Nutrient Intake, Body Composition, and Lipid Deposition among Subjects with a Fatty-Acid Oxidation Disorder and Matched Controls

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CERTIFICATE OF APPROVAL

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List of Abbreviations

ACD	Acyl-CoA dehydrogenase
ATP	Adenosine-triphosphate
CACT	Carnitine-acylcarnitine Translocase
CPT1	Carnitine Palmitoyltransferase-1
CPT2	Carnitine Palmitoyltransferase-2
DAG	Diacylglycerol
EMCL	Extramyocellular Lipid
FAO	Fatty Acid Oxidation
FAOD	Fatty Acid Oxidation Disorder
FFA	Free Fatty Acids
GLUT4	Glucose Transporter 4
HFD	High-fat diet
HFHC	High-fat, high-calorie diet
HFP	High-fat, high-protein diet
HGI	High-glycemic index
^{1}H-MRS	Proton Magnetic Resonance Spectrometry
HOMA-IR	Homeostatic Model Assessment Insulin Resistance
HSL	Hormone-sensitive lipase
IHL	Intrahepatic lipid
IMCL	Intramyocellular lipid
IRS-1	Insulin Receptor Substrate 1
IVGTT	Intravenous glucose tolerance test
KO	Knockout mice
LCHAD	Long-chain 3-Hydroxy Acyl-CoA dehydrogenase
LCT	Long-chain triglyceride
LGI	Low-glycemic index
MCT	Medium-chain triglyceride
MUFA	Monounsaturated fatty-acid
PI3-kinase	Phosphoinositide 3-kinase
PKC-θ	Protein Kinase C
^{31}P-MRS	Phosphorus Magnetic Resonance Spectrometry
PUFA	Polyunsaturated fatty-acid
RQ	Respiratory Quotient
SAT	Subcutaneous Adipose Tissue
SCD1	Stearoyl-CoA Desaturase-1
SFA	Saturated fatty-acid
TFP	Trifunctional protein
VAT	Visceral Adipose Tissue
VLCAD	Very-long chain acyl-CoA dehydrogenase
WT	Wild-type mice

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Abstract

Previous investigators have studied the interactions of various diets and lipid deposition in healthy, physically-fit subjects, providing insight into the relationship between lipid deposition and diet. While high-fat diets have been associated with increased intramyocellular lipid (IMCL) deposition and high-carbohydrate diets have been shown to influence intrahepatic lipid deposition (IHL), high-protein diets may be related to decreased IHL deposition. Although it is understood that excess free fatty-acid (FFA) influx contributes to tissue lipid deposition, the influence of diet remains unresolved. Studying the interaction of diet and lipid deposition in subjects with an inherited defect in the fatty acid oxidation pathway provided a unique opportunity to examine relationships between mitochondrial function, accumulation of fatty-acid oxidation intermediates, differences in dietary intake and the effects of these changes on body composition. Preliminary data demonstrated that patients with Long-chain 3-Hydroxy Acyl-CoA dehydrogenase (LCHAD) deficiency had higher total body fat and extramyocellular (EMCL) lipid deposition, but similar levels of IMCL and IHL compared to controls. This study addressed whether impaired fatty acid oxidation (FAO) influences the deposition of IHL and IMCL.

This was a primary sub-analysis of an ongoing prospective randomized cross-over study. Fifteen subjects with a confirmed diagnosis of very-long chain acyl-CoA (VLCAD), LCHAD, or Carnitine Palmitoyltransferase-2 (CPT2) deficiency were recruited for this study. In addition, 15 controls were recruited to participate in the study. Subjects were counseled to consume a low-fat diet. All participants completed three-day diet records that were analyzed using ESHA Food Processor for average total energy, macronutrients, and medium-chain triglyceride (MCT). Subjects completed ^{1}H-MRS

measurements of liver and soleus muscle lipid content during an inpatient admission at Oregon Health and Science University.

Impaired FAO, observed in subjects with a long-chain FAO disorder, was not shown to influence lipid deposition, compared to controls. There were no significant differences in energy or macronutrient intake between groups. Five of the subjects with FAO disorders reported daily MCT supplementation. A significant positive relationship between total fat intake and IMCL deposition was observed in controls. Although not significant, this relationship was negative in subjects with FAO disorders. There were no additional relationships observed between other diet variables and lipid deposition in either group.

Although patients with FAO disorders are commonly instructed to restrict LCT and replace it with MCT, subjects that reported daily MCT supplementation did not have significantly different LCT intake from FAO subjects who did not report MCT supplementation. Trends observed in preliminary data may have not been repeated in this current study, due to differences in age of the subjects and low power resulting from a small number of pairs recruited. Opposite relationships related to fat intake and IMCL deposition observed in controls and FAO disorders may suggest having a FAO disorder may change the relationship between dietary intake and subsequent muscle lipid accumulation.

Introduction and Specific Aims

Increased intrahepatic (IHL) and intramyocellular lipid (IMCL) deposition are associated with the development of insulin resistance. However, the role of dietary intake such as total energy, fat, protein, and carbohydrate compared to the role of metabolism, including flux through the fatty acid oxidation (FAO) pathway on IHL and IMCL accumulation, is not fully understood. This project examined the associations between dietary intake, energy metabolism, and substrate oxidation on IHL and IMCL deposition.

Previous investigators have studied the interactions of diet and lipid deposition in healthy, physically-fit subjects and subjects with existing insulin resistance. While these studies have provided insight into the relationship between lipid deposition and diet, studying patients with long-chain FAO defects allowed us to separate the effects of mitochondrial dysfunction, decreased fatty acid oxidation and dietary intake.

Patients with FAO disorders were studied to examine relationships between mitochondrial function, accumulation of FAO intermediates, differences in dietary intake and the effects of these changes on body composition. Preliminary data demonstrated that patients with Long-chain 3-Hydroxy Acyl-CoA dehydrogenase (LCHAD) deficiency had higher total body fat and extramyocellular (EMCL) lipid deposition, but similar levels of IMCL and IHL compared to controls. This study addressed whether impaired fatty acid oxidation influences the deposition of IHL and IMCL, and if dietary intake of medium-chain triglyceride (MCT) oil or a low-fat diet affects tissue lipid deposition.

The overall goal of this project was to evaluate the relationship between differences in dietary intake and lipid deposition between subjects with an inherited defect in the fatty acid oxidation pathway and matched controls. This study used 3-day diet records and body composition measurements to address the following specific aims:

1. To compare IHL and IMCL deposition among adult patients with long-chain FAO disorders and matched controls.

   Hypothesis: Subjects with long-chain FAO disorders will have increased IHL, but similar IMCL deposition.

2. To determine the correlation of dietary intake of total energy and macronutrient distribution (%CHO, % total fat, % MCT, and % protein) and IHL and IMCL among patients with long-chain FAO disorders and matched controls.

   Hypothesis: A low-fat, high CHO diet will be associated with increased IHL, but not IMCL.

   Hypothesis: High total fat intake will be associated with higher IMCL.

This project provided a unique opportunity to investigate the role of fatty acid oxidation and its interaction with diet on IHL and IMCL deposition. Results may provide insight on health outcomes for patients with long-chain fatty acid oxidation disorders and expand our understanding of factors influencing IHL, IMCL and the development of insulin resistance.

