GROWTH AND CARNITINE STATUS OF CHILDREN TREATED WITH THE KETOGENIC DIET FOR INTRACTABLE EPILEPSY

By

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CERTIFICATE OF APPROVAL

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Abstract

Background Long-term treatment with the Ketogenic Diet among children with intractable epilepsy may negatively impact growth, especially linear growth, body composition, and plasma carnitine concentrations. Treatment with the Ketogenic Diet also results in an increased demand for carnitine, which may lead to hypocarnitinemia. As 90% of carnitine is stored in the muscle, the amount of muscle mass in children prior to starting dietary treatment may play a role in the carnitine status of these children. Limited research exists describing the potential relationship between, and potential protective effect of, greater muscle mass and changes in carnitine status in patients treated with the Ketogenic Diet.

Objective This study was designed to investigate the relationship between growth, muscle mass, and plasma carnitine concentration in patients with intractable epilepsy receiving treatment with the Ketogenic Diet.

Participants/setting Data from pediatric patients in an inpatient and outpatient setting who had intractable epilepsy and began treatment with the Ketogenic Diet.

Methods In addition to a medical record review, we collected growth measurements and plasma carnitine concentrations taken during Ketogenic Diet initiation and at regularly scheduled outpatient appointments one, three, six, nine and 12 months after diet initiation.

Results No significant changes were seen in mean weight or height z-scores among patients less than two years of age who were treated with the Ketogenic Diet. A significant increase in weight z-score ($p=0.02$), but not height or BMI z-score, were seen

among patients older than two years of age after diet initiation. No significant correlations between baseline weight, height, or BMI z-score and change in carnitine concentrations were observed; however, a greater increase in weight z-score was significantly associated with less of an increase in acyl/free carnitine concentration ratio ($p=0.003$).

Conclusions Although not significant, these results suggest that treatment with the Ketogenic Diet results in lower weight, height and BMI z-scores. These results also suggest that treatment with the Ketogenic Diet results in lower free-, increased acyl- and increased total carnitine concentrations, as well as increased acyl/free carnitine concentration ratios in most, but not all, patients with intractable epilepsy. No relationship was seen between baseline growth parameters and change in carnitine concentration.

Clinical implications As a majority of carnitine is stored in muscle tissue, the lower free carnitine concentration seen after dietary treatment among patients with a lower baseline weight z-score may theoretically be due to less carnitine initially stored in the muscle, therefore contributing to lower plasma free carnitine concentrations. This conclusion should be considered with caution due to the difficulty in taking accurate growth measurements in this patient population. It may be beneficial for anthropometric measurements to be standardized, and for them to be taken by one clinician to improve accuracy in clinical settings and in future studies.

We hope for this study to serve as a foundation for future studies describing the relationship between growth and/or body composition and carnitine concentrations.

Chapter 1: Introduction and Significance

Epilepsy

Epilepsy is a neurological disease associated with recurrent seizures. It affects approximately 345,000 children nationwide; 2,500 of whom reside in Oregon (1). The total direct and indirect cost of epilepsy in the United States is estimated to be $15.5 billion dollars per year (2).

A seizure is defined as a single occurrence and sudden disruption of the brain's normal electrical activity accompanied by altered consciousness and/or other neurological and behavioral manifestations. The International League Against Epilepsy defines an epileptic seizure as "a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain" (3). More specifically, epilepsy is considered to be a disease of the brain defined by the presence of at least two unprovoked seizures occurring at least 24 hours apart, one unprovoked seizure after two unprovoked seizures occurring over the next ten years, or the diagnosis of an epilepsy syndrome (4).

An individual is considered to have intractable epilepsy when he or she fails to respond to three or more antiepileptic drugs (AEDs) (5). For response to an AED to be considered a failure, the AED must have been used at a maximum tolerated level (6). Approximately 20-30% of children with epilepsy become refractory to AED medications and are characterized as having intractable epilepsy (5). In addition to AED use, medical treatment for epilepsy includes: surgery (typically therapeutic); vagal nerve stimulation,

which may increase free gamma-aminobutyric acid concentrations in the cerebrospinal fluid; and the Ketogenic Diet (7, 8).

The Ketogenic Diet, an extremely high fat, low carbohydrate diet, has been used to treat epilepsy in children since 1921 and is an established, effective, nonpharmacological treatment for intractable childhood epilepsy (5). The Ketogenic Diet has been shown to reduce seizures in 50-90% of individuals after just three months of treatment (9). When seizure frequency and severity is reduced, continued use of the Ketogenic Diet may help minimize or prevent cognitive deterioration and behavioral disturbances (5). However, an unintended consequence of the Ketogenic Diet is that it may increase demand for carnitine, a vitamin-like compound required for fatty acid metabolism. Insufficient/deficient carnitine status may reduce the antiepileptic response to the Ketogenic Diet.

Treatment with the Ketogenic Diet is associated with reduced seizure frequency and severity, as well as use of fewer AEDs to achieve seizure control. However, long-term treatment with the Ketogenic Diet may negatively impact growth and body composition, specifically linear growth and possibly muscle mass. To better understand the relationships between carnitine status, body composition and the impact of the Ketogenic Diet on reducing seizure frequency, we explored the following questions:

1. Does treatment with the Ketogenic Diet impact growth and carnitine concentrations in children with intractable epilepsy?

2. Does weight, height, or BMI z-score before initiating the Ketogenic Diet predict change in plasma carnitine concentrations and seizure frequency one, three, six, nine and twelve months after Ketogenic Diet initiation?
3. Does change in weight, height, or BMI z-score after initiation and treatment with the Ketogenic Diet correlate with change in plasma carnitine concentrations one, three, six, nine and twelve months after baseline?

To address these questions, we carried out the following specific aims:

1. We measured and described growth before and one, three, six, nine and twelve months after initiation and treatment with the Ketogenic Diet among children with intractable epilepsy, and we compared these values to standard pediatric reference data.
2. We determined if weight, height or BMI z-score before Ketogenic Diet initiation predicts change in plasma carnitine concentrations (plasma free, acyl-, and total carnitine concentrations and the acyl/free carnitine concentration ratio) and seizure frequency one, three, six, nine and twelve months after initiation of Ketogenic Diet treatment among children with intractable epilepsy.
3. We determined if change in growth, specifically weight, height and BMI z-scores, correlated with change in plasma carnitine concentrations one, three, six, nine and twelve months after initiation and treatment with the Ketogenic Diet among children with intractable epilepsy.

Chapter 2: Background

The Ketogenic Diet

The Ketogenic Diet is designed to mimic a fasting or starvation state when circulating ketones are used for energy. The Ketogenic Diet is very high in fat and very low in carbohydrate. It is prescribed as a ratio, which describes the amount of fat the diet will provide compared to the amount of protein and carbohydrate combined. The most extreme form of the diet is prescribed as a 4:1 ratio and provides about 90% of energy from fat and 10% of energy from protein and carbohydrate, combined (5). Historically, treatment with a Ketogenic Diet was initiated as part of a hospital admission after a 48-hour fast (5). Fluid intake was restricted to 80-90% of daily requirements to reduce plasma volume and increase blood ketone concentration. This more extreme protocol is no longer deemed necessary unless a quicker response time from diet initiation to reduced seizure frequency is medically indicated (5).

In the classical Ketogenic Diet, dietary fats are typically long-chain triglycerides, the amount of protein consumed is enough to maintain adequate growth, and carbohydrate intake is severely restricted (5). Alternative Ketogenic Diets replace long-chain triglycerides with medium-chain triglycerides, which may increase ketogenic potential and require lower amounts of total fat to initiate and maintain ketosis (5). Medium-chain triglycerides are more efficiently absorbed and metabolized by the liver and yield more ketones per kilocalorie of energy than long-chain triglycerides, which is thought to heighten their ketogenic response (5). However, the traditional medium-chain triglyceride Ketogenic Diet provides 60% of energy as medium-chain triglycerides,

which may result in gastrointestinal distress (5, 10). To lower the risk of gastrointestinal distress, a modified medium-chain triglyceride diet may be initiated, in which 30% of energy is derived from medium-chain triglyceride and 30% of energy is derived from long-chain triglycerides (5).

