the low-or high-complex-carbohydrate dietary groups were "balanced" according to age, sex, BMI, and fasting total cholesterol and triglyceride concentrations at baseline using a covariate adaption randomization procedure. Initial testing of these variables between dietary groups suggested no signifiant differences between groups and that the balancing was successful. The energy-matching protocol ensured that participants consumed the same average percent estimated energy requirement between dietary groups during the 6-week controlled dietary intervention, which further eliminated differentiation based on relative energy intake between dietary groups. All participants were considered "healthy" at baseline – free of major disease or other ailments controlled for by the inclusion and exclusion criteria. Blood samples were collected at multiple time points (baseline, week 2, 4, 6, and 18) and included concentrations for 21 acylcarnitines for each participant. Body mass, lean mass, and fat mass were collected at multiple time points (baseline, week 6 and 18) using DEXA, which is considered the gold standard for measuring body composition.

Mixed models were used to test both fixed and random effects of diet, time, and their interaction on specific acylcarnitine concentrations. Between and within dietary group effects

were also tested using mixed models instead of multiple paired and unpaired t-tests. The use of mixed models reduced the likelihood of commiting a type I statistical error and was more appropriate for this exploratory analysis compared to Analysis of Variance (ANOVA). This is because ANOVA could mask significant changes in specific acylcarnitine concentrations even with the use of post-hoc analysis.

Limitations

The limitations of this secondary analysis include the assumption that all participants adhered to their assigned dietary intervention during the 6-week controlled dietary intervention phase and the 12-week home intervention phase. Participants completed daily surveys to monitor adherance, which suggested strong adherance to the study protocol. Participants in the low-carbohydrate dietary group also tested twice daily for increased urine ketone concentrations using urine ketone strips, which also suggests high-compliance among this group. Although it is still remains plausible for participants to consume foods outside of the meals provided by the GCRC and urine ketone strips are not as realiable for measuring elevated ketone concentrations as a blood test. In addition, participants receiving a low-carbohydrate diet were provided with foods containing higher amounts of dietary carnitine than those consuming the high-complex-carbohydrate diet. The composition of a low-carbohydrate diet contains a higher proportion of total energy from protein, and most protein-rich foods were derived from aminal sources, where carnitine is naturally high. Consequently, we were unable to control for the amount of carnitine provided to participants in each dietary group. This may have increased or maintained total carnitine concentrations among partipicants receiving the low-carbohydrate dietary intervention differently from those receiving the high-complex-carbohydrate dietary intervention. Since current nutrient databases do not contain information on the carnitine content of foods, we were not able to retrospectively analyze the average

amount of dietary carnitine provided to participants in the low-and high-complex-carbohydrate dietary groups. Due to an unrealible method, we were also unable to accurately quantify free carnitine concentration in participants' blood samples and therefore were not able to include free carnitine in our analyzes.

The purpose of this thesis was to provided exploratory and descriptive data on changes in acylcarnitine concentrations after consumption of two distinct dietary interventions and resulting changes in body composition. Our sample size was not statistically powered to see differences in change in acylcarnitine concentrations. However, our sample size was powered to see differences in change in body mass and BMI. Due to the small sample size and higher level of significance (0.10) used during the "alerting" mixed modeling phases, any statistical significance should be interpreted with caution and only be used for descriptive purposes in change in acylcarnitine concentrations. The participants in our study were mostly healthy, middle-aged, Caucasian adults (primarily female) with obesity yet free of comorbities. Therefore, the generalizability of our results are limited to adults with similar demographic characteristics as our study participants.

Conclusions

The primary objective of this secondary analysis was to provide preliminary and descriptive data for future statistically powered, controlled dietary intervention studies evaluating the impact of low-and high-complex-carbohydrate diets on body mass, fat mass, lean mass, and acylcarnitine concentrations. Our sample size was not powered to conduct explicit hypothesis testing on changes in acylcarnitine concentrations or to provide conclusive evidence with external validity to larger populations. Yet, the results from this analysis do endorse the previous research describing the effect of low-and high-complex-carbohydrate diets and body composition changes, as well as offers original insight on how these dietary interventions affect

acylcarnitine concentrations during a controlled feeding study and home implementation protocol. With that said, this secondary analysis provides novel evidence that individual acylcarnitine concentrations differ by time, dietary intervention, and/or the synergistic interaction of time:diet in healthy adults with overweight and obesity over time. We found significant within and between dietary group differences in the concentrations of C4, C5, C5-DC, and C18 acylcarnitines over time. We also reported no observable associations between change in lean mass and any of these 4 acylcarnitine concentrations among participants receiving either low-or high-complex-carbohydrate diets. However, we did describe an association between change in fat mass and change in C4 acylcarnitine concentrations among individuals consuming a low-carbohydrate diet, as well as an association between change in fat mass and change in C18 acylcarnitine concentrations among individuals consuming either a low-or high-complex carbohydrate dietary intervention (no effect of diet). These associations may be due to an increased reliance on FAO with high-fat, high-protein diets, resulting in increased mobilization of NEFAs from adipose tissue and increased ketone production from FAO.

Future Directions

To our knowledge, there are no other studies published to date that are similar in study design and research inquires. The majority of dietary studies investigating change in individual acylcarnitine concentrations over time do not describe changes in body composition, and the association between changes in body composition and changes in individual acylcarnitine concentrations. Mihalik et al.,[2] Newgard et al.,[4] and Floegal et al.[33] have all reported elevated mean concentrations of various carnitine species including mean free carnitine and C3, C5, C6, C8:1, C10:1, C14:1, C14-OH, C16, C16-OH, C18, and C18:1 acylcarnitine concentrations in adults with obesity compared to lean controls. Mihalik et al.[2] observed similar elevated concentrations of mean free carnitine, C5, C10:1, C14:1, C14-OH, C16, C16-OH, C18, and C18:1 acylcarnitine

concentrations in adults with obesity and in adults with obesity and T2DM. These findings suggest that individuals with obesity may share similar defects in the early phases of lipid metabolism as individuals with obesity and T2DM.

Although the low-and high-complex carbohydrate dietary interventions in our study did not significantly lower mean C5 or C18 acylcarnitines concentrations, our findings did highlight the important role that dietary intervention may have on changes in individual acylcarnitine concentrations among individuals with obesity. Dietary intervention-induced changes in individual acylcarnitine concentrations may still have an important role in preventing insulin resistance among individuals with obesity. It is feasible that we did not observe decreases in mean C5 or C18 acylcarnitine concentration because our study was not statistically powered to detect changes in individual acylcarnitine concentrations. Additional controlled dietary intervention studies with larger sample sizes that are statistically powered to detect differences in body composition and individual acylcarnitine concentrations after low- and high-complex-carbohydrate dietary interventions are needed to confirm or reject our findings. Future research in this area should also include dietary interventions with controlled dietary carnitine content in order to prevent variations in total carnitine concentration solely explained by an imbalance in dietary carnitine content. Lastly, larger, longer-term controlled feeding trials are needed to further establish evidence supporting our preliminary findings.

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APPENDICES

Appendix A – Evidence Table

Original Dietary Approaches to Stop Hypertension (DASH) on Blood Pressure Studies

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes
Appel et al., 1997 (49)	• Randomized controlled feeding trial	• 3-week run-in (control diet) • 8-week intervention	• n=459 overweight/obese M/F adults with or without HTN • Mean age 44 y/o • Age range (22+ y/o) • Mean BMI 28 kg/m^2 across all groups	• Change in SBP and DBP	1. Typical American diet (control diet, n=154) 2. Typical American diet + high fruits & vegetables (n=154) 3. DASH diet (combo diet, n=151) * Diets designed for weight stability	• DASH diet ↓ SBP and DBP more in participants with and without HTN than the control diet (-5.5 mmHg SBP, -3.0 mmHg DBP, p<0.001 for each). • DASH diet ↓ BP more than the high fruits & vegetables dietary group when both groups were compared to the control diet. • Weight remained stable as intended.
Sacks et al., 2001 (50)	• Randomized controlled feeding trial w/ crossover, parallel-group design	• 2-week run-in period on high sodium (control) diet • 90-day intervention (30 consecutive days on each sodium level per diet group)	• n=412 M/F adults with stage 1 HTN or high BP • Mean age 47 y/o & BMI 29 kg/m^2 for DASH group • Mean age 49 y/o & BMI 30 kg/m^2 for control group	• Change in SBP/DBP	1. Typical American diet (control group, n=204) 2. DASH diet (n=208) *All participants received high (3 gm/d), intermediate (2.3 gm/d), and low (1.5 gm/d) sodium intake via a step-wise fashion * Diets designed for weight stability	• ↓ sodium intake significantly ↓ SBP/DBP in a stepwise fashion in the control and DASH diet groups in all participants • Compared to the control group, DASH diet group ↓ SBP at every sodium level and ↓ DBP at high and intermediate sodium levels. • Low sodium DASH diet produced greater ↓ in SBP/DBP than either the DASH diet alone or a reduction in sodium alone, compared to control diet. • Weight remained stable as intended.