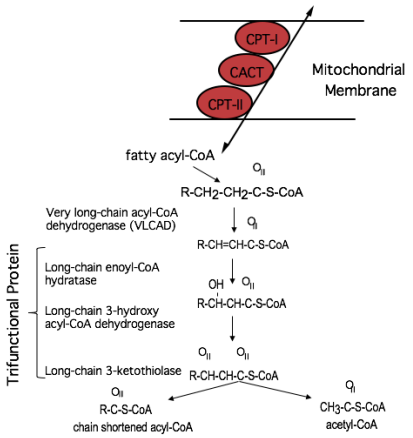
Background

Overview of FAOD

Normal Metabolism of Long-chain fatty-acids

In healthy individuals, FAO primarily functions as an endocrine-mediated response to fasting and increased energy demands that result from glycogen depletion, such as periods of fasting or increased muscular activity.[1]

Figure 1: Fatty Acid Oxidation Pathway



Long and medium chain FAO provides acetyl-coA and reducing equivalents for the TCA and the electron transport chain

During periods of fasting and stress, decreased circulating levels of glucose and insulin allow triglycerides stored in adipose tissue to be hydrolyzed into free-fatty acids (FFA) through activation of hormone-sensitive lipase (HSL). The non-esterified fatty acids mobilize through circulation bound to albumin to become available to provide energy to peripheral tissues through FAO.[2,3]

Following entry into cells and the mitochondria, FAO occurs in a repeating four-reaction cycle, resulting in one molecule of acetyl-CoA and a fatty-acid chain shortened by two carbons (Figure 1).[1]

Long-chain fatty-acids require the carnitine cycle to enter the mitochondria. Between the outer and inner mitochondrial membrane, Carnitine Palmitoyltransferase-1 (CPT-1) catalyzes the exchange of CoA for carnitine to form acylcarnitine.[4] This is the rate-limiting step for FAO in insulin-sensitive tissues, such as the liver and muscle. Increased glucose uptake in the post-prandial state leads to increased concentrations of acetyl-CoA, and subsequent conversion to Malonyl-CoA. High intracellular levels of Malonyl-CoA inhibit CPT-1 and prevent transport of fatty acids into the mitochondria.[1] Alternatively, during periods of fasting, cystolic acetyl-CoA concentrations decrease followed by a decrease in

Malonyl-CoA, reducing the inhibition of CPT1 and allowing transport of fatty acids into the mitochondria.[1]

Carnitine-acylcarnitine translocase (CACT) facilitates the transfer of the acylcarnitine across the impermeable inner mitochondrial membrane. Carnitine palmitoyltransferase-2 (CPT2) catalyzes the exchange of carnitine for CoA, reforming the fatty acyl-CoA.[4]

After import into the mitochondrial matrix, chain-specific dehydrogenases oxidize fatty acyl-CoA to begin the four-step process, beginning with a specific acyl-CoA dehydrogenase (ACD). Tri-functional protein (TFP) is responsible for catalyzing the hydratase, dehydrogenase, and thiolase reactions to ultimately produce one acetyl-CoA molecule and a shortened fatty-acid chain cleaved by two carbons. In most tissues, acetyl-CoA is completely oxidized and produces adenosine triphosphate (ATP) through the Krebs Cycle and Electron Transport Chain. In the liver and to a lesser extent in the kidney, acetyl-CoA is used to generate ketone bodies to be used an alternative energy source for tissues.[5]

CPT 2 Deficiency

As mentioned previously, CPT2 is located on the inner mitochondrial membrane and is responsible for catalyzing the exchange of carnitine for CoA to reform the long-chain fatty acyl-CoA to be used as the substrate for fatty-acid oxidation.[6]

Patients with CPT2 deficiency have inherited mutations in the CPT2 gene that substantially impair the function of the protein, however small amounts of residual CPT2 activity remain. CPT2 deficiency results in accumulation of acylcarnitines in the mitochondria and impaired energy production. [1]

VLCAD Deficiency

Very-long chain acyl-CoA (VLCAD) is bound to the inner mitochondrial membrane and catalyzes the first reaction of FAO for fatty acids with a chain length of 14 to 20

carbons. This dehydration reaction generates a double bond between the second and third carbons of the acyl-CoA, forming 2-enoyl-CoA.[7]

Similar to CPT2, patients with inherited defects in the VLCAD gene, ACADVL, have significantly reduced FAO, but maintain some small amounts of residual enzyme activity. VLCAD deficiency results in impaired energy production during periods of fasting and stress, and potentially toxic effects of long-chain acyl-CoA intermediate accumulation in the mitochondria.[7]

LCHAD/TFP Deficiency

LCHAD deficiency is a FAO disorder that is caused by a mutation in the HADHA gene.[5] The HADHA gene encodes for the alpha subunit of TFP. TFP is responsible for catalyzing the hydratase, 3-hydroxy-acyl CoA dehydrogenase, and thiolase reactions of long-chain FAO in the mitochondrial matrix.[5] In isolated LCHAD deficiency, the dehydrogenase reaction is most severely impaired, affecting the conversion of long-chain 3-hydroyxlacyl-CoA esters into 3-ketoacyl-CoA esters.[8] In TFP deficiency, there is decreased activity of all three enzymes.[5]

LCHAD/TFP deficiency results in impaired energy production, accumulation of long-chain 3-OH acyl CoA intermediates in the mitochondria, and hypoglycemic crises during prolonged fasting or increased energy demands.[5]

<u>Lipotoxicity Hypothesis</u>

Although the role of mitochondrial function in lipid-induced insulin resistance remains unresolved, the lipotoxicity hypothesis links impaired mitochondrial FAO with the development of insulin resistance. This theory suggests that mitochondrial dysfunction results in impaired capacity to oxidize fatty acids, leading to accumulation of cystolic lipid by-products, including diacylglycerols (DAG), ceramides, and triglycerides in skeletal

muscle and liver. These cystolic lipid intermediates have been shown to activate PKC-θ, leading to a serine/threonine phosphorylation cascade and increased serine phosphorylation of IRS-1, causing decreased tyrosine phosphorylation of IRS-1 upon insulin stimulation, decreased activity of PI3-kinase, and decreased activation of GLUT4, affecting insulin-mediated glucose disposal.[9]

The storage of triglycerides in non-adipose tissue, such as muscle and liver can be referred to as ectopic fat.[10] Research has shown that increased IMCL and IHL are associated with insulin resistance.[11-13]

In this model, insulin sensitivity would be improved by reducing ectopic fat. One potential pathway to reduce ectopic fat would be to increase FAO and oxidize lipid stores.

The relationship between diet, lipid deposition, and insulin resistance

Intramuscular Lipid (IMCL)

Skeletal muscle plays an integral role in insulin-stimulated glucose homeostasis. Insulin facilitates glucose control through glucose uptake into skeletal muscle and inhibition of glucose production in the liver.[14]

Insulin resistance has been associated with adipose tissue dysfunction and a reduced lipid-buffering capacity, leading to an increased influx of triglycerides and FFAs to non-adipose tissue. As FFAs enter the myocytes, they face three fates: FAO, deposition in IMCL, or synthesis of lipid intermediates.[14] The impaired capacity of skeletal muscle to oxidize fat in combination with increased lipid supply and uptake causes excess fat storage in skeletal muscle, referred to as IMCL.[15] IMCL can potentially be metabolized to DAGs, which may activate PKC, subsequently impairing insulin signal transduction.[16]

IMCL deposition is determined by a balance between FFA influx and expenditure in skeletal muscle, thus increased FFA influx into muscle and/or impaired FAO due to

mitochondrial dysfunction may cause IMCL accumulation.[17] This accumulation of IMCL has been shown to correlate to insulin resistance in skeletal muscle.[18]

Although it is understood that excess FFA influx contributes to IMCL deposition, the influence of diet remains unresolved. The literature provides evidence that excess calories, but also starvation leads to increased IMCL.[19] Furthermore, diets high in fat and low in carbohydrate contribute to IMCL deposition, but diets with excess calories from carbohydrates have similarly been shown to increase IMCL stores.[19] These inconsistencies in lipid deposition consequently produce opposing results in relation to insulin sensitivity. With such conflicting evidence, it is difficult to determine which aspects of diet truly influence excess FFA influx and subsequent IMCL deposition. Differences in study methods, particularly length of intervention diet and measurement of IMCL and insulin sensitivity contribute to these contradictory results.