A "Modified Atkins Diet" may also be used to treat intractable epilepsy. The Modified Atkins Diet is based on similar principles as the classical Ketogenic Diet; however the ratio of grams of fat to grams of protein and carbohydrate combined is lower at approximately 1:1, with less carbohydrate restriction (5). Patients typically consume about 10 grams of carbohydrate per day when first initiating a modified Atkins diet and slowly increase carbohydrate intake to 15-20 grams per day after one to three months (15).

To achieve the required carbohydrate restriction to induce ketosis, the Ketogenic Diet severely restricts consumption of high carbohydrate containing foods such as milk, fruit, vegetables, and cereals and grain products. Elimination of these foods/food groups from the diet severely reduces the intake of essential micronutrients. To ensure adequate micronutrient intake, supplementation with a carbohydrate-free, broad-spectrum multivitamin/mineral supplement is needed.

An Overview of Fat Metabolism

When a Ketogenic Diet is consumed, triacylglycerides are oxidized to generate acetyl CoA, which enters the Krebs cycle to be used as the primary source of energy. As illustrated in Figure 1, beta-oxidation of long-chain triacylglycerides, in this case, palmitic acid, a 16-carbon fatty acid, undergoes four enzyme-catalyzed reactions within

the mitochondrial matrix resulting in the oxidative removal of two-carbon units from the carboxyl end of the fatty acyl chain in the form of acetyl-CoA. First, acyl-CoA dehydrogenase produces a double bond

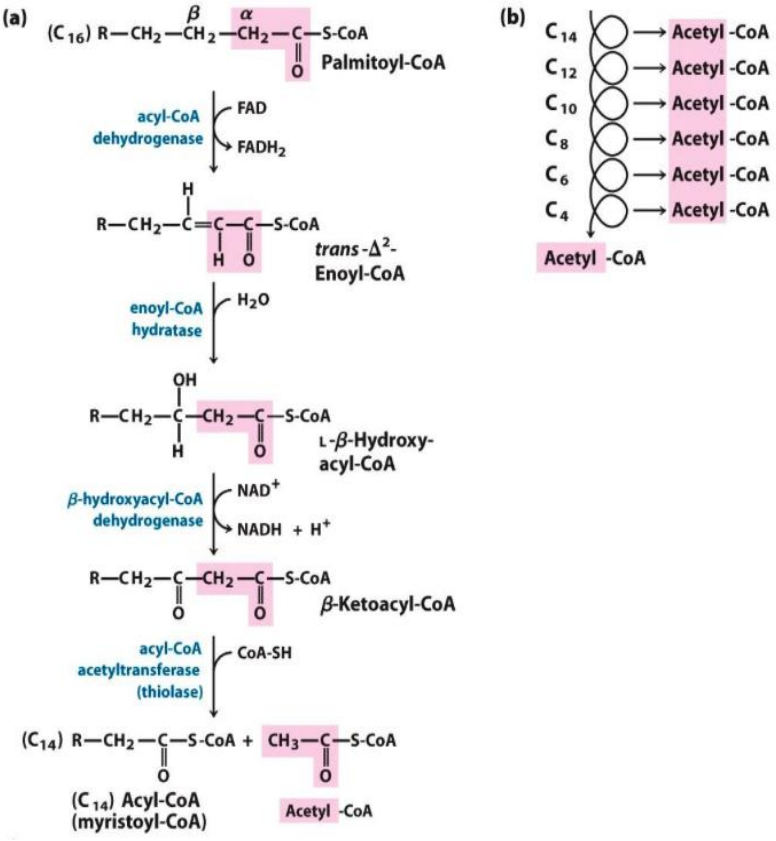

Figure 1: Beta-oxidation of Palmitic Acid

between the second and third carbons in the chain, C-2 and C-3, yielding a trans-Δ^2-enoyl-CoA. Electrons removed from the fatty acyl-CoA are transferred to flavin adenine dinucleotide (FAD) to generate reduced $FADH_2$. The reduced form of the dehydrogenase then donates its electrons to an electron carrier in the mitochondrial membrane. Enoyl-CoA hydratase catalyzes the second reaction where water is added to the double bond of trans-Δ^2-enoyl-CoA and L-β-hydroxyacyl-CoA is formed. The third step involves the dehydrogenation of L-β-hydroxyacyl-CoA to form β-ketoacyl-CoA via β-hydroxyacyl-CoA dehydrogenase with nicotinamide adenine dinucleotide (NAD) as the electron acceptor. The fourth and final step is catalyzed by acyl-CoA acetyltransferase, or thiolase. β-

ketoacyl-CoA and a molecule of free coenzyme A from the carboxyl-terminal two-carbon fragment of the original fatty acid are cleaved and together form acetyl-CoA. Eight molecules of acetyl-CoA are formed during the complete oxidation of palmitic acid. In order for B-oxidation to occur, fatty acids must be transported across the inner mitochondrial membrane into the mitochondrial matrix. This process occurs by a series of carnitine-dependent translocation steps.

The Role of Carnitine in Fatty Acid Oxidation

Carnitine is a small water-soluble, vitamin-like substance that is mainly synthesized in the liver and kidneys from the amino acids lysine and methionine (Figure 2) (16, 21). The L- isomer of carnitine is the biologically active form of this molecule (16, 17).

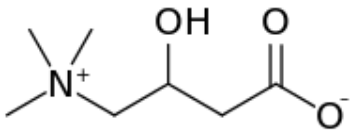

Figure 2: L-Carnitine Molecule

In addition to endogenous production, carnitine can also be consumed in the diet. Main dietary sources of carnitine include meat and dairy products (16, 17, 21). About 54-87% of dietary carnitine is absorbed by the small intestinal mucosa via active transport and then slowly released into circulation (18). Carnitine circulates in free- and acylated forms, and approximately 90% of the body's total carnitine supply is stored in muscle (18, 22).

Carnitine's role in fatty acid oxidation was first discovered in 1955, and because of its function in fatty acid transport into the mitochondrial matrix, it plays an essential role in fat metabolism and ketone production. Carnitine acts as a cofactor to transfer long-chain fatty acids as acylcarnitine esters across the inner mitochondrial membrane (19). Acyl-CoA synthase catalyzes the formation of acyl-CoA by combining intracellular

free fatty acids and coenzyme A in the cytosol. The acyl moiety of acyl-CoA is transferred across the inner mitochondrial membrane in a series of three carnitine-dependent enzymatic reactions. Together, this multi-step process represents the carnitine shuttle system, which is illustrated in Figure 3 (17).

As shown in Figure 3, carnitine-palmitoyltransferase I (CPT I) is the first enzyme in the carnitine shuttle system. CPT I catalyzes the reversible transesterification of long-chain acyl-CoA with carnitine to form an acylcarnitine ester. Acylcarnitine esters are then transported through the inner mitochondrial membrane via a carnitine/acylcarnitine translocase. The acyl moiety of the carnitine ester is then transferred to CoA to form acyl-CoA by carnitine-palmitoyltransferase II (CPTII) on the matrix side of the inner mitochondrial membrane. The mitochondrial matrix is the site where beta-oxidation occurs (17).