Appel et al., 2003 (48)	• Randomized controlled trial	• 6-month intervention • Follow up 18-months from baseline	• n=810 M/F adults with stage 1 HTN or high BP • Mean age 50 y/o & mean BMI 33 kg/m^2	• Change in SBP, DBP, and HTN status	1. "Established" intervention group = tx plan + counseling (n=268) 2. "Established "intervention group + DASH diet" (n=269) 3. "Advice-only" comparison group = 1 time initial 30-minute appt. w/ no counseling (n=273) *18 total counseling sessions over 6-month intervention	• Mean 4.3 mmHg ↓ in SBP ($p<0.001$) and 2.6 mmHg ↓ in DBP in the established + DASH group ($p<0.001$). • The established group + DASH diet ↓ an average 5.8 ± 5.8 kg compared to 4.9 ± 5.5 kg for the established group alone, and 1.1 ± 3.2 kg for the advice only group. • 34.3% of subjects on the established + DASH ↓ >6.8 kg of baseline body weight over 6-months compared to 6.2% in the advice only group ($p<0.001$ for difference). • Statistically significant difference between the established + DASH vs advice only group ($p<0.001$) but not the established vs established + DASH ($p=0.07$).

High-Carbohydrate Diet, Weight Loss, and Body Composition

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes
Saris et al., 2000 (57)	• Multi-center randomized controlled ad libitum feeding trial	• 5-week run-in phase • 6-month intervention • Diets provided between 75-125% of EER	• n=398 M/F healthy obese adults free of major diseases • 316/398 included in analyses after dropouts • Mean age 39 y/o • Mean BMI 30.4 kg/m^2	• Change in body weight, body composition, and blood lipids	1. Seasonal control group (no intervention, n=80) 2. Typical American diet group (control group, n=77) 3. Low-fat, high simple carbohydrate diet group (n=76) 4. Low-fat, high complex carbohydrate diet group (n=83)	• Mean weight ↓ was 1.8 ± 3.2 kg in the low-fat high-complex-carbohydrate diet group ($p<0.001$) and↓ 0.9 ± 3.6 kg ($p<0.05$) in the low-fat high-simple-carbohydrate diet group, compared to control group. • Fat mass ↓ by 1.8 ± 3.9 kg among the low-fat high-complex-carbohydrate diet group ($p<0.001$) and ↓ 1.3 ± 3.6 kg ($p<0.01$) in the low-fat high simple-carbohydrate diet group, compared to control group.
Hays et al., 2004 (55)	• Randomized controlled ad libitum feeding trial	• 1-week run-in phase on isoenergetic mixed diet designed for weight stability • 12-week intervention providing 150% of EER	• n=34 older adults with impaired glucose tolerance completed study (20 males & 14 females) • Mean age 66 y/o • Mean BMI 30 kg/m^2	• Change in body weight, body composition, & fat distribution	1. High-complex carbohydrate, low-fat diet alone (n=11) 2. High-complex carbohydrate, low-fat diet with 4x/week aerobic exercise (n=11)) 3. Control diet alone (n=12)	• Participants on the high-complex carbohydrate, low-fat diet without exercise ↓ 3.2 ± 1.2 kg body weight ($p=0.02$) and ↓ 2.2 ± 1.2% body fat, compared to the control group ($p=0.049$). • Participants on the high-complex-carbohydrate, low-fat diet with exercise ↓ 4.8 ± 0.9 kg body weight ($p=0.003$) and ↓ 3.5 ± 0.7% body fat compared to the control group ($p=0.01$). • The control group ↓ 0.1 ± 0.6 kg body weight and ↓0.2 ± 0.6% body fat.

Poppitt et al., 2002 (56)	• Randomized controlled ad libitum feeding trial	• 1-month run-in phase on control diet • 6-month intervention	• n=39 overweight/obese M/F adult subjects with ≥ 3 metabolic syndrome risk factors completed trial • Mean age 46 y/o • Mean BMI 32 kg/m^2	• Change in body weight, BMI, blood pressure, & blood lipids	1. Control diet (n=11) 2. Low-fat, high-complex-carbohydrate diet (n=14) 3. Low-fat, high-simple-carbohydrate diet (n=14) * ≥60% of total energy provided via research grocery store & other eaten at subject's home	• Between baseline and the 6-month intervention, body weight ↑ 1.03 kg (NS) in the control group, ↓ 4.25 kg ($p<0.01$) in the low-fat, high-complex-carbohydrate diet group, and ↓ 0.28 kg (NS) in the low-fat, high-simple-carbohydrate diet group. • There was only significant body weight ↓ ($p<0.01$) and change in BMI ($p<0.001$) over time in the low-fat, high-complex-carbohydrate diet group, when each diet was modeled separately. • BMI and waist circumference significantly ↓ during the 6-month intervention only in subjects who ↓ ≥3% total body weight ($P < 0.05$).

Low-Carbohydrate Diet, Weight Loss, and Body Composition

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes
Dansinger et al., 2005 (54)	• Randomized controlled trial	• 12-month intervention (all participants were asked to follow dietary assignment until 2-month assessment, but were encouraged to continue until 12-months)	• n=160 M/F overweight/obese adults with known HTN, dyslipidemia, or fasting hyperglycemia • 93 completed entire study • Mean age 49 y/o • Mean BMI 35 kg/m^2	• Change in body weight & cardiac risk factors	1. Atkins low-carbohydrate diet (n=40) 2. Zone diet (n=40) 3. Weight watchers diet (n=40) 4. Ornish diet (n=40) *All participants were encouraged to take multivitamin, obtain at least 60-minutes of exercise weekly, and avoid commercial support services *Diet-specific advice provided to each group, meeting for 1 hour on 4 occasions during the first 2 months of the study.	• Body weight ↓ at 12-months was ↓ 2.1 ± 4.8 kg for Atkins (53% of participants completed) ↓ 3.2± 6.0 kg for Zone (65% of participants completed) ↓ 3.0 ± 4.9 kg for Weight Watchers (65% of participants completed) and ↓ 3.3 ± 7.3 kg for Ornish (50% of participants completed) (p-value for trend of 0.40). • Among the participants in the Atkins group, the greatest amount of body weight ↓was during the first 2-months of the dietary intervention (↓3.6 ± 3.3 kg body weight).
Shai et al., 2008 (43)	• Randomized controlled trial • Lunch provided in self-service cafeteria at workplace (lunch was biggest meal of the day)	• 24-months	• n=322 obese M/F adults • 272 completed entire study • Mean age 52 y/o • Mean BMI 31 kg/m^2 • Adherence to study diets was 95.4% at 1 year and 84.6% at 2 years	• Change in body weight, BMI, and blood lipids	1. Low-fat, restricted-energy diet (n=104) 2. Mediterranean, restricted -calorie diet (n=109) 3. Low-carbohydrate, non-restricted calorie diet (n=109) *All groups met with a dietitian for a total of 18, 90 minute sessions & subjects with poor adherence received 6, 10-15 minute motivational telephone call from a different dietitian	• Maximum weight ↓ occurred within the first 6-months in all three diet groups with a maintenance weight loss phase between 7- and 24-months in all groups. • All diet groups ↓ weight but the weight ↓ was greatest in the low-carbohydrate, non-restricted and the Mediterranean diet groups (p<0.001). • Mean weight change among study completers at 24-months was ↓ 3.3 ± 4.1 kg for the low-fat group, ↓ 4.6 ± 6.0 kg for the Mediterranean diet group, and ↓ 5.5 ± 7.0 kg for the low-carbohydrate group (p=0.03 for comparison between the low-fat vs low-carbohydrate groups at 24-months).