Short-term, high-fat, excess calorie diets have been shown to increase IMCL by 30-52% and reduce whole-body insulin sensitivity in healthy, lean men.[20,21] Interestingly, Brons et al studied a similar population of men consuming a high-fat, excess calorie diet for the same duration and observed no change in IMCL deposition and no effect on whole-body insulin resistance, but did observe hepatic insulin resistance. [22] It is important to consider the type of lipid identified in the skeletal muscle, as increased IMCL doesn't always indicate elevated DAG level, and this may affect whether or not insulin sensitivity of the muscle is affected.[16] All three papers employed use of the hyperinsulinemic-euglycemic clamp to measure insulin sensitivity, however Lundsgaard et al and Gemmnick et al used ^{1}H-MRS to measure IMCL and EMCL in a defined muscle relative to the water peak, while Brons et al performed ^{31}P-MRS on the forearm flexor and tibialis anterior muscles.[22] Although ^{31}P-MRS is useful in assessing metabolite content within tissues, it is not as sensitive of a measure

as ^{1}H-MRS.[23] Additionally, differences in muscle fiber type content of the various muscles measured may have contributed to conflicting results.

Johannsen et al studied the effect of an 8-week high-fat, high calorie diet in healthy men. ^{1}H-MRS was performed on the soleus and tibialis anterior and a two-step hyperinsulinemic-euglycemic clamp was used to measure insulin sensitivity. There was no observed change in IMCL deposition, however there was a significant increase in IHL. Although IMCL was unaffected, both liver and muscle tissue became more insulin resistant after 8 weeks.[24]

Excess caloric intake, in combination with high-fat consumption appears to be related to increased lipid deposition. It is difficult to determine if this effect is due to the excess calories, the increased fat, or a combination of both. To answer this question, researchers studied the effect of a high-fat, weight-maintaining diet on lipid deposition. Kakehi et al and Sakurai et al followed healthy men and women on a 3-day, high-fat, isocaloric diet. Both studies performed ^{1}H-MRS on the tibialis anterior and soleus and reported a significant increase in IMCL. Hyperinsulinemic-euglycemic clamp was used to measure peripheral insulin sensitivity. While Kakehi et al observed insulin resistance, Sakurai et al reported no change in insulin sensitivity despite the increase in IMCL.[16,17]

When studying a high-fat, weight-maintaining diet in overweight men for 3 weeks, van Herpen et al found no change in vastus lateralis IMCL, but a significant increase in IHL. The results demonstrated no change in insulin sensitivity, but decreased metabolic flexibility as evidenced by a smaller change in RQ upon insulin stimulation.[25]

Ryberg et al observed IMCL in the soleus and IHL in obese, postmenopausal women after a 5-week, high-fat, low-carbohydrate, weight-maintaining diet. Consistent with results produced by van Herpen et al, IMCL concentration did not change, IHL increased,

and there was no effect in insulin sensitivity. It is important to note that this study included a physical activity regimen which may have affected results independent of diet.[26]

Dietary interventions such as low-glycemic diets have shown improvements in insulin sensitivity in obese, insulin-resistant subjects.[27] Thus, studying the glycemic index in addition to a low-carbohydrate diet, is worth consideration. Haus et al assigned obese, sedentary individuals to a 7-day low-glycemic, high-fat diet or high-glycemic, low-fat diet and measured IMCL concentration in the soleus. The low-glycemic, high-fat diet increased IMCL, while the high-glycemic, low-fat diet exhibited no change in IMCL. The low-glycemic group demonstrated improved insulin sensitivity, however this was independent of diet and was shown to be related to increased physical activity during the study period.[28]

Considering there is a relationship between high-fat diets and IMCL accumulation, studying the association of different types of dietary fats and IMCL is of interest. Jans et al observed the effect of high-fat liquid meals of differing fatty-acid compositions in the vastus lateralis of obese, insulin-resistant women. The subjects were assigned to a liquid meal composed of high proportions of PUFA, MUFA, or SFA. There was no observed effect in IMCL composition after all three of the meals, however the PUFA meal improved post-prandial insulin sensitivity and reduced FA uptake into muscle, compared to the SFA and MUFA meals. The authors suggest that the PUFA meal improves insulin sensitivity through reduction of lipid overflow and promotion of FAO, as measured through RQ.[15]

A systematic review conducted by Ahmed et al highlighted that weight-maintaining high-carbohydrate diets decreased IMCL, however diets with excess calories from carbohydrate increased IMCL. Excess carbohydrate intake when coupled with excess calories may result in reduced insulin sensitivity as a result of energy surplus.[20] Diets high in carbohydrate and low in fat have shown stable concentrations of IMCL and DAG, with increased glucose uptake and improved insulin sensitivity.[20] While starvation diets have

been shown to increase IMCL stores, hypocaloric diets without weight loss demonstrate no change in IMCL.[19]

Johnson et al assigned physically-fit men to either a high-fat, low-carbohydrate diet or a starvation diet for 3 days to study the differences in vastus lateralis IMCL and insulin sensitivity. The researchers observed increased IMCL and insulin resistance in both the low-carbohydrate and starvation groups, with no significant differences between groups. This study design did not include the use of a hyperinsulinemic-euglycemic clamp, using an intravenous glucose-tolerance test (IVGTT) to measure insulin sensitivity instead.[29]

Similarly, Green et al studied the effects of a 3-day starvation diet using IVGTT and ^{1}H-MRS of the vastus lateralis, but compared this diet to a carbohydrate-restricted, high protein diet and a mixed-carbohydrate diet. Both Green et al and Johnson et al defined starvation diet as a water-only diet. Consistent with the results of Johnson et al, starvation caused increases in IMCL stores and insulin resistance. Differences were not observed between the low-carbohydrate, high-protein group and the mixed carbohydrate group, however both diets resulted in an increased IMCL to water ratio.[30]

Carbohydrate restriction and starvation diets result in mobilization of endogenous lipid stores and concurrent elevation of plasma FFA. This elevation of plasma FFA can contribute to excessive influx of FFA, leading to IMCL deposition and reduced insulin sensitivity.[30]

Lindeboom et al observed IMCL in the tibialis anterior and IHL using ^{1}H-MRS in lean, healthy subjects after a high fat meal with protein (HFP) or without protein (HF). Although both the HFP and HF meals were associated with increased IHL deposition, no changes in IMCL concentration were observed. After both meals, plasma FFA concentrations were not elevated. Studies that have shown a rapid increase in IMCL after lipid infusion reported increased plasma FFA concentrations.[31,32] Lack of elevation in plasma FFA suggests that

skeletal muscle may be more sensitive to elevated plasma FFA concentration, than elevated triglyceride concentrations, with respect to lipid deposition.[10]

Intrahepatic Lipid (IHL)

The liver plays a key role in regulation of carbohydrate and lipid metabolism. Research has shown that high fat diets contribute to hepatic lipid accumulation through excess plasma FFA. The increased influx of FA stimulates gluconeogenesis, subsequently increasing plasma glucose. In the presence of excess FFA, triacylglycerol, ceramides, and DAG accumulate inside the hepatocytes, interfering with the insulin signaling pathway.[33]

The literature provides varying evidence on the effect of diet on IHL deposition. Studies have shown that a high-carbohydrate diet in combination with excess calories contributes to increased IHL deposition.[10,20,24] This effect is exaggerated in diets dominated by simple carbohydrates and diets with a high glycemic index.[34,35] This increased IHL concentration is associated with decreased hepatic insulin clearance, decreased insulin sensitivity, and increased plasma triglyceride levels.[20,24] However, there is also research to support that a weight-maintaining, high carbohydrate diet decreases IHL deposition, with no effect on insulin sensitivity.[25,26]

Potential mechanisms to explain high carbohydrate intake resulting in decreased insulin sensitivity and increased IHL include increased insulin secretion and decreased ability to adapt fuel oxidation to fuel availability.[36]