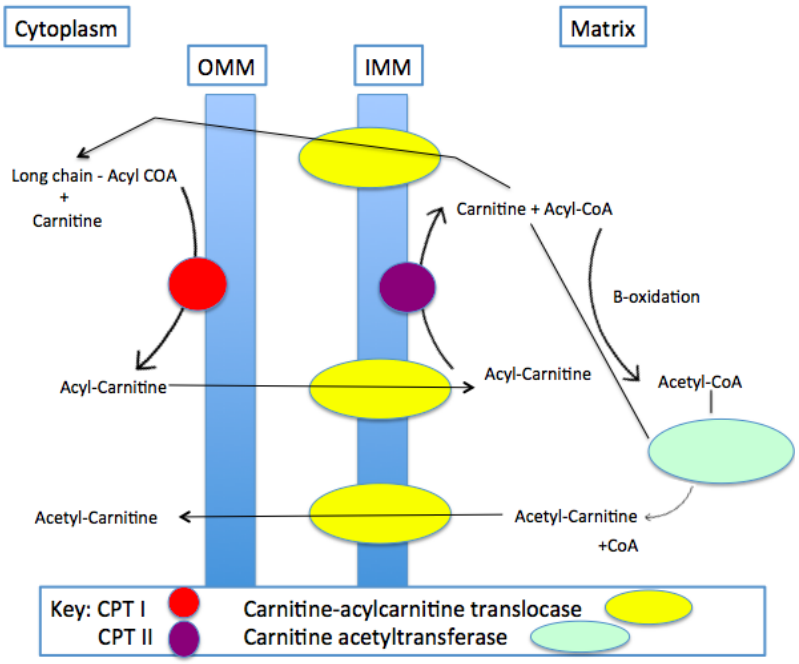

Figure 3: The carnitine shuttle system

Carnitine, primarily as acetylcarnitine, is shuttled back across the inner mitochondrial membrane from the matrix into the cytosol and is excreted or reused to

transport other long-chain fatty acids across the inner mitochondrial membrane (20). Following beta-oxidation of long-chain fatty acids, acetyl-CoA may enter the Krebs cycle where it is further oxidized to carbon dioxide and water, or it can be used for ketone synthesis in the mitochondria of hepatocytes.

Ketone Formation and Utilization

In a fasting or starving state when glucose availability is limited, such as when a low carbohydrate Ketogenic Diet is consumed, fatty acid oxidation increases and ketone bodies are formed and used as an alternative energy source by peripheral tissues, specifically the brain.

Ketogenesis occurs in the mitochondria of hepatocytes where the ketone bodies acetoacetate, β-hydroxybutyrate, and acetone are formed from acetyl-CoA. As illustrated in Figure 4, acetyl-CoA is converted to acetoacetate via 3-ketothiolase, HMB CoA synthase and HMB CoA lyase (26). Acetoacetate undergoes spontaneous decarboxylation to form acetone. Acetoacetate can also be reduced to beta-hydroxybutyrate in a reversible reaction catalyzed by the NADH-dependent mitochondrial enzyme, beta-hydroxybutyrate dehydrogenase.

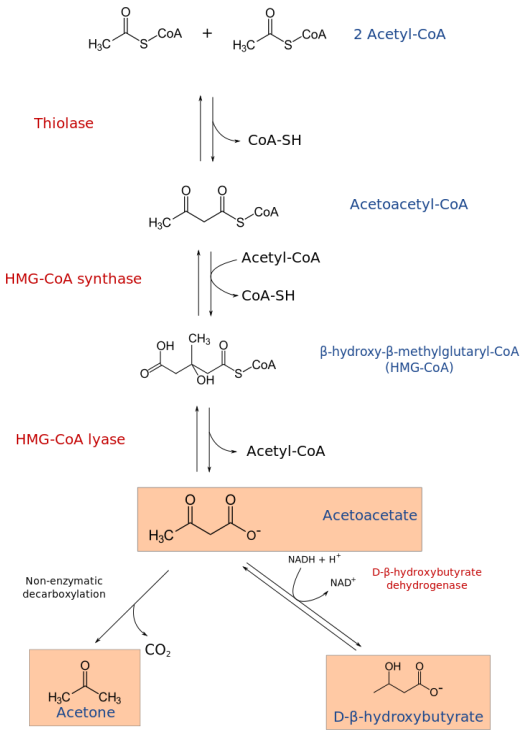

Figure 4: Formation of ketone bodies from long chain fatty acids (11)

Acetoacetate and beta-hydroxybutyrate can be transported across the blood-brain barrier via monocarboxylic acid transporters. Once in the brain, ketones are used as alternative energy sources, and although the exact mechanism remains unclear, elevated serum ketone concentrations are associated with reduced seizure frequency (22,5).

Carnitine and Muscle

As previously mentioned, approximately 90% of the body's total carnitine supply is stored in muscle, making it an important component of fat-free mass, and muscle carnitine concentrations differ by muscle type (22, 23). Cederblad et al. measured carnitine concentrations in muscle biopsies of the rectus abdominis, vastus lateralis, gracilis and gastrocnemius leg muscles after an overnight fast. Carnitine concentrations were higher in leg muscles than in the rectus abdominus and there was no relationship between carnitine concentration in muscle tissue and plasma (23). However, other studies describe potential associations between skeletal muscle and plasma carnitine concentrations (24, 25).

Hiatt et al. explored the relationship between skeletal muscle metabolism during exercise and changes in carnitine metabolism in six healthy, active men. During high-intensity exercise, muscle carnitine concentrations decreased while plasma carnitine concentrations increased (24). Specifically, mean plasma short-chain acylcarnitine concentration significantly increased from 7.0 ± 1.2 μM at rest to 10.2 ± 1.4 μM 30 ($p<0.05$) (24), mean plasma long-chain acylcarnitine concentration increased from 5.7 ± 0.4 μM at rest to 7.0 ± 0.5 μM ($p<0.05$), and mean plasma total carnitine concentration

increased from 63.2 ± 3.9 μM at rest to 69.9 ± 3.6 μM 30 minutes after exercise ($p<0.05$) (24).

Morita et al. described a relationship between hypocarnitinemia, though not defined, and poor muscle volume in 25 disabled individuals treated with sodium valproate or another anticonvulsant medication. Lean body mass was estimated by calculating arm muscle circumference, which was a major determinant of serum carnitine concentrations. The authors concluded that patients treated with anticonvulsants with poor muscle volume are at greater risk of developing hypocarnitinemia (25).

The studies described above suggest that carnitine is released from muscle into blood during high-intensity exercise. They also suggest that individuals with lower amounts of muscle mass are more likely to develop hypocarnitinemia when treated with anticonvulsant medications. Our study built on these findings to determine if changes in plasma carnitine concentrations are associated with changes in weight and BMI z-scores. As we didn't have an accurate method for measuring muscle mass, z-scores served as a determinant for loss or gain of muscle mass from baseline to twelve months after Ketogenic Diet initiation. A relationship between the change in plasma carnitine concentrations and changes in weight and/or BMI z-scores may imply that muscle carnitine stores may preserve plasma carnitine concentrations, maintain higher circulating ketone concentrations and reduce seizure frequency and severity in children with intractable epilepsy who are treated with the Ketogenic Diet. Another condition

that might influence circulating carnitine concentrations is weight loss, and as a result, loss of muscle mass.

Carnitine and Weight Loss

A study conducted by Schooneman et. al described plasma free- and acylcarnitine profiles before and after weight loss in 60 obese subjects who were randomized to one of three 12-week weight loss interventions (38). Between baseline and day 84, an overall mean weight loss of ~4.5 kg ($p<0.05$) was seen. Fat mass decreased throughout the intervention, although the greatest loss was seen after 24 days. Fat-free mass also decreased (~0.6 kg, $p<0.05$), but only between baseline and day 28. After 84 days of intervention, free carnitine concentrations were significantly higher ($p<0.05$). There were also statistically significant increases in C2-, C4OH-, C10- C14:1, C16, and C18:1-carnitine concentrations after 28 days of intervention ($p<0.05$). Although these carnitine concentrations were lower after 84 days of intervention, they were still higher compared to baseline (38).

Mechanisms of the Ketogenic Diet in Seizure Reduction

Treatment with the classical Ketogenic Diet requires severe carbohydrate restriction, which shifts the primary metabolic fuel source from glucose to ketones.