Volek et al., 2004 (53)	• Randomized controlled crossover trial	• Females participants consumed each diet in random crossover fashion for 30-days & males consumed each diet for 50-days	• n=15 healthy, overweight/obese males (mean age 33.2 y/o & BMI 34.1 kg/m^2) & 13 premenopausal females (mean age 34 y/o & BMI 29.6 kg/m^2) completed both dietary interventions	• Change in body weight, fat mass, lean mass, & trunk fat (via DEXA)	• Hypoenergetic low-fat diet (↓500 kcals of energy/day from REE) • Hypoenergetic very-low-carbohydrate, ketogenic diet (↓500 kcals of energy/day from REE) *All subjects received extensive initial instruction and follow-up by dietitians if needed	• Within group comparisons showed that both men and women had significantly greater ↓ in body mass, total fat mass, and trunk fat mass when on the very-low carbohydrate, ketogenic diet compared to the low-fat diet ($p<0.05$). • Between group comparisons of subjects showed that both men and women participants experienced a greater ↓in the ratio of trunk fat/total fat when on the very-low carbohydrate, ketogenic diet versus the low-fat diet. However, the ↓ in body mass, total fat mass, and trunk fat were only significantly greater for men and not women ($p<0.05$). • Note: did not report quantities lost.

Hashimoto et al., 2016 (52)	• Meta-analysis of 14 randomized controlled trials	• Studies included ranged from 2- to 24-months	• n=1416 overweight/obese M/F adults from 8 very-low-carbohydrate studies and 7 mild-low-carbohydrate studies	• Changes in body weight & fat mass (via DEXA or BIA)	1. Control diet 2. Low-carbohydrate diet	• The low-carbohydrate diets were associated with a greater mean change of body weight (↓ 0.70 kg; 95% CI: -1.07, -0.33 kg, p<0.05) and mean change in fat mass (↓ 0.82 kg; 95% CI: -1.22, -0.42 kg, p<0.05) when compared to a control diet. • Data from 6 studies (770 participants) found that a diet intervention of >12-months found that a low-carbohydrate diet was associated with a ↓in fat mass (↓0.57 kg; 95% CI: -1.05, -0.09 kg, p<0.05) but not body weight when compared to the control group (p>0.05). • Data from 8 studies (666 participants) found that a diet intervention of <12-months found the low-carbohydrate diet was associated with both a ↓ in body weight (↓ 0.89 kg; 95% CI: -1.43, 0.35 kg, p<0.05) and fat mass (↓ 0.98 kg; 95% CI: -1.60, -0.36 kg, p<0.05) when compared to the control group. • Data from 8 studies (831 participants) found an association between a very-low carbohydrate diet and greater ↓ in body weight (↓ 1.00 kg; 95% CI: -1.54,-0.45 kg) and fat mass (↓ 0.97 kg; 95% CI: -1.50, -0.44 kg) when compared to the control group. • Data from 7 studies (584 participants) found no statistical differences in the change in body weight or fat mass among participants on a mild-carbohydrate diet and changes in body weight or fat mass compared to a control diet.

High-versus Low-Carbohydrate Diet, Weight Loss, and Body Composition

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes
Sacks et al., 2009 (58)	• Randomized controlled trial w/ 2 x 2 factorial design	• 6-month intervention • 24-months of follow-up	• 811 overweight/obese M/F adults (645 completed the entire study) • Mean age 51 y/o • Mean BMI 33 kg/m^2	• Change in body weight, blood lipids, blood pressure, & insulin sensitively	1. Low-fat average protein diet (n=204) 2. Low-fat, high protein diet (n=202) 3. High-fat, average protein diet (n=204) 4. High-fat, high-protein diet (n=201) *Each diet group had a ↓ 750 kcals of energy/day deficit from their REE *Goal of 90-minutes of moderate exercise per week • Group sessions were held once a week, 3 of every 4 weeks during the first 6 months and 2 of every 4 weeks from 6 months to 2 years; individual sessions were held every 8 weeks for the entire 2 years.	• Among the participants who completed the 24-month study, there were no statistically significant mean differences in the amount of weight ↓ among participants on a low-versus high-protein diets (mean difference ↓ 0.9 kg, 95% CI: -2.1 to 0.2, p=0.11), a low-versus high-fat diet (mean difference ↑ 0.2 kg, 95% CI: -1.0 to 1.3 kg, p=0.76), or a low versus high-carbohydrate diet (mean difference ↑ 0.7 kg, 95% CI: -0.9 to 2.3 kg, p=0.37). • The most weight was ↓ during the 6-month intervention (mean ↓ 6 kg between all dietary groups). After 12-months, on average, all participants in all dietary groups slowly regained weight.
Foster et al., 2003 (59)	• Multi-center randomized controlled trial	• 12-month intervention	• n=63 obese M/F (43 females & 20 males) • 37 subjects completed entire study (49 by 3-months, 42 by 6-months, & 37 by 12-months) • Mean age 44 y/o • Mean BMI 34 kg/m^2	• Change in body weight, blood lipids, blood pressure, & insulin sensitively	1. Low-carbohydrate diet (n=33) 2. High-carbohydrate, low-fat, low-calorie (conventional) diet (n=30) *All study participants met with dietitian prior to starting intervention	• Participants lost the most weight after 6-months (mean percent weight change ↓ 7.0 ± 6.5% for the low-carbohydrate diet group & ↓ 3.2 ± 5.6% for the conventional diet group, p-value=0.02 between group difference). • After 12-months, the mean percent weight change for the low-carbohydrate diet group was ↓ 4.4 ± 6.7% & ↓ 2.5 ± 6.3% for the conventional died group (p=0.26 for between group differences at 12-months).

Samaha et al., 2003 (60)	• Randomized controlled trial	• 6-month intervention	• n=132 obese M/F adults (83% had DM or metabolic syndrome) • 79 subjects completed entire trial • Mean age 55 y/o • Mean BMI 43 kg/m^2	• Change in body weight, blood lipids, blood pressure, glycemic index, & insulin sensitively	1. Low-carbohydrate diet (n=64) 2. Convectional low-fat diet (n=68) *The low-fat diet group restricted calories by ↓ 500 kcals of energy/day w/ <30% of total kcals of energy from fat. *Low-carbohydrate diet group = <30 grams carbohydrates/day *Diet groups met in weekly counseling sessions for 4 weeks, followed by 5 monthly sessions.	• Subjects on the low-carbohydrate diet ↓more weight during the 6-month study than the low-fat diet (mean weight ↓ 5.8 ± 8.6 kg vs. ↓ 1.9 ± 4.2 kg, 95% Cl for the difference in weight loss between groups, –1.6 to –6.3; P=0.002). • The difference in weight ↓ between the groups remained significant after adjustment for baseline variables alone (P=0.002). • No significant difference was seen after including baseline weights of those who dropped out. • Weight ↓ >10% of baseline weight occurred in 14% of subjects on the low-carbohydrate diet compared to 3% of subjects on the low-fat diet (P=0.02).
Stern et al., 2004 (61)	• Continuation of randomized controlled trial	• 6-month follow up after a 6-month dietary intervention	• n=132 obese M/F adults (83% had DM or metabolic syndrome) • 87 subjects completed the 12-month study. • Follow up weights obtained from 126/132 original participants at 12-months • Mean age 55 y/o • Mean BMI 43 kg/m^2	• Change in body weight, blood lipids, blood pressure, glycemic index, & insulin sensitively	1. Low-carbohydrate diet (n=64) 2. Conventional low-fat diet (n=68) *The low-fat diet group restricted calories by ↓ 500 kcals of energy/day w/ <30% of total energy from fat. *Low-carbohydrate diet group = <30 grams carbohydrate/day *Diet groups met in weekly counseling sessions for 4 weeks, followed by 11 monthly sessions.	• Participants on the low-carbohydrate diet maintained most of their 6-month weight ↓, whereas those on a conventional diet continued to ↓ weight throughout the year. • By 12-months, the mean weight change for subjects consuming the low-carbohydrate diet was ↓5.1 ± 8.7 kg compared to ↓ 3.1 ± 8.4 kg for subjects consuming the conventional diet (p-value=0.195 for difference between groups).