The addition of protein to a high-carbohydrate meal has been shown to maintain insulin sensitivity, despite increases in IHL.[10] However, subjects on a long-term, high-protein, low-carbohydrate, weight-maintaining diet demonstrated a decrease in IHL, compared to subjects assigned a high-carbohydrate, low-protein diet.[37]

High-calorie, high-fat diets have also been shown to increase IHL deposition and reduce hepatic insulin sensitivity, while whole-body insulin sensitivity remained

unchanged.[22] Alternatively, the previously mentioned study conducted by Lundsgaard et al reported that high-calorie, high-unsaturated fat diets demonstrate improved hepatic glucoregulation and decreased de novo lipogenesis.[20]

Garbow and colleagues fed mice a ketogenic diet for 6 weeks and reported the mice developed hepatic steatosis, but a preserved systemic insulin response.[38] However, similar mice studies have shown a blunted insulin-mediated suppression of hepatic glucose production in response to a ketogenic diet for 5 weeks.[39] These results may be explained by reduced lean body mass in ketogenic-fed mice and disparate phenotypes between liver and muscle in ketogenic-fed mice.[38]

MCT Supplementation

Although MCT supplementation is often used in the treatment of FAOD, the effects of long-term MCT use on lipid deposition in humans is not fully understood. Tucci et al fed VLCAD-knockout mice (KO) a long-chain triglyceride (LCT) diet, MCT diet, or LCT diet plus MCT bolus over five weeks. The KO mice fed the MCT diet developed severe hepatic steatosis, while the wild-type (WT) and KO mice fed the LCT diet did not show IHL accumulation. The mice fed a LCT diet with bolus of MCT displayed increases in IHL, but not to the extent of the MCT diet.[40]

In a follow-up study, Tucci et al fed KO and WT mice either a LCT diet or LCT diet with MCT for one year and measured insulin sensitivity, IMCL, and IHL concentration. Both WT and KO female mice fed MCT displayed insulin resistance, evidenced by increases in HOMA-IR, while male mice exhibited a milder insulin resistance. There was a 40% increase in IMCL in KO and WT female mice fed the MCT diet and severe hepatic steatosis in the KO female mice. Tucci et al proposed sex-specific differences are related to decreased hepatic stearoyl-CoA desaturase 1 (SCD1) activity in females, resulting in more severe injury and systematic oxidative stress in response to MCT supplementation.[41]

Diet and Lipid Deposition in Fatty-Acid Oxidation Disorders

Because increased ectopic lipid deposition and insulin resistance have been associated with impaired mitochondrial FAO, studying subjects with an inherited defect in FAO is of interest, as it provides insight into the relationships between mitochondrial function and the effects on body composition and glucose metabolism.

Treatment for long-chain fatty acid oxidation disorders includes dietary restriction of LCT, replacement of LCT with MCT supplementation, and increased intake of carbohydrate and protein to provide alternate fuel sources for energy production.[42] As a result, individuals with FAO disorders typically consume very high carbohydrate diets and eat frequently to avoid fasting.[42] While high-carbohydrate, high-calorie diets have shown insulin resistance in physically-fit and overweight subjects with efficient FAO, no individual with LCHAD deficiency and glucose intolerance has been reported in the literature.[43]

Gillingham et al studied body composition, lipid deposition, and glucose tolerance, measured through OGTT, in adolescents with LCHAD deficiency and matched controls. Subjects with LCHAD deficiency displayed greater EMCL deposition and total body fat compared to controls, however IMCL and IHL were similar between groups.[43] LCHAD-deficient subjects demonstrated characteristics commonly related to insulin resistance such as lower energy expenditure, increased body fat, increased long-chain acylcarnitine levels, and lower HMW adiponectin levels, however they exhibited normal insulin sensitivity.[43]

Summary of the Literature

The present literature provides insight into the degree diet affects IMCL and IHL lipid deposition and insulin resistance. Although the literature presents varying evidence, it appears that extremes in excessive fat, carbohydrate, and energy increase IMCL and IHL deposition. However, the extent to which excess calories, high-carbohydrate, or high-fat individually affects lipid deposition is not fully known. The effects of lipid deposition relative

to insulin sensitivity seem to depend on the study population, length of the intervention diet, and differences in measurement of tissue and insulin sensitivity.

Many of the studies reviewed include specific populations, such as physically-fit men or overweight and/or obese individuals, that are difficult to generalize to the general population, as well as the FAOD population. Studies observing lipid deposition and diet specifically in the FAOD population are extremely limited. It is difficult to compare the results across studies, as the length of intervention duration varied extensively from single PO loads, to short-term diets and long-term diets. Additionally, muscle tissue selected to observe IMCL composition varied across studies and contributed to differences in results. No research to date has looked at the effects of MCT intake on muscle or hepatic lipid deposition in humans.

Methods

Population Description

Study Participants and Recruitment

This is a primary sub-analysis of an ongoing prospective randomized cross-over study. Approval was obtained for this research from the OHSU Institutional Review Board (eIRB#11258). Participants were consented prior to the first admission and medical records were reviewed to document that the participants met the inclusion criteria.

Subjects with a fatty acid oxidation disorder

Inclusion Criteria: 15 subjects with a confirmed diagnosis of VLCAD, LCHAD/TFP, or CPT2 deficiency were recruited for this study. Subjects are ≥ 18 years of age, and were willing to complete 2 CRC admissions for hyperinsulinemic euglycemic clamp studies and muscle and adipose tissue biopsies. Subjects with disorders in FAO

Table 1. Participant Selection Criteria

FAO		**Control**	
Inclusion criteria	**Exclusion criteria**	**Inclusion criteria**	**Exclusion criteria**
Confirmed CPT2, VLCAD, LCHAD, or TFP	Hgb <10g/dl INR>1.20 PTT>36 seconds Platelets<150 K/mm^3	Same gender and similar age/BMI as subject with FAO disorder	Hgb <10g/dl INR>1.20 PTT>36 seconds Platelets<150 K/mm^3
≥18 years	Pregnant /lactating females	≥18 years	Endocrine disorder such as diabetes, or untreated thyroid disease
Ability to travel to OHSU	Endocrine disorder such as diabetes, or untreated thyroid disease	Good health, exercise < 30 min, 3 x per week for 2 weeks prior to study visits.	Cardiovascular disease or elevated plasma lipids
Ability and willingness to complete protocol	Cardiovascular disease or elevated plasma lipids	Ability and willingness to complete protocol	Taking lipid lowering meds
	Regularly taking meds that strongly affect bleeding, bruising or platelets.		Regularly taking meds that strongly affect bleeding, bruising or platelets

were recruited to participate in this study through announcements on the FAO family support network website, through OHSU's clinical population, and through letters sent to metabolic specialists across the US. Inclusion and exclusion criteria are in **Table 1.**

Confirming the Diagnosis: Each subject has a confirmed diagnosis of VLCAD, LCHAD/TFP, or CPT-2. Diagnosis of disorders in fatty acid oxidation were confirmed by obtaining medical record results of acylcarnitine profiles, fatty acid oxidation probe studies in cultured fibroblasts, and/or mutation analysis. Establishing the diagnosis of these disorders is a complex process that varies across metabolic centers in the US. Not all potential subjects had a skin biopsy and FAO probe studies in cultured fibroblasts. In many cases mutation analysis can identify only one recognized mutation in the sequenced exons of the gene. Thus, a combination of methods is most often used to establish the diagnosis. If two of the three diagnostic tests suggested a diagnosis of VLCAD, LCHAD/TFP, or CPT2 deficiency the subject was identified as eligible to participate in the trial (**Table 2**).