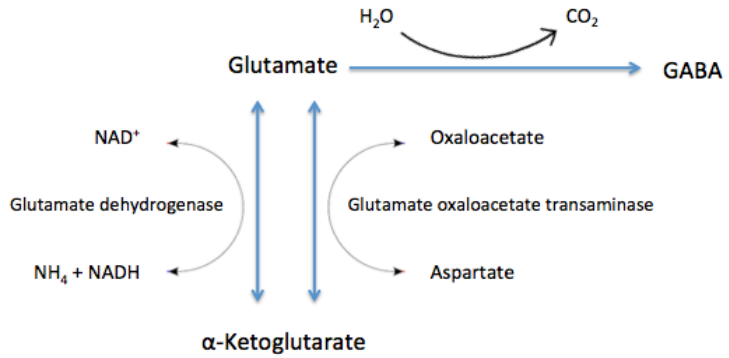

Figure 5: Conversion of alpha-ketoglutarate to glutamate and GABA

Using ketones instead of glucose to produce energy is thought to alter neurotransmitter concentrations in the brain, conferring anticonvulsant effects. When dietary carbohydrate intake is severely limited, the demand to maintain serum glucose concentration causes oxaloacetate to be shunted from the Krebs cycle to the pathway for gluconeogenesis (11). When oxaloacetate concentrations are limited, the Krebs cycle cannot handle the high levels of acetyl-CoA generated from fat metabolism as it can when adequate glucose is available. As a result, alpha-ketogluterate builds up and, as illustrated in Figure 5, alpha-ketoglutarate is converted to glutamate, which functions either as an excitatory neurotransmitter, or as the precursor of the inhibitory neurotransmitter, gamma amino butyric acid (GABA) (11).

Ketone production and the subsequent increase in neuronal GABA concentrations is one potential mechanism through which the Ketogenic Diet may reduce the frequency and severity of seizures in children with intractable epilepsy (10). Low dietary carbohydrate intake associated with the

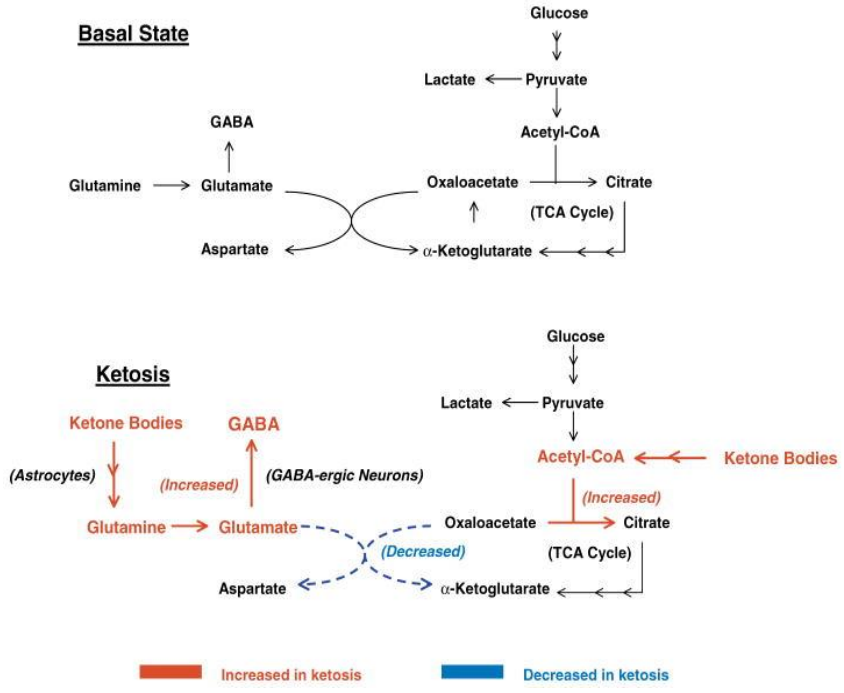

Figure 6: Increased GABA production associated with ketosis

classical Ketogenic Diet shifts metabolism toward high levels of glutamate in the cytosol of neurons in the brain, which results in more efficient glutamate released into the synaptic cleft in the presence of ketone bodies, as illustrated in Figure 6. This may improve GABA synthesis for inhibitory neurotransmission more than it would affect glutamate repackaging for use as an excitatory neurotransmitter (27, 12).

Another potential anticonvulsant mechanism of the Ketogenic Diet results from elevated concentrations of acetoacetate in neurons, as illustrated in Figure 7. When glutamate is not used as a precursor to GABA, it can be transported from the cytosol into presynaptic vesicles by a vesicular chloride-dependent glutamate transporter (VGLUT). When acetoacetate concentrations are high, these negatively charged molecules compete with cytoplasmic chloride at the VGLUT binding site, which inhibits the transport of glutamate into presynaptic vesicles. This results in a lower concentration of glutamate released into the synaptic space and reduced glutamate binding to glutamate receptors on the post-synaptic neuron. This mechanism is thought to ultimately reduce neuronal excitability in the brain (12, 28).

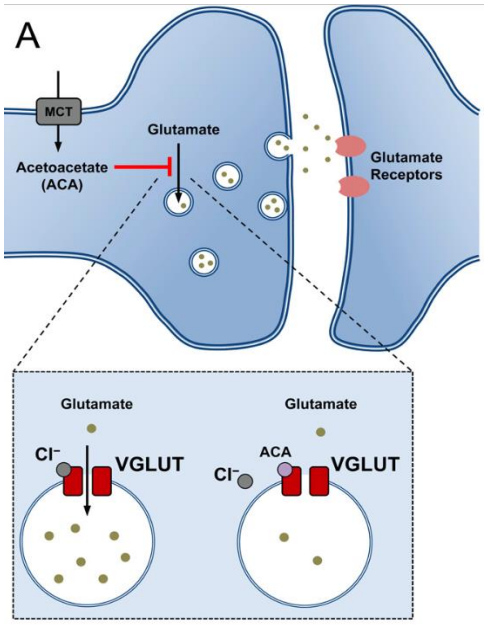

Figure 7: Inhibition of glutamate transport in the presence of acetoacetate (12) Abbreviations: ACA, acetoacetate; Cl-, Chloride

Both proposed mechanisms, higher GABA production and reduced glutamate release into the synaptic space between neurons in the presence of high concentrations of acetoacetate, may be associated with the reduced seizure frequency and severity among individuals treated with the Ketogenic Diet for intractable epilepsy (12).

Increased Demand of Carnitine with the Ketogenic Diet

Treatment with the classical Ketogenic Diet, from which long-chain triglycerides make up the primary source of dietary fat and energy, requires a greater demand for carnitine to shuttle higher amounts of fatty acids into the mitochondria to undergo oxidation. As a result, as mentioned above, treatment with the Ketogenic Diet may deplete carnitine stores through various mechanisms.

First, endogenous carnitine production may be unable to keep up with the increased need for carnitine due higher amounts of long-chain fatty acids that need to be oxidized. Second, the consumption of high protein foods such as dairy, eggs and meat, which are the primary exogenous sources of carnitine, may be insufficient to provide adequate carnitine, as the high fat to protein plus carbohydrate ratio of the Ketogenic Diet would restrict intake. Third, there may be increased urinary excretion of carnitine as acylcarnitine conjugates because of increased filtered load associated with a high fat diet (22). Lower carnitine availability may limit the ability to achieve and maintain high rates of ketosis due to the impaired ability to metabolize fatty acids and synthesize ketone bodies. Reduced ability to reach and maintain high levels of ketosis may then interfere with the seizure-reducing effect of the diet (22).

Total circulating carnitine concentrations have been shown to decrease after a few months of Ketogenic Diet therapy and then normalize within 12-24 months (29). A study conducted by Berry-Kravis et al. investigated the association between the Ketogenic Diet and carnitine deficiency among 38 patients with intractable epilepsy (29). Carnitine deficiency was defined as plasma total carnitine concentrations <31 μM for males and <25 μM for females (29). Serial blood samples were analyzed for carnitine concentrations in 26 patients. Three patients developed hypocarnitinemia and required carnitine supplementation after one month, and two patients required carnitine supplementation after six months of Ketogenic Diet treatment. The average plasma total carnitine concentration in patients who did not require carnitine supplementation was significantly lower one month (40.5 $\pm$ 12.4 μM, $p<0.01$) and six months (33.2 $\pm$ 5.4 μM, $p<0.0001$) after Ketogenic Diet initiation than before (49.9 $\pm$ 10.9 μM). The average total carnitine concentration then normalized 12 and 24 months after Ketogenic Diet initiation despite ongoing Ketogenic Diet therapy (42.4 $\pm$ 5.4 μM, $p<0.05$ and 48.3 $\pm$ 7.8 μM, $p<0.0001$, respectively) (29).

Interestingly, children consuming fat in the form of medium-chain triglycerides do not typically experience the associated carnitine deficiency seen among those consuming a Ketogenic Diet primarily composed of long-chain triglycerides. This is likely due to the ability of medium-chain triglycerides ability to bypass the carnitine shuttle and enter directly into the mitochondria for oxidation (9).