Meckling et al., 2004 (62)	• Randomized controlled trial	• 10-week intervention	• n=31 healthy overweight/obese M/F subjects completed trial (22 females & 9 males) • Mean age 42 y/o and BMI 32.2 kg/m^2	• Change in weight, blood lipids, blood pressure, insulin sensitivity, fat mass, & lean mass (via BIA)	1. Energy-restricted low-fat diet (control) (n=16) 2. Energy-restricted low-carbohydrate diet (n=15) *All participants met weekly for a diet consultation *Low-carbohydrate diet = 50-70 gms carbohydrate/day by day 5. *Energy intake was matched based on the low-carbohydrate diet group's intake	• No difference in the pattern of weight ↓ over time between the two groups. • Subjects on the low-fat diet ↓ an average weight of ↓ 6.8 kg (no SD) compared to ↓ 7.0 kg (no SD) in the low-carbohydrate diet ($p<0.05$ compared to baseline values). • Significant ↓ in fat mass were observed in both diet groups [(low-fat diet, ↓ 5.4 kg (no SD); low-carbohydrate diet, ↓ 4.1 kg (no SD), $p<0.05$ compared to baseline] • The low-fat diet group better preserved lean body mass than the low-carbohydrate diet group. • A significant ↓ in lean mass was observed in the low-carbohydrate diet group [(↓ 1.9 kg (no SD), $p<0.05$ compared to baseline values)] but not the low-fat group [(↓ 1.0 kg (no SD)]. However, both groups had improvements in body composition when controlling for total body weight changes.

Noakes et al., 2006 (67)	• Randomized controlled trial	• 12-week intervention (8-weeks of isoenergetic weight loss diet intervention & 4-weeks of same diet intervention in energy balance)	• n=83 overweight/obese M/F subjects with at least 1 cardiovascular risk factor • 67 completed entire trial (12 males & 55 females) • Mean age 48 y/o & mean BMI 33 kg/m^2	• Change in body weight, fat mass, lean mass (via DEXA), blood lipids, blood pressure, insulin sensitivity, inflammatory markers, folate, homocysteine, and vitamin B12	1. Very-low-carbohydrate diet (<20 gm carbohydrate per day) (n=24) 2. Very-low-fat diet (n=22) 3. High-unsaturated fat diet (n=21) *First 8-weeks = isoenergetic version of each diet (30% energy restriction) followed by energy balance version of each diet during last 4-weeks *All subjects received detailed instructions for each diet and met with a dietitian every 2 weeks	• Each diet group ↓ weight over the 8-week energy restriction period and maintained their weight ↓ during the 4-week energy balance phase. • No statistically significant differences in absolute mean weight ↓ by diet group with net weight ↓ of ↓ 8.0 ± 0.6 kg for very-low-carbohydrate diet group, ↓ 6.7 ± 0.7 kg for very-low-fat diet group, and ↓ 6.4 ± 0.6 kg for high-unsaturated fat diet group (p=0.18). • However, percent change in weight from baseline differed significantly by diet (P = 0.034) with the very-low-carbohydrate diet group resulting in a greater weight ↓ of 9.2% compared to the very-low-fat group (↓ 7.3%) and high-unsaturated fat group (↓ 7.0%). • Lean mass ↓ as a proportion of weight ↓was significantly greater in the very-low-carbohydrate diet group (↓ 31%) and the very-low fat group (↓ 31%), compared to the high-unsaturated fat diet group (↓ 21%) (p<0.05). • Percent change in fat mass ↓ at baseline compared to 10-weeks of dietary intervention was not statistically different between diets (↓ 4.5 ± 0.5 kg in very-low-carbohydrate diet group, ↓ 4.0 ± 0.5 kg in very-low-fat diet group, ↓ 4.4 ± 0.6 kg in high-unsaturated fat diet).

Free-and Acyl-Carnitine Concentrations in Individuals with Obesity

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes	Major Carnitine Species	Assessment Method of Carnitine Species
Newgard et al., 2009 (4)	• Cross-sectional metabolomic study	n/a	• 73 healthy M/F obese adults enrolled from local weight loss programs and 67 lean (controls) adults from local community • Obese: Median age 52 y/o & BMI 36.6 kg/m^2 • Lean controls: Median age 50 y/o & 23.2 kg/m^2	• Dietary intake (via Block FFQ), physical activity (via IPAQ), weight, height, waist circumference, lean and fat mass (via DEXA), REE, serum acylcarnitines, insulin sensitivity, hormones, blood lipids, FFAs, ketones, cytokines, amino acids, and organic acids	• n/a	• Results from a self-administer Block FFQ revealed that obese participants had a ↑ mean dietary intake of fat (42.2% versus 35.7%, p<0.0001), a lower mean intake of carbohydrate (43.4% versus 50%, p=0.0005), and a non-statistically significant ↑ trend in protein consumption (15.5% versus 14.3%, p=0.072) with ↓ levels of reported physical activity (p=0.0957) compared to lean controls. • C3, C5, C6, C8:1 acylcarnitine concentrations were all significantly ↑ among the obese subjects compared to controls (p<0.009). • Valine, leucine, isoleucine) were all dramatically ↑ in the obese compared to lean controls (p<0.007). • Strongest differences between obese and lean groups was in leucine, isoleucine, valine, methionine, glutamate, glutamine, phenylalanine, tyrosine, and C3 and C5 acylcarnitines (p<0.001).	• 37 acylcarnitine species	• Tandem mass spectrometry of serum

Mihalik et al., 2010 (2)	• Cross-sectional metabolomic study	• n/a	• n=14 healthy, non-diabetic obese M/F adults (mean age 43 y/o & BMI 34.3 kg/m^2) • 10 adults with T2DM (mean age 45 y/o & BMI 34.2 kg/m^2) • 12 lean sedentary adult controls (mean age 47 y/o & BMI 23.9 kg/m^2)	• Free-and acyl-carnitine concentrations	• n/a	• Mean plasma free carnitine, C5, C10:1, and individual long-chain acylcarnitines (C14:1, C14-OH, C16, C16-OH, C18, C18:1) concentrations were similarly ↑ in both the obese group and the T2DM group and all statistically higher compared to the lean controls ($p<0.05$) • Relative to the obese and lean groups, T2DM participants had significantly ↑ mean C3, C4-OH, C5, C5-OH, C6-OH, C8 acylcarnitine concentrations ($p<0.05$), with a larger contribution of ↑ C3 & C5 acylcarnitine concentrations in T2DM males. • Participants in the T2DM group but not the obese group had significant elevations in C4 and C6 acylcarnitines concentrations compared to lean participants ($p<0.05$), although the mean concentrations for C4 were nearly identical in the obese and T2DM groups. • C4-DC acylcarnitine concentration was significantly ↑ among the T2DM group compared to both the obese ($p<0.0001$) and lean groups ($p<0.002$).	• Free carnitine • Short-acylcarnitines (C2, C3, C4, C4-OH, C5, C5-OH) • Medium-chain acylcarnitines (C6, C6-OH, C8, C10:1) • Long-chain acylcarnitines (C14:1, C14-OH, C16, C16-OH, C18, C18:1)	• Tandem mass spectrometry of dried plasma blood spots

Ramos-Roman et al., 2012 (77)	• Feeding trial	• 10-day intervention (on weight maintaining diet 3-days before admission #1 & 7-days before admission #2)	• n=12 female and 4 male non-diabetic overweight/obese adults with wide range of insulin sensitivities • Mean age 45.8 y/o & BMI 35.4 kg/m^2	• Fasting and postprandial (6-hours post-meal) concentrations of acylcarnitines • Fat mass & lean mass (via DEXA) • Insulin sensitivity & substrate oxidation (via IC)	• Weight maintaining diet (formulated to resemble usual food intake pattern as assessed by a FFQ & 3-day food diary) *33.5 ± 5.4% calories from fat; 48 ± 5.9% from carbohydrates; 18.5 ± 3.9% from protein *High-fat (~50% of total calories) lunch meals was provided on day 2 of admission #2 contained labeled palmitate FA (providing 38% daily energy needs) after an 18-hour fast.	• Lean body mass was positively associated with fasting plasma C3, C3-DC, C4, C5, C5:1, and C10:1 acylcarnitine concentrations, whereas fat mass was not associated with any acylcarnitine species (all $p<0.05$). • Fat oxidation was positively associated with fasting plasma C12:1, C14, C14:1, C14:2, and C16:1 acylcarnitine concentrations (all $p<0.05$). • The fasting rate of appearance of plasma free fatty acids was negatively associated with the fasting concentration of C12:2, C14:1, and C18:2 acylcarnitines concentrations. • Free carnitine and all medium and long-chain acylcarnitines were ↓ after the post-prandial meal except the short-chain acylcarnitines-C3, C4, and C5. • Although not statistically significant, C2, C3-DC, and C4-DC acylcarnitine concentrations were 20-30% ↓, whereas C3 & C4 tended to ↑ by 15% post-meal ($p=0.07$).	• Free carnitine & short-medium-, and long-chain acylcarnitines	• Liquid chromatography with tandem mass spectrometry of plasma