Table 2: Medical Records to Confirm the Diagnosis

Disorder	Acylcarnitine Profiles	Fatty acid oxidation Probe studies	Mutation analysis
VLCAD	↑ C14:0 or C14:1	↓ FAO flux with ↑ disease specific acylcarnitines	1 or 2 known mutations in specific gene
LCHAD/TFP	↑ OH-acylcarnitines		
CPT2	↑ C16:0 or C16:1		

Exclusion Criteria: Subjects with CPT2, VLCAD, LCHAD/TFP deficiency could not be actively participating in another research project that prohibited their participation in this study, such as a project that requires a specific supplement that must be consumed daily. All subjects were screened for anemia prior to study participation. Female subjects of childbearing potential were screened for pregnancy. Anemic (Hgb < 10 g/dl) or pregnant subjects were excluded from the study. Subjects regularly taking medications that strongly

affect bleeding, bruising, or platelets such as Coumadin, Plavix, Aggrenox, Ticlid, Agrylin, Xagrid, Aricept, Namenda, Exelon, Razadyne, or aspirin were excluded from the study. Subjects were excluded if they were diagnosed with diabetes or were on medications to treat diabetes. We did not anticipate any subjects with CPT2, VLCAD, or LCHAD/TFP to have diabetes, as it has never been reported. However, several cases of diabetes have been mentioned on disease specific list serves among subjects with MCAD deficiency.

Matched Control Subjects:

Inclusion Criteria: In addition, 15 age-, sex-, and BMI-matched controls were recruited to participate in the study through local newspaper and OHSU website announcements. Control subjects were required to be a similar age ($\pm$2 years), sex, and BMI ($\pm$2 kg/m^2) as a study subject with a FAO disorder, complete and comply with the protocol, and be in good health. Subjects had to be weight stable ($\pm$5 lbs) for the 3 months prior to each study visit and exercise < 30 minutes, three times a week (low activity) for two weeks prior to each study visit.

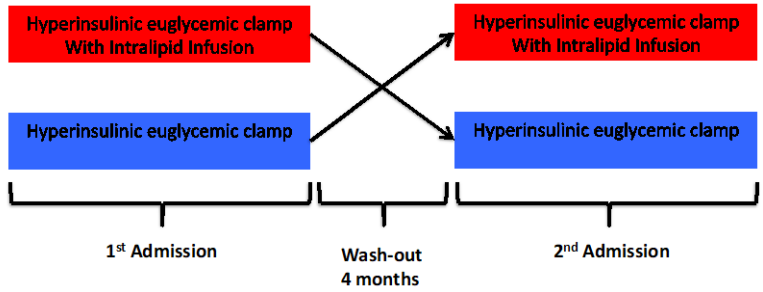
Exclusion Criteria: Control subjects could not be participating in another study that altered their macronutrient intake, such as a low carbohydrate diet. All subjects were screened for anemia prior to study participation. Female subjects of childbearing potential were screened for pregnancy. Anemic (Hgb < 10 g/dl) or pregnant subjects were excluded from the study. Subjects regularly taking medications that strongly affect bleeding, bruising, or platelets such as Coumadin, Plavix, Aggrenox, Ticlid, Agreylin, Xagrid, Aricept, Namenda, Exelon, Razadyne or aspirin were excluded from the study. Subjects could not have diabetes or be on medications to treat diabetes. Control subjects could not have a history of an endocrine disorder such as thyroid disease, renal disease or cardiovascular disease.

Inclusion of Children, Women, and Minorities: All participants were adults able to provide informed consent. We believe the complex full-day testing schedule on Day 2 of each inpatient admission, including two biopsies, is best conducted in the adult population. Both genders were included in this research project. Because FAO disorders appear to occur equally in males and females, we included similar numbers of males and females. Women of child-bearing potential are included in this research. Pregnant women were excluded because the DEXA scan could increase risk to the fetus and pregnancy could affect insulin sensitivity. Recruitment resulted in study demographics similar to that of the national disease demographics. Caucasians compromised the majority of subjects in this study. To date, the majority of patients with fatty acid oxidation disorders have been of Caucasian ancestry. We welcomed subjects from other racial backgrounds, but anticipated that more, if not all, subjects would be white.

Study Design

This is a randomized cross-over study in which all participants completed 2 inpatient admissions with a 4 month washout period in between visits (**Figure 2**). Participants were consented prior to the first admission and medical records were reviewed to document the participants met the inclusion criteria. Most of the subjects with an FAO disorder were traveling from other parts of the US to participate. Travel arrangements were made and the first inpatient visit was scheduled once the consent was complete and the participant met all of the

Figure 2: Randomized cross-over study design



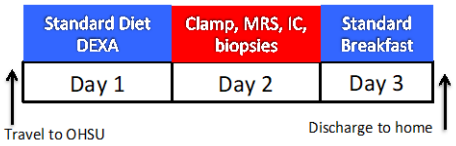
Participants completed 2 inpatient admissions with a 4 month wash-out period in between.

inclusion criteria. The outline for each inpatient visit is provided in **Figure 2**. Subjects living locally were consented in person at an outpatient screening visit while the others were consented by mail.

All subjects kept a record of foods consumed during the three days prior to inpatient admission. The food record was reviewed and confirmed by the study coordinator and graduate student during inpatient admission.

Participation included two admissions to the OHSU CTRC inpatient unit (**Figure 3).** Subjects were admitted the day before the clamp for measurement of body composition by DEXA scans. A standardized diet (20% of total calories from fat, 20% from protein, and 60% from carbohydrates) was fed to subjects prior to the clamp procedure (Day 1).

Figure 3: Inpatient visit schedule



Subjects will travel to OHSU if necessary prior to day 1. Day 1 they will be fed a standardized diet and have a DEXA scan. On day 2, a hyperinsulinemic, euglycemic clamp with MRS, indirect calorimetry and tissue biopsies will be completed. The following day, subjects will consume a standard breakfast and then be discharged to home.

DEXA: Whole body and regional fat, lean, and bone mass were measured by DEXA (Lunar iDXA, GE Healthcare Lunar, Madison, WI) through the OHSU Clinical and Translational Research Center (CTRC) Bionutrition Unit.

The following day, subjects completed a hyperinsulinemic euglycemic clamp beginning at approximately 8:00 AM and ending at about 4:00 PM (Day 2). The order of the intralipid vs. glycerol/saline infusion during HEC was randomly assigned. Subjects returned 4 months later and repeated the admission with the alternative clamp procedure. For the purpose of this sub-analysis, data from the DEXA, MRS, MRI, and 3-day diet records were used to address the hypotheses.

^{1}H MRS Procedure: of the liver and muscle were performed prior to (-100 min) of the clamp. Each subject completed 2 MRS measurements of liver and muscle lipid content prior to the HEC. Image-guided, ^{1}H localized, MRS following high-resolution T-weighted spin-echo imaging was performed on a Siemens 3T whole-body system.

MRI of the abdomen to quantify visceral and subcutaneous fat: Although not a primary outcome, these measures were acquired using a Siemens Magnetom TIM Trio 3T system.

Study Procedures

A list of all study procedures is found in **Table 3**. Data from the bolded procedures were used in this analysis.

Table 3. Schedule of Study Procedures

	Screening Visit	Prior to Admit	Day 1	Day 2	Day 3
Informed Consent/HIPAA Authorization	X	X			
Inclusion/Exclusion Assessment	X	X	X		
Demographics and Medical History	X	X			
CBC with platelets, INR, PTT			X		
Pregnancy Test (if applicable)			X		
Vital Signs and Weight	X		X	X	X
Height	X		X		
Physical Exam			X		
Standard FAO Low Fat Diet		X	X	X	X
Low Fat Diet Instruction	X	X			
3-Day Diet Diary		X			
DEXA Scan			X		
Intravenous (IV) Catheter Placement			X		
Indirect Calorimetry				X	
Magnetic Resonance Spectroscopy (MRS)				X	
Hyperinsulinemic-Euglycemic Clamp				X	
Muscle Biopsy				X	
Fat Biopsy				X	
Discharge from CTRC					X

Statistical Analysis

A summary of the statistical analyses performed is found in **Table 4.** Descriptive statistics were collected and expressed as the mean and standard deviation for subject characteristics, dietary intake, and lipid deposition data. A Shapiro-Wilk test was performed to test for normality. Although the diet data was normally distributed, ^{1}H-MRS data was not and normality was not improved with transformations. Therefore, non-parametric tests were used when analyzing ^{1}H-MRS data.