A pilot study conducted by Riebold et al. evaluated the impact of the Ketogenic Diet on plasma carnitine concentrations in 22 children, mean age 3.9 ± 4.8 years,

treated for intractable epilepsy (30). Mean plasma free carnitine concentration was significantly lower after one month of dietary treatment than before (n=5, 24.38 ± 9.63, vs 34.9 ± 3.31 µmol/L, $p<0.05$), as well as after 3 months (n=3, 16.7 ± 11.7, vs 22.4 ± 14.2 µmol/L, $p<0.05$) and 5-8 months (n=5, 20.3 ± 7.51 µmol/L, $p<0.05$). Mean plasma total carnitine concentrations were significantly higher than baseline after 1 month (n= 3, 41.1 ± 11.1 vs 54.5 ±11.4 µmol/L, $p<0.05$) and 5-8 months of Ketogenic Diet treatment (n=3, 61.6 ± 12.5 µmol/L, $p<0.05$). Increased concentrations of plasma acylcarnitine and the acyl/free carnitine ratio were also observed.

Impact of the Ketogenic Diet on Growth and Body Composition Parameters

Body composition analysis is used to describe the amount of fat mass and fat-free mass in the body and allows researchers and clinicians to quantify changes in these components over time.

The World Health Organization Global Database on Child Growth and Malnutrition uses the z-score or standard deviation classification system to assess anthropometric data. Weight, height and BMI z-scores less than -2 SD and -3 SD are used to classify low weight-, height- and BMI-for age among children with moderate and severe undernutrition. Conversely, weight, height and BMI z-scores greater than +2 SD are used to classify high weight-, height-and BMI-for age among children with overweight/obesity.

Few studies have reported how the Ketogenic Diet impacts growth and body composition among children treated for intractable epilepsy. Two studies suggest that long-term treatment with the Ketogenic Diet results in reduced linear growth. One

retrospective study followed fifty-seven subjects who started the Ketogenic Diet between 1995 and 2001. Results indicate that while not statistically significant, average height-for-age percentile decreased from the 42nd percentile at baseline to the 38th percentile at 6 months and to the 24th percentile at 12 months after Ketogenic Diet initiation. Weight-for-age percentiles were also affected and results indicate that average weight-for-age percentiles decreased from the 45th percentile at baseline to the 42nd percentile after 6 months and to the 38th percentile 12 months after Ketogenic Diet initiation. A statistically significant decrease in mean height-for-age *z*-score from baseline was seen among those treated with the Ketogenic Diet for 12 months (-0.30 $\pm$ 1.19 to -0.99 $\pm$ 1.13; $p \leq 0.005$) (37). The same study also reported that patients who reached and maintained a high level of ketosis, defined as blood ketone concentrations between 80-160 mg/dL, experienced a statistically significant greater reduction in mean height-for-age *z*-scores (-0.45 $\pm$ 1.28 to -1.1 $\pm$ 1.23, $p \leq 0.005$) than those with only a mild or moderate level of ketosis.

A study reported by Groleau et al. in 2014 demonstrated a decrease in fat-free mass three months following Ketogenic Diet initiation. Twenty-four children were included in the study and after three months, mean fat mass increased by 0.4 $\pm$ 0.7 kg ($p=0.01$), while mean fat-free mass decreased by 0.1 $\pm$ 0.7 kg. Fifteen of the 24 participants responded to the Ketogenic Diet and remained in the study for the full 15 months. In these participants, mean fat mass and fat-free mass increased significantly from baseline by 1.1 $\pm$ 0.8 kg ($p<0.001$) and 1.2 $\pm$ 0.6 kg ($p<0.001$), respectively, as would be expected among growing children (36).

Another study conducted by Groleau et al. evaluated the long-term impact of the Ketogenic Diet on growth in 24 children with intractable epilepsy. After 15 months of Ketogenic Diet treatment, mean height-for-age *z*-score decreased -0.6 ± 1.3, *p*=0.001 while mean weight-for-age *z*-score remained unchanged. The study also estimated fat mass and free-fat mass from skinfold thickness measurements from four areas: tricep, bicep, suprailiac, and subscapular skinfold thickness measurements using specific age and sex-specific equations (35). Three months after Ketogenic Diet initiation, mean fat-free mass was -0.1 ± 0.7 kg ($p<0.01$) lower than baseline (15.3 ± 4.3 kg) and mean fat mass was 0.4 ± 0.7 kg higher than baseline 2.9 ± 1.5 kg ($p<0.05$). Mean fat-free mass and fat mass were significantly higher by 1.2 ± 0.6 ($p<0.001$) and 1.1 ± 0.8 kg ($p<0.001$), respectively, 15 months after Ketogenic Diet initiation than before, which again is expected among growing children (36).

In order to investigate changes in and the relationships between weight, height and BMI z-scores and carnitine concentration among children being treated with the Ketogenic Diet for intractable epilepsy, the following methodology was carried out.

Chapter 3: Materials and Methods

General Design

We completed a medical record review and collected data to describe the impact of the Ketogenic Diet on growth (weight gain and linear growth), plasma carnitine concentrations and seizure frequency in pediatric patients with intractable epilepsy. Measurements were taken as part of the standard of care during regular clinic visits in the Ketogenic Diet Clinic at Doernbecher's Children's Hospital. Measurements are typically taken on the first day of the inpatient admission for Ketogenic Diet initiation and at regularly scheduled outpatient appointments one, three, six, nine and twelve months after diet initiation. Electronic medical record audits of historical variables were conducted to describe the study sample and to identify specific attributes associated with patients who respond positively to the Ketogenic Diet. For comparison and consolidation purposes, much of the methodology proposed for this pilot study is similar to the methods used by Ms. Jane Riebold, MS, RD for her thesis research which was also carried out at Oregon Health & Science University and Doernbecher's Children's Hospital (published electronically through the OHSU library, 2015) (30).

Inclusionary Criteria

To be considered for Ketogenic Diet initiation for the treatment of intractable epilepsy at OHSU, patients must have:

1) Failed at least 2 AEDs (meaning they were unable to achieve optimal seizure control with those medications)
2) Been referred by a pediatric neurologist, and

3) Been less than 18 years of age (the typical patient on Ketogenic Diet treatment at Doernbecher's Children's Hospital is younger than 10 years old).

Patient Population and Selection for Medical Record Review

During the time frame of this study, patients who initiated the Ketogenic Diet were asked to continue diet therapy for at least three months to assess whether the diet was effective at reducing the number and/or severity of seizures. Although some patients discontinued the Ketogenic Diet before the three-month mark, we were able to review the medical records of 34 patients who met the following criteria for our primary analysis:

1) Patients who began Ketogenic Diet therapy between January 2010 and July 2016
2) Patients who were treated with the Ketogenic Diet at a ≥ 3:1 goal ratio with at least 75% of total calories derived from fat and at least 25% of calories derived from protein plus carbohydrate combined.
3) Patients who did not receive supplemental carnitine
4) Patients who had growth data and plasma carnitine concentration values recorded at baseline and at least one month after Ketogenic Diet initiation.

Institutional Review Board Approval, Assent/Consent, and Privacy

A modification to the original research protocol was approved by the OHSU Institutional Review Board. All measurements were obtained as part of standard medical care. When possible, parental/guardian written informed consent was obtained and child written informed assent was obtained before extracting data from the child's medical record. All information obtained for this study was kept strictly

confidential. All data associated with patient identifiers was stored on secure, password-protected, OHSU networked computers. Cloud storage was used to exchange secure information among investigators when appropriate. Randomly generated number identifiers were used to identify any data stored in other locations.