Patel et al., 2013 (1)	• Cross-sectional analysis	• n/a	• n=500 overweight/obese M/F adults who participated in the Weight Loss Maintenance (WLM) Trial • All selected participants met eligibility requirements lost at least 4-kg in the Phase 1 of the WLM trial and were randomized into phase 2, & had blood samples at all time points • Mean age 55.9 y/o & BMI 33.9 kg/m^2	• Fasting plasma concentrations of 69 small-molecule intermediary metabolites taken at baseline before any initial weight loss occurred during the WLM trial (total FFAs, ketones, β-hydroxybutyrate, 45 acylcarnitines, & 15 amino acids)	• n/a	• C14, C16, C18:1, C16:1-OH/C14:1-DC acylcarnitine concentrations were ↑ among Caucasians compared to African Americans ($p<0.0001$) • C14, C16, C18:1, C16:1-OH/C14:1-DC acylcarnitine concentrations (all $p<0.01$), Ci4-DC/C4-DC, C6-DC, C10-OH/C8-DC, C12:1, C12-OH/C10-DC, C14:1-OH/C12:1-DC, C14-OH/C12-DC, C8:1-DC acylcarnitine concentrations (all $p<0.0001$), amino acids related to branched-chain amino acids (alanine, proline, valine, leucine/isoleucine, methionine, phenylalanine, tyrosine, glutamine, glutamate, ornithine, all $p<0.0001$) were also ↑ among males compared to females.	• Free carnitine • 45 short-chain, medium-chain, & long-chain acylcarnitine species	• Tandem mass spectrometry of plasma

Floegal et al., 2014 (33)	• Cross-sectional analysis	n/a	• n=50 males & 50 females randomly drawn subcohort of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam • Mean age 49.8 y/o & BMI 26.1 kg/m^2	• 127 serum metabolites (17 acylcarnitines, 14 amino acids, 95 choline-containing phospholipids, and 1 hexose)	n/a	• Using Gaussian graphical modeling to create partial correlation coefficients, acylcarnitines were positively associated with obesity. • Free carnitine, C3, and C8:1 acylcarnitine concentrations were positively correlated with BMI (0.3-0.4 multivariable adjusted partial correlation coefficient). • Free carnitine, C3, C8:1, C16, and C18:1 acylcarnitine concentrations were positively correlated with waist circumference (0.3-0.4 multivariable adjusted partial correlation coefficient). • Free carnitine and C3 acylcarnitine concentrations were linked as a separate pair away from the acylcarnitine metabolite network and showed a partial correlation of 0.6-0.8. • Ornithine, proline, and C9 acylcarnitine concentration were not correlated to any other metabolites. *No p-values indicating significance reported.	• Free carnitine • Short-acylcarnitines (C2, C3, C3-DC, C5-DC, C5-OH) • Medium-chain acylcarnitines (C6-OH, C7-DC, C8:1, C9, C10, C10:2) • Long-chain acylcarnitines (C14:1, C14:2, C16, C16:2, C18, C18:1, C18:2)	• Mass spectrometry of serum

High-and Low-Carbohydrate Diets and Changes in Free-and Acyl-Carnitine Concentrations and no Weight Loss

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes	Major Carnitine Species	Assessment Method of Carnitine Species
Seccombe et al., 1978 (71)	• Feeding trial	• 2 days of intervention	• n=18 male White Wistar rats delivered at 21 days gestation and weaned onto free access standard rat chow • All rats were 20-22 days old when 2-day diet intervention was initiated	• Fasting serum free carnitine and total-, free-, and acylcarnitine concentrations after two days of dietary intervention • Effect of starvation over 96-hours on serum total-, free-, and acylcarnitine concentrations *Acylcarnitine concentrations estimated by subtracting free carnitine from total carnitine concentration	1. "High-fat" long-chain triglyceride diet using cotton seed oil (n=8) 2. Medium-chain triglyceride diet using coconut oil (n=10) 3. High-carbohydrate diet using dextrose (n=8) *All diets contained the same amount of dietary carnitine	• Free carnitine significantly ↓ after 24-hours of fasting ($p<0.01$ compared to baseline) but gradually ↑ to maximum concentration by 96-hours ($p<0.05$ compared to baseline). • Mean acylcarnitine concentration significantly ↑ by 24-hours of fasting ($p<0.05$ compared to baseline) and reached its maximum concentration by 48-hours ($p<0.05$ compared to baseline & $p<0.01$ compared to 24-hours). • Mean total carnitine significantly ↓ after 24-hours of fasting ($p<0.01$ compared to baseline) but gradually ↑ to maximum concentration by 96-hours ($p<0.01$ compared to baseline). • The ratio of acylcarnitine-to-free carnitine ↑ by 24-hours ($p<0.01$ compared to baseline) and continued to ↑ until 48-hours ($p<0.05$ compared to baseline). • Mean serum free carnitine was ↓ in rats on the long-chain and medium-chain triglyceride diets compared to the high-carbohydrate diet ($p<0.0001$ when each diet compared to high-carbohydrate diet). • Mean serum acylcarnitines were significantly ↑ in rats on the medium-chain triglyceride diet compared to both the high-carbohydrate diet ($p<0.0001$)	• Serum total-, free-, and acylcarnitine concentrations	• Simplified radioisotopic assay (method developed by McGarry & Foster. J Lipid Res 1976; 17:277-81)

						and the long-chain triglyceride diet ($p<0.0001$). • Mean total serum carnitine was ↑ in the high-carbohydrate diet group, ↓ in the long-chain triglyceride diet group ($p<0.0001$ compared to the high-carbohydrate diet) and ↑ in the medium-triglyceride diet group compared to the long-chain triglyceride group ($p<0.0001$). • Serum acyl-to-free carnitine ratio was significantly ↑ in rats fed the high-fat diets compared to those fed the high-carbohydrate diet ($p<0.001$).		

Bell et al., 1982 (70)	• Randomized controlled crossover feeding trial	• All monkeys started on a high-carbohydrate, low-fat diet. • 90-day intervention on low-carbohydrate, high-fat diet, followed by a 76-day intervention on high-carbohydrate, low-fat diet	• n=21 male stumptail monkeys (*Maca arctoides*) • ~6-10 years old	• Changes in fasting serum triglycerides, free-, total-, and acyl-carnitines concentrations	1. High-carbohydrate, low-fat diet *Purina monkey chow (~10% of total energy from fat) *Fed at 10am daily 2. Low-carbohydrate, high-fat diet *Custom-mixed chow (~45% total energy from fat) *Fed at 10am & 4:30pm daily *All 24-48 hour fasting data obtained from eight monkeys maintained on low-fat diet for ~6-months.	• The ratio of esterified/unesterified carnitine ↑ significantly within 62 days of initiating the high-fat diet (0.22 ± 0.03 nmol/ml at baseline to 0.35 ± 0.02 nmol/ml, $p<0.05$) and remained significantly ↑ compared to baseline until the low-fat diet was initiated at 90-days. • The ratio of esterified/unesterified carnitine ↓ significantly to 0.20 ± 0.03 nmol/ml ($p<0.05$) within 3-days of starting the low-fat diet and then stabilized to 0.24 nmol/ml from day 93 to 166. • Fasting for 24-hours resulted in a 270% ↑ in esterified carnitine and a 410% ↑ in esterified carnitine after 48-hours compared to baseline. • Free carnitine ↑ to a lesser degree (45% ↑ at 24-hours and 31% at 48-hours, no p-value reported).	• Changes in serum free-(unesterified), total-, and acyl-carnitines (esterified)	• Simplified radioisotopic assay (method developed by McGarry & Foster. J Lipid Res 1976; 17:277-81)