The primary outcome for the first aim of this sub-study was to compare IHL and IMCL deposition among subjects with long-chain FAO disorders and matched controls. Averages for visit 1 and visit 2 were calculated for all subjects' percent fat mass, percent fat free mass, IHL, IMCL, and EMCL. Means were compared between long-chain FAOD subjects and controls using a Mann-Whitney test.

Averages for visit 1 and visit 2 were calculated for all subjects' total energy, expressed as kcal per kilogram, and grams of carbohydrate, total fat, long-chain fat, medium-chain fat, and protein. Means were compared between long-chain FAOD subjects and controls using an unpaired t-test.

The second aim of this sub-study was to evaluate the relationship between dietary intake of total energy and macronutrients and IHL and IMCL among subjects with long-chain FAO disorders and matched controls. The strength of association between calories per kilogram and grams of macronutrients and IMCL and IHL were analyzed using a Spearman non-parametric test, where $p < 0.05$ was considered statistically significant. The strength of association between dietary factors and IHL and IMCL was analyzed using a least squares multiple linear regression.

Statistical analyses were performed in R Studio version 1.1.453 and Prism 8.

Table 4. Statistical Analysis Summary		
Specific Aim	**Hypothesis**	**Statistical Test**
1. To compare IHL and IMCL deposition among adult patients with long-chain FAO disorders and matched controls.	We hypothesized that subjects with long-chain FAO disorders will have increased IHL, but similar IMCL deposition, compared to matched-controls.	Averages of % FM, % FFM, IHL, IMCL, and EMCL were compared between FAO subjects and matched controls using a Mann-Whitney test.
2. To determine the correlation of dietary intake of total energy and macronutrient distribution (% CHO, % total fat, % MCT, and % protein) and IHL and IMCL among patients with long chain FAO disorders and matched controls.	We hypothesized that a low-fat, high carbohydrate diet will be associated with increased IHL, but not IMCL.	The strength of association between diet and lipid deposition was analyzed using a multiple regression analysis.
	We hypothesized that high total fat intake will be associated with higher IMCL.	The strength of association between total fat intake and IMCL was analyzed using Spearman non-parametric correlation analysis, where $P < 0.05$ was considered statistically significant.

Results

Subject characteristics

Subject characteristics are summarized in **Table 5.** Thirty subjects were included in this sub-analysis. Subject characteristics were similar between groups. This was expected, as control subjects were recruited to match age (±2 years), sex, and BMI of subjects with a long-chain FAO disorders. There was no significant difference in fat-free mass or fat mass between subjects with long-chain FAO disorders (35.9%±7.4, 64.1%±7.4) and controls (34.5%±8.6, 65.4%±8.6).

Table 5
Subject characteristics

	FAOD	Control
n	15	15
Age (years)	31.8 ± 11.3	35.4 ± 11.4
Height (cm)	175.1 ± 8.2	174.9 ± 13.1
Weight (kg)	85.1 ± 12.8	85.9 ± 12.0
BMI (kg/m^2)	27.8 ± 4.6	28.2 ± 4.1
Fat mass (%)	35.9 ± 7.4	34.5 ± 8.6
Fat-free mass (%)	64.1 ± 7.4	65.4 ± 8.6

Data are expressed as means ± standard deviation of the mean. BMI, body mass index.

Dietary intake

All 30 subjects completed at least one three-day diet record. A summary of dietary intakes of subjects is found in **Table 6.** Two subjects in the FAO disorder group only completed a three-diet record from visit 1, one of which dropped out of the study early. Seven subjects in the control group completed 2, three-day diet records. Eight subjects in the control group will have only completed visit 1 during this sub-analysis, resulting in completion of 1 three-day diet record.

Table 6
Dietary intakes of subjects

Variables	FAOD	Control	*p* value
Energy (kcal)	1932 ± 287	1813 ± 297	0.4460
kcal/kg body weight	23.4 ± 5.9	21.6 ± 5.8	0.4545
Carbohydrate, g (% kcal)	254 (52.1) ± 79.1	244.4 (53.9) ± 63.3	0.6818
Protein, g (% kcal)	85.1 (17.9) ± 10.4	93.2 (20.3) ± 29.0	0.1685
Fat, g (% kcal)	57.6 (27.4) ± 22.2	48.3 (24.4) ± 15.4	0.4947
SFA, g (% kcal)	14.2 (6.6) ± 6.7	16.1 (7.9) ± 6.8	0.4997
MUFA, g (% kcal)	10.2 (4.8) ± 4.4	11.2 (5.5) ± 6.0	0.6405
PUFA, g (% kcal)	5.8 (2.7) ± 2.0	5.8 (2.7) ± 4.9	0.9953

All continuous variables are presented as means ± standard deviation of the mean. Independent t-tests were used to compare differences between continuous variables. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

The diet records were analyzed using ESHA Food Processor and averages were calculated for total energy, macronutrients, SFA, PUFA, MUFA, LCT, and MCT. Under-reporting was suspected in two control subjects, who demonstrated an average caloric intake less than 1000 calories. In order to preserve diet data from all participants, an under-reporting rule was established. Under-reporting was defined as consuming < 75% of estimated energy requirements (EER). On days where the participant reported consuming <75% of their EER, diet data was removed from the analysis. Data from two days was dropped for both control subjects that under-reported. Each subject had one acceptable day, reporting >75% of their EER to include in analysis.

There was no significant difference between groups in average energy intake (FAOD 1932 kcal, Control 1813 kcal, $p = 0.3202$). There was no significant difference between

groups in average macronutrient, SFA, MUFA, or PUFA intake. Dietary intake for each group is presented in **Figure 4.**

MCT supplementation among subjects with FAO disorders is summarized in **Table 7.** Five subjects with a long-chain FAO disorder reported daily MCT supplementation, with an average intake of 23.2 grams MCT (10.1% of total energy) and 45.3 grams LCT (21.4% of total energy). Although it is recommended to replace LCT with MCT, subjects who reported MCT supplementation did not have different LCT intake than subjects with a FAO disorder who did not report MCT supplementation.

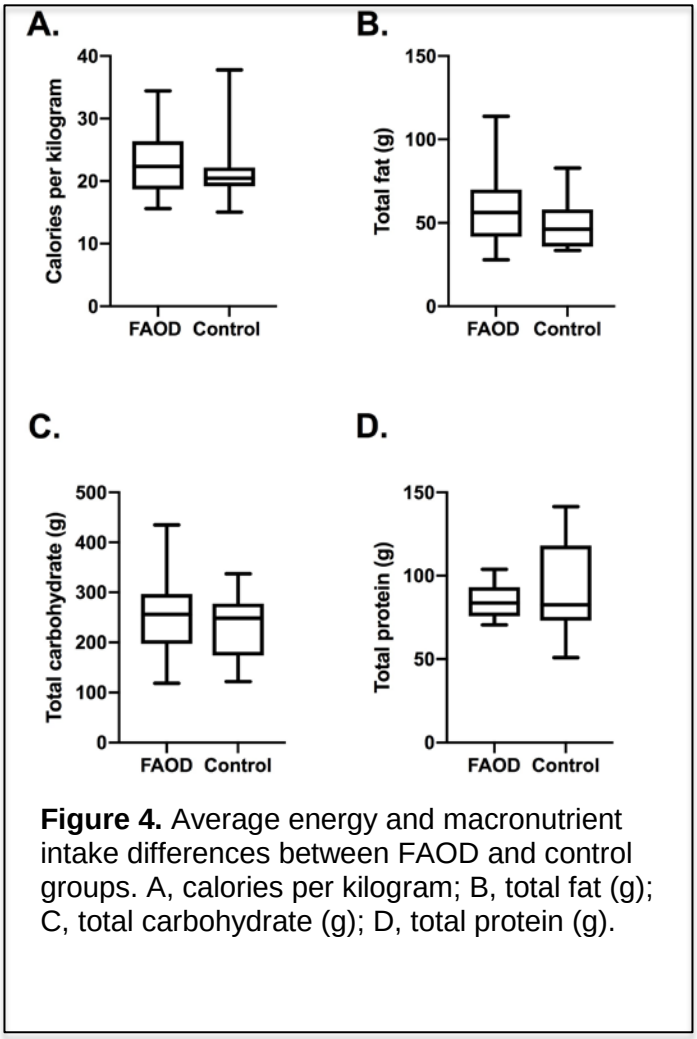


Figure 4. Average energy and macronutrient intake differences between FAOD and control groups. A, calories per kilogram; B, total fat (g); C, total carbohydrate (g); D, total protein (g).