Doernbecher Children's Hospital Ketogenic Diet Administration Protocol

Diet therapy using the classic Ketogenic Diet is initiated in accordance with Doernbecher Children's Hospital policy as medically appropriate. Diet initiation occurs over a three-day hospital admission. Standard protocol at the Ketogenic Diet Clinic does not include fasting or fluid restriction at initiation. Each subject consumes a diet designed by the pediatric dietitian who staffs the Ketogenic Diet Clinic at Doernbecher Children's Hospital. Diet ratios, typically a 4:1 fat-to-combined protein and carbohydrate, are calculated using the Ketocalculator. Commercial formulas used for patients requiring enteral feeding include: KetoCal 4:1 liquid (Nutricia, Gaithersburg, MD) and RCF (Abbott, Abbott Park, IL). Duocal (Nutricia, Gaithersburg, MD), Beneprotein (Nestle, Florham Park, NJ) and Liquigen (Nutricia, Gaithersburg, MD) may be used as additives to meet the energy and macronutrient ratio goals, if needed. Duocal is a high-calorie nutritional supplement that can be added to food and beverages. Beneprotein is a whey protein powder, and Liquigen is an emulsion of approximately 50% medium chain triglyceride oil and 50% water. Following discharge from the hospital, the Ketogenic Diet is prepared and served by the parents/guardians of the patients as instructed by the pediatric dietitians.

Anthropometric Measurements

Length, Height and Weight

Length (or height) and weight were measured and recorded at each routine clinic visit by the medical assistant who staffs the Ketogenic Diet Clinic at Doernbecher Children's Hospital. Infants under 24 months of age had length measured in a recumbent position on an infant stadiometer. Height was assessed using a stadiometer or estimated using recumbent length or arm span measurements in participants who were able to stand unassisted, when possible.

Infant weight was measured using a Scale-Tronix (Welch Allyn, Skaneateles Falls, NY), Model 4800 infant scale. Infants were weighed without clothes and while wearing a dry diaper. Older children who were unable to stand were weighed in light clothing while sitting in a wheelchair on the wheelchair scale. The wheelchair was then weighed independently and the difference in weight was recorded in kilograms.

Calculations and Statistical Analysis

Percentiles and z-scores were calculated according to CDC reference data (40). Means and 95% confidence intervals of continuous study variables were calculated before and one and three months after Ketogenic Diet initiation. To describe outcomes for the first specific aim, difference in means (from baseline) were calculated and significance of differences were assessed using a generalized estimating equations (GEE) model. Differences in mean percent change from baseline were determined using the GEE model. Linear regression analysis was performed for continuous study variables. Cox regression was used to determine correlation between baseline weight, height, BMI

z-scores and response to KD therapy. Microsoft Excel (version 14.4.8, Redmond, WA), StataCorp LP STATA/IC (version 13.1, College Station, Texas), and SPSS (version 24, Armonk, North Castle, NY) were used for data analysis. Statistical output was considered significant at $p<0.05$.

Doernbecher Carnitine Status Assessment Protocol

Historically, carnitine sufficiency was assessed through acylcarnitine profile analysis. Since February 2014, all patients receiving dietary treatment at the Ketogenic Diet Clinic have also had their carnitine status assessed through free, total, and acyl carnitine concentrations as well as acyl-to-free carnitine ratios.

Biochemical Assessment of Carnitine and Anthropometric Data Collection

Plasma free-, acyl- total and acyl/free carnitine concentrations and anthropometric measurements were measured during each participant's first hospital admission before starting the KD at Doernbecher Children's Hospital. On the first day of admission, patients' weight, length, and height were obtained, and a fasting blood sample was collected to measure baseline concentrations of carnitine concentrations. Baseline and follow-up weight, height and/or length measurements, blood draws, and biochemical analyses required by the clinic were repeated at each of the patient's routine follow-up appointments.

Chapter 4: Results

Study Participants

A medical record review was conducted for 48 patients who initiated the Ketogenic Diet for treatment of intractable epilepsy at Doernbecher Children's Hospital. Figure 1 illustrates the patient selection process. Patients were excluded if: they were prescribed a Ketogenic Diet ratio of less than three grams of fat for every one gram of combined carbohydrate and protein (n=2); they were prescribed L-carnitine supplementation at baseline or during Ketogenic Diet treatment (n=10); they had a weight z-score <-5 (n=1); they had no baseline weight z-score data available (n=1); and/or they had no baseline carnitine concentration values at least one month after Ketogenic Diet initiation (n=26). Of the 48 patients who initiated dietary treatment with the Ketogenic Diet, 34 patients were included in the analyses represented in Figures 1-5 and in Figure 14, and eight patients were included in all other figures.

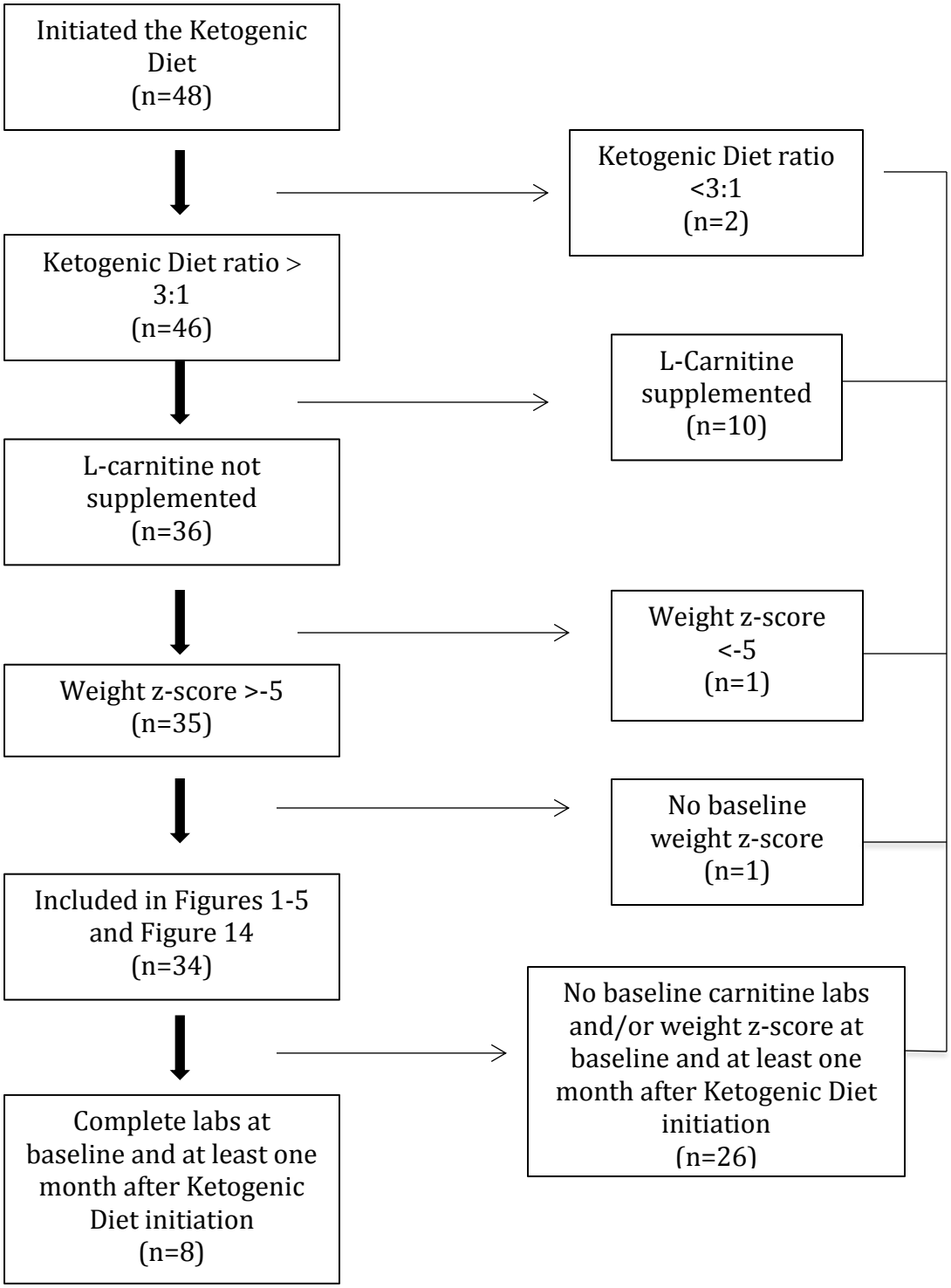

Figure 1: Patient selection process. Horizontal arrows indicate number of patients excluded for various reasons.