Cederblad et al., 1987 (68)	• Randomized controlled crossover feeding trial	• 2-week intervention (4-days on one diet intervention, 3-day break, & 4-days on other diet intervention)	• n=7 healthy male adults • Median age 33 y/o • Median BMI 20.4 kg/m^2	• Changes in fasting total plasma carnitine concentration, free carnitine, and urinary excretion of free- and acyl-carnitines.	1. Isoenergetic high-carbohydrate, low-fat diet (~30% of total energy from fat, 51% of total energy from carbohydrates, 19% of total energy from protein) 2. Isoenergetic low-carbohydrate, high-fat diet (~54% of total energy from fat, 29% of total energy from carbohydrates, 17% of total energy from protein) *Both diets contained same amount of carnitine-rich foods	• Plasma concentrations of total carnitine and free carnitine ↑ significantly by day 3 and 5 on the low-carbohydrate, high-fat diet compared to high-carbohydrate, low-fat diet ($p<0.05$ for between group comparison). • The low-carbohydrate, high-fat diet group significantly ↑ the total carnitine concentration by day 5 (day 5 vs day 1 within group comparison, $P<0.05$). • Both diets significantly↑ the plasma acylcarnitine concentrations and the acylcarnitine-to- free carnitine ratio by day 5 (day 5 vs day 1, $p<0.05$ for changes within diet group). However, these changes did not significantly differ between diet groups after each dietary intervention period. • There was a progressive, statistically significant ↑ in urinary excretion of total-, free-,and acyl-carnitine concentrations between days 3-5 on the low-carbohydrate, high-fat diet compared to the high-carbohydrate, low-fat diet ($p<0.05$ for between group variation). The mean excretion of total-and acyl-carnitine was also ↑ between days 4-5 compared to days 1-5 in the low-carbohydrate, high-fat group ($p<0.05$ for within group comparison). • No significant changes in body weight were noted.	• Changes in plasma and urine concentrations of free-, total-, acyl-carnitine concentrations, and acyl-to-free-carnitine ratio	• Radioenzymatic assay

Davis et al., 1990 (69)	• Feeding trial	• 2-week initiation diet providing 1000-2000 kcals of energy/day • 2-month intervention	• n=10 obese female adults part of an outpatient weight management clinic • Liquid formula group: mean age 33 y/o & 152 ± 7.2% IBW • Meat/Fish/Poultry group: mean age 28 y/o & 161 ± 8% IBW	• Changes in plasma and urinary excretion of total-, free-, - and acyl-carnitine concentrations	1. Liquid formula very-low carbohydrate diet (n=5) **Providing 420 kcals of energy/day (70 gms protein, 30 gms carbohydrates, 4.4 μmol carnitine/day) 2. Lean Meat/Fish/Poultry very-low-carbohydrate diet (n=5) **Providing 500-600 kcals of energy/day (60-70% of total energy from protein, 30-40% of total energy from fat, <10 gm carbohydrates, 375 μmol carnitine/day) **Only lean meat diet supplemented with MVI w/ minerals ***All participants reported to clinic weekly for weight loss instruction.	• Plasma total carnitine was ↑ among subjects receiving the meat/fish/poultry diet compared to the liquid diet over the intervention period ($p<0.05$). Plasma total carnitine slightly ↑ in the meat/fish/poultry group but ↓ by 21% in subjects of the liquid formula diet. • Plasma short-chain acylcarnitine concentration ↑ and free carnitine ↓ significantly ($p<0.05$) over the intervention period in both diet groups. • No significant effect of either diet on long-chain acylcarnitine concentration over the intervention period. • At 2-months, participants in the liquid formula (low dietary carnitine) group excreted significantly ↓ urinary free-and acyl-carnitine than those receiving the meat/fish/poultry diet ($p<0.05$).	• Total carnitine • Free carnitine • Short- and long-chain acylcarnitines	• Radioisotopic assay

Mathew et al., 2015 (72)	• Secondary analysis of data collected from a controlled feeding trial	• 21-days of intervention	• 13 obese M/F adults with treated HTN and stable heart failure & preserved ejection fraction & comorbities completed the study • Mean age 72 y/o & BMI 35.5 kg/m^2	• Changes in body weight and 152 serum metabolites (amino acids, free-, total-, and acyl-carnitines, phospholipids, diglycerdies, FFAs, triglycerides, & cholesterol esters).	• Sodium-restricted DASH diet (n=13)	• Block FFQ & 3-day food records displayed mean changes in percent of total energy from fat went from 37.45 ± 4.38% to 28.85 ± 3.56%, from 45.92 ± 7.82% to 52.58 ±3. 56% of total energy from carbohydrate, and from 17.58 ± 3.93% to 18.53 ± 2.05% of total energy from protein • The majority of acylcarnitines had a non- significant ↑ during the dietary intervention period except for C8:1, C10:1, C12, C12:1, C14:2, C16, C16-OH, C18, C18:2, C20:2, C20:3, and C20:4 acylcarnitine concentrations ,which all ↓. • The only statistically significant change in carnitine species between baseline and post-dietary intervention were in the C2, C3, C4, (all $p<0.03$), and C10 acylcarnitine concentrations $p=0.04$).	• Total carnitine • Free carnitine • Short-acylcarnitines (C2, C3, C4, C5, C5-DC) • Medium-chain acylcarnitines (C6, C8, C8:1, C10, C10:1, C12, C12:1, C12-OH • Long-chain acylcarnitines (C14, 14:1, C14:2, C14-OH, C16, C16:1, C16-OH, C16:2, C18, C18:1, C18:2, C18:2-OH, C20, C20:1, C20:2, C20:3, C20:4)	• Targeted liquid chromatography-mass spectrometry

High-and Low-Carbohydrate Diets, Changes in Free-and Acyl-Carnitine Concentrations, and Weight Loss

First Author, Year (Reference)	Design	Duration	Participants	Methods	Intervention	Outcomes	Major Carnitine Species	Assessment Method of Carnitine Species
Lien et al., 2009 (81)	• Prospective analysis of pooled data from phase 1 (non-randomized) and phase 2 (randomized & blinded) controlled trial (trial still active for additional 24-months at time of analysis)	• 6-month intensive behavioral intervention for weight loss (phase 1) • 6-months of follow-up during the 30-month phase 2 portion of the Weight Loss Maintenance Study	• 27 obese M/F participants part of the phase 2 of the Weight Loss Maintenance Study completed the trial • Mean age 51 y/o. & BMI 32.6 kg/m^2	• Change in body weight, fat mass and lean mass (via DEXA) • Change in fasting plasma acylcarnitine & amino acid concentrations • Changes in other molecules (blood lipids, hormones, cytokines, insulin resistance, etc).	• **Phase 1 of the Weight Loss Maintenance Study:** 20 weekly group sessions over 6-months with a trained interventionist focusing on reducing energy intake, increasing physical activity, and adopting a DASH diet • **Phase 2 of the Weight Loss Maintenance Study (Behavioral Maintenance Intervention):** 1. Monthly personal counseling by phone 2. Interactive website 3. No intervention (control group)	• After the 6-month intensive behavioral intervention, the participants reported eating on average ↓ 6.71% total calories from fat (95% CI:-10.47 to -2.81%, p<0.001), ↑ 6.53% total calories from carbohydrates (95% CI: 3.32 to 11.64%, p<0.0001), and ↑ 0.98% total calories from protein (95% CI: -2.08 to 2.12%, p=0.5787) • The mean weight change from baseline to 6-months was ↓ 13.90 lbs (95% CI: -18.65 to -8.00 lbs, p<0.0001) with mean fat mass change of ↓ 3.78 kg (95% CI: -5.51 to -1.38 kg, p=0.0001), and mean lean mass change was ↓ 1.43 kg (95% CI: -2.53 to 0.27 kg, p=0.0121) • At 12-months, weight reverted towards baseline values. • Both C8:1 and C10:3 acylcarnitine concentrations displayed small ↑ mean trends over the 6-month period. • The only significant changes from baseline was seen at week 4, when C8:1 displayed a 0.03 uM ↑ (95% CI: 0.00 to 0.06 uM, p=0.02) and C10:3 displayed a 0.02 uM ↑ (95% CI: -0.01 to 0.06, p=0.0102). • C10:3 displayed a significant ↑ of 0.02 uM (p=0.0242) at 12-months compared to baseline.	• Only plasma C8:1 and C10:3 acylcarnitines included in results	• Tandem mass spectrometry