Table 7
MCT Supplementation in FAOD Subjects

	MCT Supplementation	No MCT Supplementation
Diagnosis	LCHAD (*n* = 3)	LCHAD (*n* = 3)
	VLCAD (*n* = 1)	VLCAD (*n* = 2)
	CPT2 (*n* = 1)	CPT2 (*n* = 5)
MCT, g (% kcal)	23.2 (10.1)	
LCT, g (% kcal)	45.3 (21.4)	50.9 (21.3)

All continuous variables are presented as means. LCHAD, long-chain hydroxyacyl-CoA dehydrogenase deficiency; VLCAD, very-long-chain acyl-CoA dehydrogenase deficiency; CPT2, carnitine palmitoyltransferase II deficiency; MCT, medium chain triglyceride; LCT, long chain triglyceride.

Lipid Accumulation in Liver and Muscle

Complete ^{1}H-MRS data was analyzed in 12 FAOD subjects and 11 controls. Two subjects with FAO disorders did not have ^{1}H-MRS data, due to defibrillators that prevented them from entering the MRI. Differences in IHL, IMCL, and EMCL deposition between groups are presented in **Table 8.**

Table 8
Lipid Deposition

	FAOD (n = 13)	Control (n = 12)	p value
IHL	0.1060 ± 0.27	0.0572 ± 0.11	0.5033
IMCL	0.0383 ± 0.09	0.0168 ± 0.01	0.7283
EMCL	0.0521 ± 0.07	0.0251 ± 0.02	0.3760

All continuous variables are presented as means ± standard deviations. Lipid deposition is expressed as percent of water peak. ^{1}H-MRS, Proton magnetic resonance spectrometry; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; EMCL, extramyocellular lipid.

As previously stated, ^{1}H-MRS data was not normally distributed. Several different transformations did not normalize the data distribution; thus, data was left in the original form and non-parametric analyses were used. Distributions in lipid deposition are visualized in **Figure 5.**

It was suspected that results were influenced by one subject with extremely high lipid deposition. An outlier analysis was performed, identifying one outlier in the FAOD group. Analyses were completed with and without the outlier, confirming the outlier did not affect the conclusions. For this reason, the outlier was included in all statistical analyses.

Although subjects with a long-chain FAO disorder demonstrated greater mean IHL deposition compared to controls, this was not statistically significant (FAOD 0.1060% of water peak, Control 0.0572% of water peak, $p = 0.5033$). One participant with a FAO disorder demonstrated an IHL deposition as high as 0.9905% of water peak. This has not been observed by the MRI spectroscopist or in our studies previously and suggests an extremely high level of hepatic lipid deposition. The scans were analyzed twice to confirm the results and do appear to demonstrate a truly elevated IHL in this particular subject. There were no significant correlations observed between energy, macronutrient, or MCT intake and IHL deposition in either group.

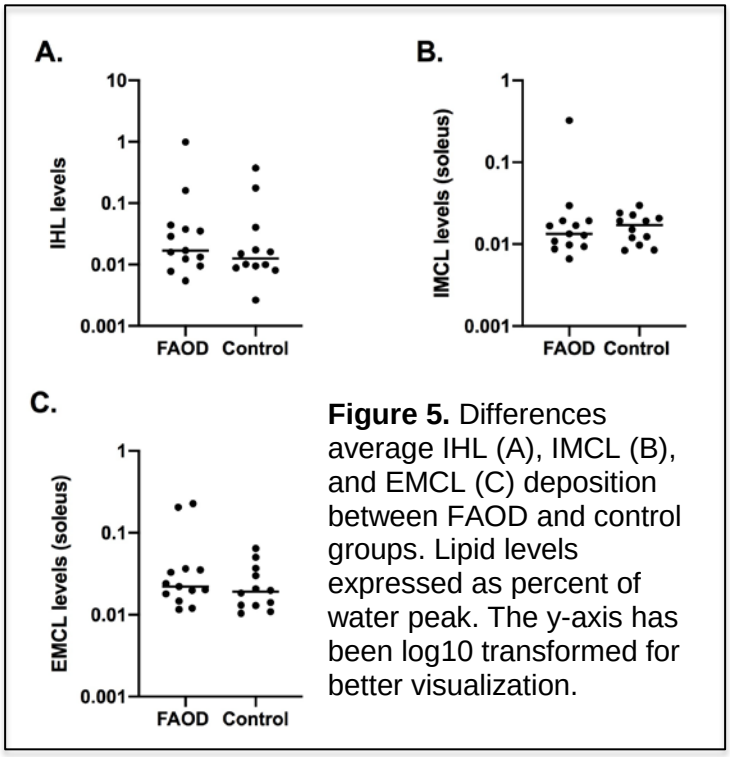


Figure 5. Differences average IHL (A), IMCL (B), and EMCL (C) deposition between FAOD and control groups. Lipid levels expressed as percent of water peak. The y-axis has been log10 transformed for better visualization.

There was not a significant difference in mean EMCL deposition in subjects with a long-chain FAO disorder (0.0521% water peak) compared to controls (0.0251% water peak), $p = 0.376$. There was no observed relationship between diet and EMCL deposition in both subjects with long-chain FAO disorders and controls.

As expected, mean IMCL deposition was similar between FAOD subjects (0.0383% water peak) and controls (0.0168% water peak) with a p-value of 0.7283. There was a significant positive correlation found in total fat intake and IMCL deposition in controls (r = 0.6434, p = 0.0278). Although not significant, an opposite relationship was demonstrated in subjects with a FAO disorder, where total fat intake appeared to be negatively associated with IMCL deposition. Correlations between IMCL deposition and total fat intake are presented in **Figure 6.** To better visualize the relationship trend of total fat intake and IMCL deposition in subjects with a FAO disorder, the outlier has been removed from Figure 6. Significant relationships between other diet variables and IMCL deposition were not detected in either group.