Patient characteristics

Table 1 describes baseline patient characteristics at the time of Ketogenic Diet initiation (n=34). Eighteen male patients (53%) and 16 female patients (47%) were included. The mean age of patients was 3.89 ± 3.90 years with a range of 0.1 – 13.9 years. Sixteen (47%) of patients were less than two years old, and 18 (53%) patients were two years of age or older. Fifteen (44%) patients received treatment with a Ketogenic Diet ratio of 3:1, three (0.09%) patients received dietary treatment with a ratio of 3.5:1, and 16 (47%) patients received dietary treatment with a ratio of 4:1. Twenty-one (62%) patients obtained nutrition through enteral formula, eight (24%) were orally fed, and five (15%) received dietary therapy from a combination of enteral formula and foods.

Table1: Patient characteristics at Ketogenic Diet initiation (n=34)

Category	**Number of Patients**
Sex	
Male	18 (53%)
Female	16 (47%)
Age (years)	
Mean ± SD	3.89 ± 3.90
Median	2.1
Range	0.1 – 13.9
Number of patients by age	
<2 years	16 (47%)
2+ years	18 (53%)
Ketogenic Diet Ratio	
3:1	15 (44%)
3.5:1	3 (0.09%)
4:1	16 (47%)
Ketogenic Diet Source	
Enteral formula	21 (62%)
Foods	8 (24%)
Enteral formula + foods	5 (15%)
Epilepsy Etiology	
Lennox-Gastout Syndrome	3 (9%)
Cerebral Palsy	3 (9%)
Infantile spasms	7 (21%)
Unclear etiology	6 (18%)
Early infantile epileptic encephalopathy	2 (6%)
Glucose transporter 1 deficiency syndrome	2 (6%)
Hypoxic ischemic encephalopathy	3 (9%)
Mitochondrial defect	1 (3%)
Trisomy 13 syndrome	1 (3%)
Chromosomal deletion	1 (3%)
MECP2 duplication	1 (3%)
Schizencephaly	2 (6%)
Hypoxic ischemic injury	1 (3%)
Traumatic brain injury	1 (3%)

Impact of the Ketogenic Diet on growth among children with intractable epilepsy.

The first aim of this study was to describe growth in children with intractable epilepsy from initiation of the Ketogenic Diet through 12 months of treatment. Growth information is presented for children who were less than two years of age and for children between two and 18 years of age at the time treatment with the Ketogenic Diet was initiated. Growth parameters were assessed using sex- and age-specific weight, height, and BMI z-scores. BMI z-scores were only used to describe growth in patients after they reached two years of age. To better understand factors that may contribute to changes in weight and height z-scores, we explored whether children received enteral nutrition support, and if so, when during Ketogenic Diet treatment this mode of feeding was initiated.

Growth in children less than two years of age

Changes over time in individual weight and height z-scores are illustrated in Figures 8 and 9, respectively, for patients less two years of age (n=17). As depicted in Figure 8, of the 17 patients included in this analysis, weight z-score decreased in nine patients, increased in two patients, and remained relatively stable in seven patients after initiating the Ketogenic Diet. The average weight z-score at baseline (Time 0 months) in this subset of patients was -0.63, 95% CI [-1.22, -0.04] which decreased by an average of -0.05 units per month, 95% CI [-0.11, 0.02] following Ketogenic Diet initiation. As described in Figure 9, the average height z-score at baseline was -0.96, 95% CI [-1.59, -0.33] which decreased by an average of -0.05 units per month, 95% CI [-0.13,

-0.03] following Ketogenic Diet initiation. The downward trends observed for weight and height z-scores over the first 12 months of Ketogenic Diet treatment were not statistically significant (p=0.17 and p=0.22, respectively).

Of the 17 patients under two years of age at initiation of Ketogenic Diet therapy, seven were fed orally and ten received enteral nutrition support. Of the ten patients receiving enteral nutrition support, seven were dependent on gastrostomy, jejunostomy, or gastrojejunostomy feeding prior to Ketogenic Diet initiation, and three patients initiated nasogastric or nasojejunal tube feeding during their course of treatment.

Patients who experienced a substantial increase or decrease in weight and/or height z-scores are identified numerically (1-11) in Figures 10 and 11, respectively, and are described in more detail below.

Patient One's weight z-score was higher three months after Ketogenic Diet initiation (2.29) than at baseline (1.81) or 12 months post intervention (0.86). The lower weight z-score at 12 months likely reflects a change in dietary prescription made at three months to address prior excessive weight gain. This patient’s height z-score was also lower at 12 months post-intervention (1.4) than baseline (2.21), three months (2.29), or nine months post-intervention (1.86).

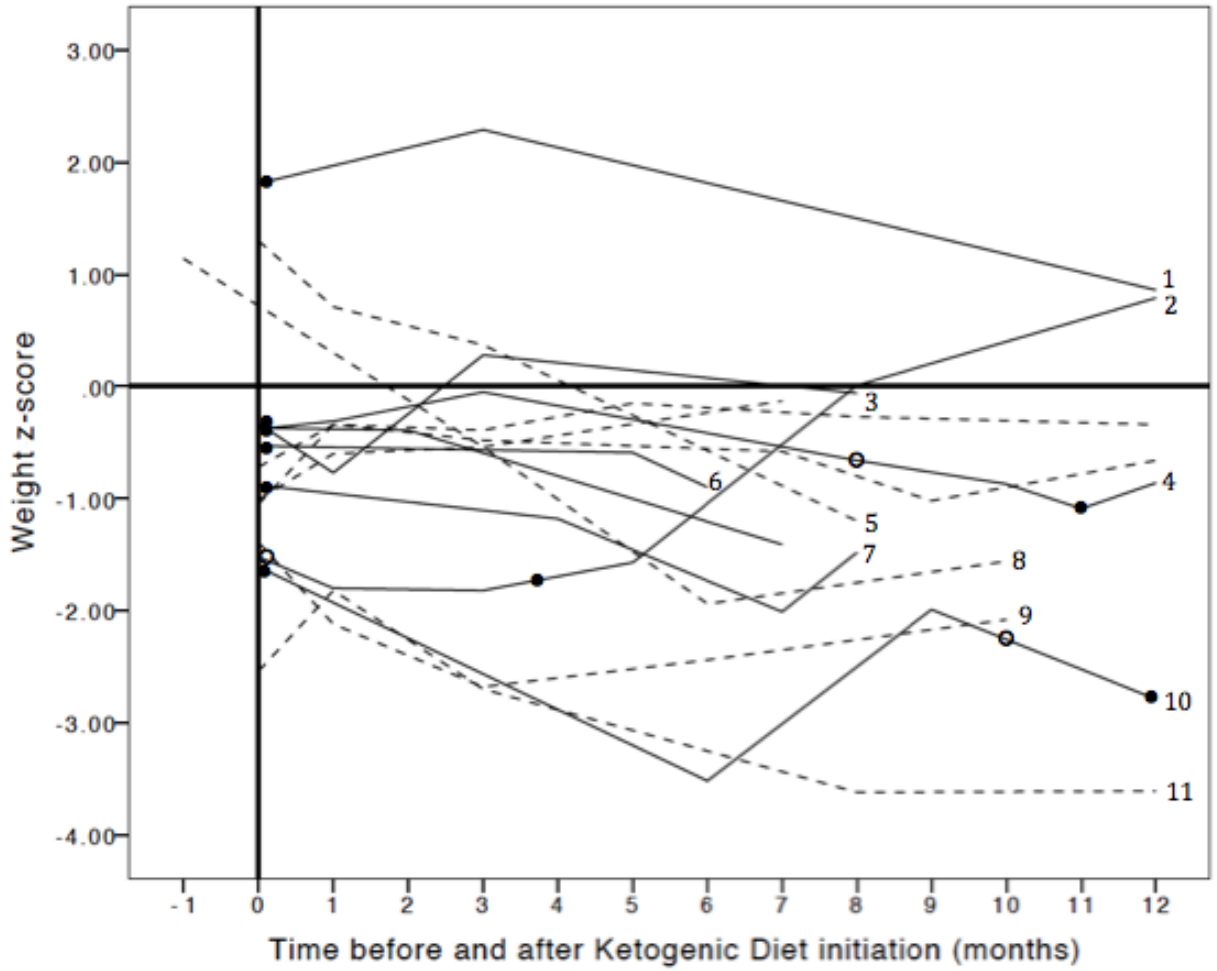

Figure 8. Individual change in weight z-scores from initiation of the Ketogenic Diet through 12 months of treatment inpatients less than two years of age with intractable epilepsy (n=17). _____ Enteral nutrition support - - - - - - - - - - Orally fed • Gastrostomy, jejunostomy or gastrojejunostomy feeding tube o Nasogastric or nasojejunal feeding tube