Redman et al., 2011 (79)	• Randomized controlled feeding trial	• 2-weeks of energy-controlled isoenergetic weight-maintaining diet at baseline • 6-months of intervention	• 35 healthy sedentary overweight M/F adults randomized and completed dietary intervention • Mean age 36.8 y/o and BMI 27.8 kg/m^2	• Changes in body weight, fat mass, fat-free mass, abdominal visceral fat, & intra-hepatic fat • Changes in fasting serum concentrations of 8 fatty acids, 15 amino acids, and 45 acylcarnitines • Changes in insulin sensitivity	• **Phase A:** Energy-controlled isoenergetic weight-maintaining diet (30% of total energy from fat, 15% of total energy from protein, and 55% of total energy from carbohydrate). • **Phase B:** 1. Controlled diet (based on AHA step 1 diet, energy intake 100% of EER, n=11) 2. Energy-restricted diet (25% energy deficit, n=12) 3. Energy-restricted diet + exercise (12.5% energy deficit + 12.5% ↑ in EE by aerobic exercise 5x/wk, n=12)	• After controlling for sex and age at baseline, fasting serum C6-DC, C8, C8:1, C10, C10:1, C10:2, C10:3, C10-OH/C8-DC, C12, C12:1, C12-OH/C10-DC, C14:1, C14:1-OH, C14:2, C16:2, and C20-OH/C18-DC) acylcarnitines were positively associated with percent body fat (R2=0.75, p=0.0001). • The 25% energy deficit by energy-restriction alone and by energy-restriction + exercise resulted in equivalent losses of body weight (ER: ↓10 ± 1%; ER + EX: ↓ 10 ± 1%), fat mass (ER: ↓24 ± 3%; ER + EX: ↓25 ± 3%), abdominal visceral (ER: ↓28 ± 4%; ER + EX: ↓ 27 ± 3%) and subcutaneous fat stores (ER: ↓ 26 ± 4%; ER + EX: ↓ 28 ± 3%). • Despite similar weight loss, there was a significant ↑ in fasting serum C2 and several medium- and long-chain acylcarnitines (Ci4-DC/C4-DC, C6-DC, C8, C10, C10:1, C10:2, C10-OH/C8-DC, C12, C12:1, C12-OH/C10-DC, C14, C14:1, C14:2, C14:1-OH, C14-OH/C12-DC, C16, C16:1, C16:2, C18:1, C18:2) in the energy-restricted group that was not seen to the energy-restricted + exercise group (p=0.01). • C2, C14:1, C16, and C18:1 acylcarnitine concentrations were all uniquely ↑ from baseline to 3-months and baseline to 6-months on the energy-restricted diet and not on the energy-restricted diet + exercise (p<0.000, p=0.001, p=0.032, p=0.03).	• 45 short-, medium-, & long chain acyl-carnitine species in serum	• Gas chromatography/mass spectrometry and tandem mass spectrometry

Laferrere et al., 2011 (78)	• Controlled trial	• Gastric bypass group: before and 1-month after surgery • Before and after losing 10-kg on meal replacement diet	• 21 obese M/F participants from the New York Obesity Nutrition Research Center (NYONRC) • Gastric bypass group: mean age 43.3 y/o & baseline BMI 44.9 kg/m^2 • Dietary group: mean age 47.9 y/o & baseline BMI 42.8 kg/m^2	• Changes in body weight, fasting serum acylcarnitines and amino acids • Changes in fasting plasma hormones, blood lipids, inflammatory markers, markers of insulin resistance, etc.	1. Roux-en-Y gastric bypass intervention (n=10) 2. 10-kg diet-induced intervention (1000 kcals of energy/day meal replacement diet, n=11)	• Before weight loss, in both groups, acylcarnitines were negatively correlated with BMI (r= -0.521, p=0.015). • Significant weight change occurred on both groups compared to baseline (p<0.05), with faster weight ↓ in the gastric bypass surgery group (2.7 kg/week) compared to the dietary intervention group (1.3 kg/week, p=0.003). However, mean weight change between each group was not significantly different (GBP: ↓ 11.8 ± 5.3 kg, versus diet: ↓ 9.9 ± 2.3kg, p=0.303). • Serum concentrations of C3 (p=0.004), C4-DC (p=0.019), C5 (p=0.027) ↓ after gastric bypass surgery but not after dietary intervention. • Although the sum of all acylcarnitines ↑ after both types of weight loss interventions (p=0.005), the sum of C3 and C5 ↓ significantly after gastric bypass (p=0.001) but not after dietary intervention (p=0.956), reflecting the ↓ in BCAA concentrations (leucine, isoleucine, and valine, p<0.01) and related metabolites (phenylalanine, tyrosine, orthithine, and histidine, p<0.05) after gastric bypass surgery but not after dietary intervention. • C4-DC significantly ↓ only after gastric bypass surgery (p<0.05).	• 45 acylcarnitine species	• Flow injection-tandem mass spectrometry

Gu et al., 2013 (85)	• Randomized controlled feeding trial	• 8-week intervention for obese group only	• 45 healthy obese M/F adults (mean age 31.8 y/o & BMI 32.58 kg/m^2) and 30 healthy controls (mean age 28.2 y/o & BMI 21.29 kg/m^2) ***38 obese participants completed entire 8-week intervention	• Changes in body weight, insulin resistance, and 113 fasting serum metabolites (carnitine, C2, fatty acids, amino acids, etc)	• Obese participants followed a very-low-carbohydrate diet (<800 kcals of energy/day with <20 gms carbohydrate ***MVI w/ minerals provided daily	• Free carnitine was significantly ↑ at baseline in obese subjects compared to healthy controls (VIP=1.14, FC=1.25, p=2.26 x 10-4). • No statistically significant change in body weight was observed. However, mean BMI was significantly ↓ from 32.59 kg/m^2 to 30.59 kg/m^2 (p<0.05) after week 4 and further reduced to 29.88 kg/m^2 (p<0.01) by week 8. • However, after the very-low-carbohydrate diet, carnitine was altered to a less significant degree, suggesting that a very-low-carbohydrate diet may attenuate the metabolic alteration of obesity. However, it did not reach a level of significance after 4-weeks (VIP=0.57, FC-=1.12, p=3.4 x 10-2) or 8-weeks of low-carbohydrate dietary intervention compared to healthy controls (VIP=0.90, FC=1.16, p=4.45 x 10-3). • After 4-weeks of dietary intervention, C2 acylcarnitine concentration was ↑ compared to baseline (VIP=2.21, FC=1.47, p=6.89 x 10-6).	• Carnitine & C2 acylcarnitine	• Ultra performance liquid chromatography quadruple time-of-flight mass spectrometry and by gas chromatography-time-of-flight mass spectrometry

Reinehr et al., 2015 (84)	• Secondary retrospective analysis of previously collected data before and after a randomized controlled trial	• 1-year intervention	• 80 obese M/F Caucasian children with substantial weight loss on the intervention (BMI-SDS reduction >0.5) and 80 obese children with stable weight status on the intervention (BMI-SDS <0.15) • Mean age 11 y/o & BMI 29.9 kg/m^2	• Change in 14 serum metabolites previously observed to be altered in obese children (C12:1 and C16:1 acylcarnitines, proline, methionine, glutamine, & 9 phospholipids.	• All participants followed an outpatient lifestyle intervention called "Obeldicks," an optimized mixed diet (30% total energy from fat, 15% of total energy from protein, & 55% of total energy from carbohydrates) using a "traffic-light system. ***Group and individual diet, exercise, & behavior therapy.	• C12:1 and C16:1 acylcarnitine concentrations were not significantly different after 1-year compared to baseline in either the weight loss group (p=0.967, p=0.604) or the weight stable group (p=0.906, p=0.700). • Glutamine and methionine both ↑ from baseline to 1-year in the weight loss group (p=0.1013, p=0.026), but not in the weight stable group.	• Serum C12:1 & C16:1 acylcarnitines	• Flow injection mass spectrometry

Schooneman et al., 2016 (80)	• Randomized controlled trial	• 12-weeks of intervention	• 60 non-diabetic obese M/F participants recruited for an weight loss outpatient study • Mean age 40 y/o & BMI 34.8 kg/m^2	• Changes in total body weight, lean and fat mass (via DEXA), fasting plasma free carnitine, acylcarnitines, NEFAs, insulin, glucose, and REE.	1. Energy-restricted diet alone (↓ 600 kcals of energy/day, n=20) 2. Energy-restricted diet + exercise (10% energy expenditure, n=21) 3. Energy-restricted diet + sibutramine (n=19)	• Mean whole group weight ↓ 4.5 kg between baseline and day 84 and mean lean body mass ↓ 0.6 kg). • The diet alone and diet + exercise groups exhibited weight ↓ only up to day 28 with weight remaining stable hereafter. The sibutramine group displayed continued weight ↓ up to day 84. • All interventions ↑ fasting plasma acylcarnitines concentrations after 84-days, with the greatest ↑in the sibutramine group. • Mean whole group change in weight was negatively correlated with C2 ($p=0.01$), C4-OH ($p<0.001$), C14:1 ($p=0.01$), C16 ($p=0.01$), & C18:1 ($p<0.001$) acylcarnitine concentrations. • The ↑ in mean C4-OH, C16, and C18:1 overtime correlated significantly with a ↓in both total and lean body mass over time. • Mean whole group C2 and C4-OH were significantly ↑after 28-days ($p<0.05$) with a slight ↓ by 84-days that remained significantly ↑ compared to baseline ($p<0.05$). • Mean whole group free carnitine significantly ↑ from day 28-84 compared to baseline ($p<0.05$). • Mean whole group C10, C14:1, C16, and C18:1 significantly ↑ after 28 days ($p<0.05$) followed by a significant ↓ from day 28-day 84 ($p<0.05$).	• Free carnitine • Short-chain acylcarnitines (C2, C4-OH) • Medium-chain acylcarnitine (C10) • Long-chain acylcarnitines (C14:1, C16, C18:1)	• Tandem mass spectrometry