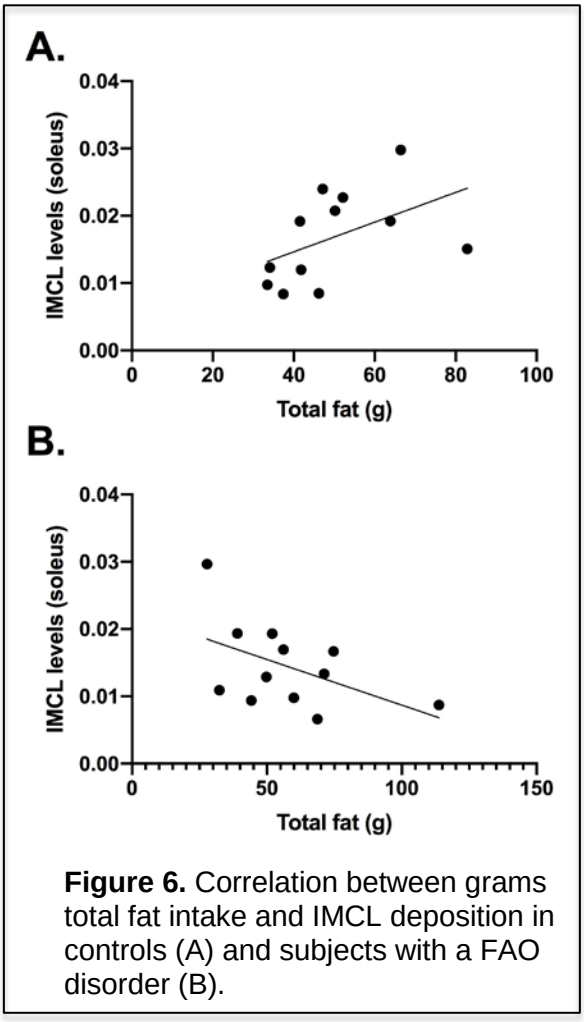


Figure 6. Correlation between grams total fat intake and IMCL deposition in controls (A) and subjects with a FAO disorder (B).

Multivariate linear regression was used to assess the associations between dietary variables and IMCL deposition. We first evaluated the impact of grams fat, carbohydrate, protein, and calories per kilogram of body weight on IMCL deposition separately, by group. Consistent with our correlation analysis, we found that total fat intake was positively associated with IMCL deposition in controls, with and without adjustments for BMI (p = 0.0372). This association was not observed in subjects with a FAO disorder.

To assess whether the presence of a FAO disorder influenced the association of fat on IMCL deposition, we conducted a second multiple linear regression incorporating both

groups, that included subject group as an independent variable, in addition to dietary intake. Dietary variables nor subject group were significantly associated with IMCL deposition.

Discussion

This study aimed to explain the relationship between differences in dietary intake and lipid deposition between subjects with a long-chain FAO disorder and controls. Subjects were counseled to consume a low-fat diet during the three-day diet recording period. While subjects with a FAO disorder consumed more fat than controls (FAOD 27.4%, Control 24.4%), both groups consumed less total fat than the average amount of fat consumed by Americans reported in the United States 2011-2014 NHANES data, 33.6% of total calories from fat.[44]

Although it is recommended that patients with long-chain FAO disorders restrict LCT and replace it with MCT, this study demonstrated that patients who supplement with MCT consume similar amounts of LCT as those who do not report MCT supplementation. This highlights that patients with FAO disorders may be misunderstanding the purpose of MCT supplementation and could provide insight into developing more efficient nutrition education materials.

Total fat intake was positively correlated with IMCL deposition in controls. This is consistent with Sakurai et al and Kakehi et al, who found a high-fat isocaloric diet significantly increased IMCL deposition in healthy men and women.[16,17] Previous studies studying high-fat, excess calories diet also found an association between fat intake and IMCL deposition.[20,21] However, we did not observe a relationship between high calorie consumption and lipid deposition. Although not significant, an opposite relationship was observed in subjects with a FAO disorder; fat intake and IMCL deposition were negatively correlated. Control subjects with the highest fat intake had higher IMCL deposition, while subjects with an FAO disorder with the lowest fat intake had the highest IMCL deposition. This suggests that having a FAO disorder changes the relationship between dietary intake and subsequent muscle lipid accumulation.

Preliminary data suggested adolescents with LCHAD deficiency had higher EMCL levels compared to controls, but similar levels of IMCL and IHL.[43] Consistent with Gillingham et al[43], this present study observed similar IMCL and IHL deposition between subjects with long-chain FAO disorders and controls. However, there was not a significant difference in EMCL deposition found between controls and subjects with a FAO disorder.

Conflicting results between studies were also observed in body composition. In the previous study, subjects with LCHADD demonstrated higher fat mass and lower fat free mass compared to controls. This was not observed in the present study.

Potential reasons for the differences between the study results include dissimilarities in subject age and FAO disorder variability. The previous study recruited subjects with LCHAD deficiency specifically, who were primarily adolescents, while this study enrolled adults that included a mixed group of FAO disorders, such as VLCAD and CPT2 deficiency in addition to LCHADD.

While analyzing three-day diet records provided insight into the dietary habits of study participants, this method relies on the participant's memory and is subject to reporting bias. Previous studies studying the association of diet and lipid deposition provided standardized, experimental diets.[10,16,17,20] We did not control dietary intake, but merely assessed what subjects were consuming through a 3-day diet record. The reported dietary intakes and lipid deposition measures were similar between groups. This may be anticipated, given previous literature looked at the effects of disparate dietary intakes on lipid deposition.

Lundsgaard et al utilized diet records at enrollment prior to initiation of the experimental diet, to determine energy content and nutrient composition from the participants' typical diet. Before starting the experimental diet, subjects consumed an isocaloric diet that reflected their habitual energy and macronutrient composition. The

control group continued this habitual isocaloric diet through the study period, while the experimental groups were provided hypercaloric diets with excess calories from unsaturated fat or carbohydrate in a controlled environment for three days.[20]

Sakurai et al and Kakehi et al provided packed meals for subjects that were prepared by an external food company.[16,17] Although this method does not ensure participants finished the same amounts of food provided, it improves variability between the subjects' dietary intake.

Lindeboom et al also controlled diet, providing one standardized meal, either high-fat or high-fat with protein, prior to lipid measurement and required that all subjects finished the meal.[10]

In this study, subjects were asked to consume a low-fat diet consisting of 20% calories from fat, 20% from protein, and 60% from carbohydrates for the three days preceding the study. The analyzed 3 day diet records suggest both subjects with an FAOD and controls consumed more fat and less carbohydrate than suggested. However, we observed dietary intakes with low overall variability among and between groups.

Previous studies that found a relationship between diet and increased IMCL and IHL deposition provided diets with intakes ranging from 60-78% energy from fat and as high as 80% energy from carbohydrate.[10,16,17,20,37] This study observed intakes of an average 24.4-27.4% energy from fat and 52.1-53.9% energy from carbohydrate, closely resembling standard diet recommendations. Relationships between diet variables and lipid deposition may have been difficult to detect in our study because of the low variability, unlike feeding studies which used extreme experimental diets. To truly study the association between diet and lipid deposition, dietary manipulation should be considered in future studies.

Although studying a unique population of patients with FAO disorders allowed insight into the effects of FAO on lipid deposition, studying a rare population resulted in a small

sample size, resulting in low power in our exploratory analysis and results that are difficult to generalize to the general population. However, previous studies that have analyzed diet and lipid deposition recruited a similar number of participants, ranging from 8-37 subjects.[10,15,20,21,26,34] Future studies may benefit from recruiting a larger, more representative pool of subjects.

Conclusion

Our results support our hypothesis that high total fat intake is associated with higher IMCL deposition, in controls. This relationship is consistent with the previous literature, which demonstrates a positive relationship between fat intake and IMCL deposition. Interestingly, high total fat intake was negatively associated with IMCL deposition in subjects with a FAO disorder which suggests that the presence of a FAO disorder may change the relationship between dietary intake and muscle lipid accumulation.

Contrary to our hypothesis, subjects with long-chain FAO disorders had similar levels of IHL deposition compared to controls. Additionally, there was not a relationship detected between high carbohydrate intake and IHL deposition.

This project allowed for the observation of the interactions between FAO, diet, and lipid deposition. Results contributed to existing literature, suggesting further that fat intake is associated with higher IMCL deposition. Dietary intake data provided insight into dietary intervention and health outcomes for patients with long-chain FAO disorders, suggesting subjects may benefit from improved education specific to LCT restriction and MCT supplementation. Furthermore, this study identified aspects in methodology, specifically dietary manipulation, that can be improved in future studies to better observe the relationship between diet and lipid deposition.

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