Smith et al., 2016 (86)	• Randomized controlled feeding trial	• Weight loss group and weight loss + high-protein group were studied until they lost 8-10% of their initial body weight and were weight stable (<2% change in body weight) for 3-4 weeks (27.8 ± 2.8 weeks & 26.4 ± 2.9 weeks) • Weight maintenance group were studied after a time-matched (27.4 ± 1.2 weeks) weight maintenance period	• 27 postmenopausal obese women • Weight loss group: mean age 58 y/o & BMI 35 kg/m^2 • Weight loss + high-protein group: mean age 58 y/o & 36 kg/m^2 • Weight maintenance group: mean age 60 y/o & BMI 36 kg/m^2	• Changes in body weight, fat mass & fat-free mass (via DEXA), plasma acylcarnitines, amino acids, FFAs, glucose, and insulin.	1. 30% energy-restricted weight loss diet (n=10) (0.8 g/kg BW/day) 2. High-protein, 30% energy restricted weight loss diet (n=10) (1.2 g/kg BW/day) 3. Weight maintenance control diet (n=7) ***All participants attended weekly counseling sessions with a dietitian	• Participants in the two weight loss groups ↓~10% of their initial body weight compared to the weight maintenance group ($p<0.05$). • The contribution of fat-free mass to total weight loss was ~45% ↓ in the weight loss high-protein group than the normal protein weight loss group alone ($p=0.03$). However, the absolute loss of fat free mass was small so that only ~ 700 grams or 1.5% of total fat-free mass was preserved by the high-protein weight loss diet compared to the normal protein weight loss group. • Mean plasma C3 and C5 acylcarnitine concentrations did not change in the weight maintenance group and tended to ↓ after weight loss in both weight loss groups, although not significant.	• Plasma C3 & C5 acylcarnitines	• Liquid chromatography-tandem mass spectrometry

Appendix B – 6-Day Cycle Menu for High-Complex-Carbohydrate Diet

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
BREAKFAST	French toast Syrup Walnuts Banana **Skim milk**	Cheerios English muffin Peanut butter Jam Banana **Skim milk**	Corn meal pancakes Syrup Frozen berries Margarine **Yogurt** Orange juice	Quaker Granola **Yogurt** Whole wheat toast Margarine **Skim milk**	Spinach **potato frittata** Whole wheat toast Margarine Jam Canned peaches Apple juice	Bagel **Cream cheese** **Yogurt** Orange juice
LUNCH	Bean soup Corn bread Margarine Romaine & spinach salad Ranch dressing Apple juice	Whole wheat bread **Tuna salad** Mozzarella cheese Romaine & spinach salad Italian dressing Orange juice	Garden burger Whole wheat bun Tomato Mayonnaise Ketchup Simple salad Fresh ginger dressing **Skim milk**	Whole wheat pita **Chicken salad** Tomato Romaine & spinach salad Italian dressing Peanuts V8 juice	Corn & **zucchini** chowder Simple salad Ranch dressing Potato chips Apple Grape juice	Whole wheat bread **Turkey** Tomato Mayonnaise Simple salad Fresh ginger dressing Fresh orange V8 juice
DINNER	**Pizza** Simple salad Balsamic dressing **Skim milk**	Beans and rice **Chicken breast** Wilted spinach, almonds, & carrots Ranch dressing Graham crackers **Skim milk**	**Tenderloin steak** Spinach, **garlic**, & mashed garbanzo bean salad Jell-O **Skim milk**	Spaghetti Veggie marinara sauce Parmesan cheese Whole wheat bread Wilted spinach & almonds Oat Maple Cookie **Skim milk**	**Cod** with pecans Scallion rice Romaine & spinach salad with mandarin oranges & red peppers Balsamic dressing **Skim milk**	Vegetable & **chicken** stir fry Rice Cashews Jell-O **Skim milk**
SNACK	Peanuts Dried apricots Fresh orange Pretzels	Pistachio nuts Dried apples Grape juice	Almonds Dried apricots Fresh orange	Celery with peanut butter Banana Apple juice	Almonds Graham crackers Hot chocolate	Raisins Carrot sticks
Bolded = Food items with ≥ 1 mg of carnitine per 100 gram or 100 mL serving. Source: Demarquoy, et al (12)						

Appendix C – 6-Day Cycle Menu for Low-Carbohydrate Diet

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
BREAKFAST	**Egg & cheese bake** *Western Family* **bacon** *Oscar Mayer* **ham slice**	**Saucy cheese & eggs** *Oscar Mayer* **pork sausage links** *Western Family* **bacon**	**Four cheese herb quiche** *Western Family* **bacon** *Oscar Mayer* **ham slice**	**Ham and Swiss eggs** *Oscar Mayer* **pork sausage links** *Western Family* **bacon**	Country **quiche** *Western Family* **bacon** *Oscar Mayer* **ham slice**	**Bacon and bleu cheese quiche** *Oscar Mayer* **pork sausage links** *Western Family* **bacon**
SNACK	*Precious* **Monterey jack cheese stick** *Shy Anne* **beef jerky** **Hard-boiled egg**	Mozzarella cheese stick *Baken-ets* **pork rinds** *Lisa's* clam dip *Shy Anne* **pepperoni stick**	*Shy Anne* **beef jerky** *Frigo* **cheddar cheese stick** **Hard-boiled egg**	*Oscar Mayer* **ham slices** *Philadelphia* **cream cheese** *Shy Anne* **pepperoni stick** *Baken-ets* **pork rinds**	Celery sticks **Cream cheese** *Shy Anne* **beef jerky** **Hard-boiled egg**	Mozzarella cheese stick *Baken-ets* **pork rinds** *Lisa's* clam dip *Shy Anne* **pepperoni stick**
LUNCH	**Hamburger with Swiss cheese** Salad with lettuce, cabbage, tomatoes Olive oil & rice vinegar Olives	No bean **chili** Shredded **cheddar cheese** *Tillamook* **sour cream** Lettuce Red cabbage *Kraft* **ranch dressing**	**Broiled chicken** *Atkins* **alfredo sauce** Mushroom soup Lettuce Red cabbage *Kraft* zesty italian dressing	**Hamburger** vegetable soup Lettuce Red cabbage Shredded **cheddar cheese** **Hard-boiled egg** *Louis Rich* **oven-roasted, fat-free turkey breast** Olives *Kraft* **ranch dressing**	**Tuna salad** Lettuce leaf **Monterey jack cheese** Lettuce salad with carrots and red cabbage *Kraft* zesty italian dressing	*Oscar Mayer* **all beef frankfurter** **Hot dog sauce** Lettuce Red cabbage *Kraft* **ranch dressing**
DINNER	Broiled **chicken breast** Greek lemon **garlic** marinade Chicken broth Cucumber slices *Kraft* zesty Italian dressing	**Feta burgers** Tomato slices Mozzarella cheese slices Rice vinegar dressing	**Broiled halibut** Lemon juice **Salted butter** Soy-ginger slaw with dressing Broccoli *Atkins* **cheese sauce**	Roasted pork loin Lettuce leaf Cucumber slices Tomato slices *Kraft* zesty Italian dressing	US **hamburger** **Cheddar cheese** Cauliflower *Atkins* **cheese sauce**	**Beef tenderloin** Sautéed mushrooms Tomato slices Cucumber slices *Kraft* zesty Italian dressing
DESSERT	Sugar-free Jello-O	Lo-carb chocolate	Sugar-free Jell-O	Sugar-free Jello-O	Lo-carb chocolate	Sugar-free Jell-O
Bolded = Food items with ≥ 1 mg of carnitine per 100 gram or 100 mL serving. Source: Demarquoy, et al (12).						