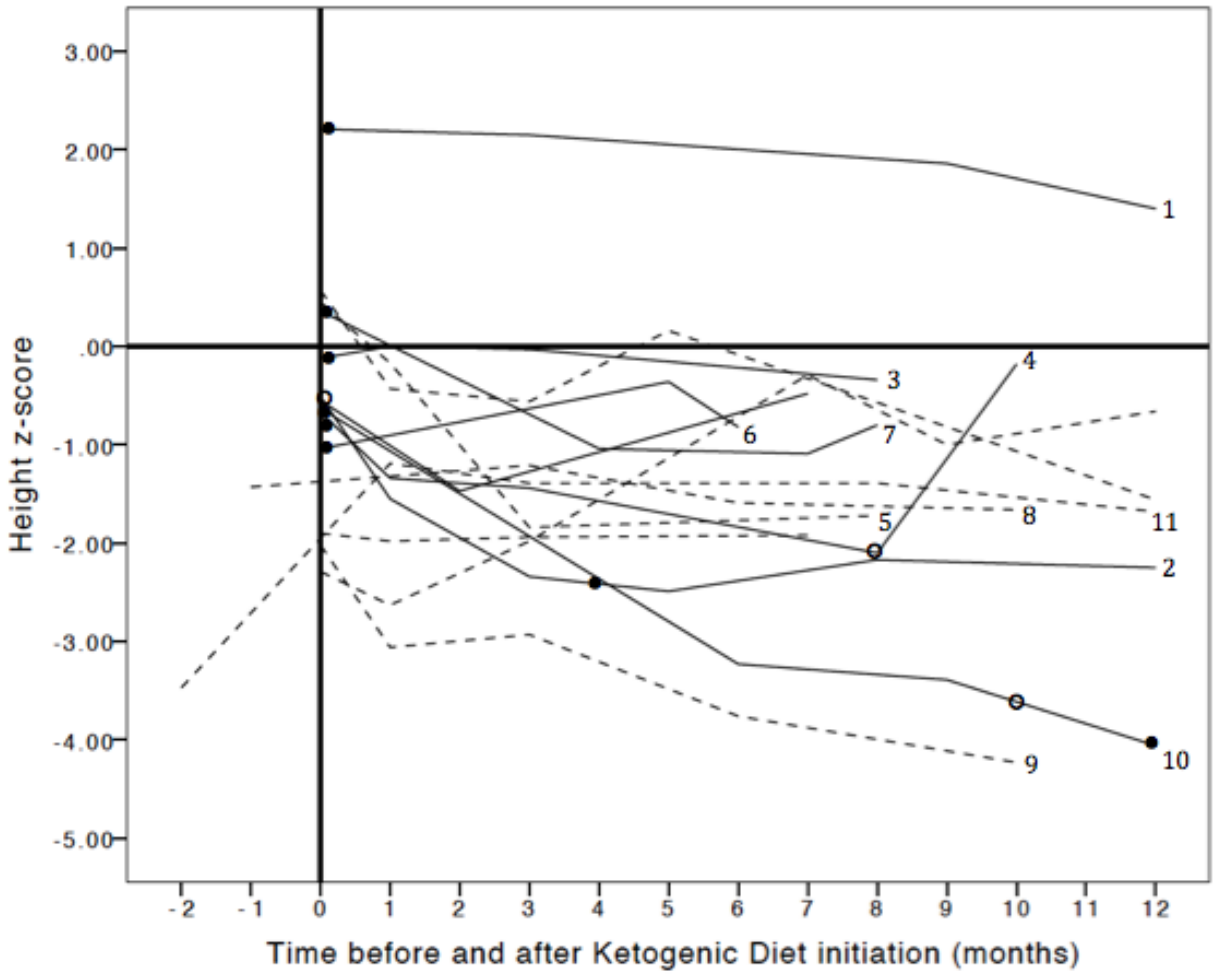

Figure 9. Individual change in weight z-scores from initiation of the Ketogenic Diet through 12 months of treatment inpatients less than two years of age with intractable epilepsy (n=17). _____ Enteral nutrition support - - - - - - - - - Orally fed • Gastrostomy, jejunostomy or gastrojejunostomy feeding tube o Nasogastric or nasojejunal feeding tube

Patient Two had a history of intermittent nasogastric tube feeding since birth and had a gastrostomy tube placed four months after Ketogenic Diet initiation. Following gastrostomy tube placement, this patient's weight z-score increased from -1.52 at baseline and -1.82 at three months to 0.01 at eight months and 0.79 at 12 months post-intervention. This patient's height z-score decreased from -0.51 at baseline to -2.34 three months after diet initiation and then stabilized after gastrostomy tube placement, so that the value at 12 months post intervention was -2.25.

Patient Three's weight z-score was lower at baseline (-0.33) and one month (-0.77) than at eight months (-0.06). The increase in weight z-score may be attributed to a modification to this patient's dietary prescription. Height z-score remained relatively stable between baseline (-0.12) and eight months (-0.34) post-intervention.

Patient Four had a nasogastric tube placed at seven months, a nasojejunostomy tube placed at eight months, and a gastrojejunostomy tube placed at 11 months after diet initiation. This patient's weight z-score was -0.38 at baseline, -0.05 at three months, and -1.09 eleven months post-intervention. Weight z-score increased to -0.86 one month after gastrojejunostomy tube placement. This patient's height z-score was -0.67 at baseline and -2.1 at eight months.

Patient Five's weight z-score was 1.3 at baseline and progressively declined to -1.2 by eight months after diet initiation. This decline in weight z-score is likely related

to discontinuing steroid medication. This patient's height z-score was also higher at baseline (0.45) than at three months (-1.84) or eight months (-1.72).

Patient Six's weight z-score was -0.53 at baseline, -0.5 at five months, and -0.9 at six months following a reported adjustment to the dietary prescription to address excessive weight gain. This patient's height z-score was higher after five months (-0.36) than either baseline (-1.04) or six months after Ketogenic Diet initiation (-0.82), which parallels the changes in weight z-score.

Patient Seven's weight z-score was higher at baseline (-0.88) than seven (-2.01) or eight (-1.68) months after diet initiation. The increase in weight z-score from seven to eight months may reflect an adjustment made to the Ketogenic Diet ratio made six months after diet initiation. This patient's height z-score was higher at baseline (0.36) than at four (-1.04), seven (-1.09), or eight (-0.8) months after diet initiation, which parallels the change in weight z-score

Patient Eight's weight z-score was higher at baseline (1.14) than three months (-0.54) or six months (-1.94) after Ketogenic Diet initiation. The decrease in weight z-score between three months and six months is likely due to refusal of formula and difficulty consuming solid food. This patient's weight z-score stabilized with improved formula and food intake and was -1.56 at 10 months. This patient's height z-score remained relatively stable throughout Ketogenic Diet treatment.

Patient Nine was on adrenocorticotropic hormone therapy prior to starting the Ketogenic Diet and experienced weight loss following discontinuation of this medication. Weight z-score was higher at baseline (-1.39) than three months (-2.68) or ten months (-2.08) after diet initiation. The increase in weight z-score between three and ten months is likely due to a change in formula concentration to increase total energy intake. This patient demonstrated a steady decline in height z-score from -2.02 at baseline to -4.23 at 10 months after diet initiation.

Patient Ten had a complicated course of feeding. This patient was gastrostomy tube dependent prior to Ketogenic Diet initiation, after which a nasojejunstomy tube was placed at ten months and a gastrojejunostomy tube was placed at 12 months. Weight z-score was higher at baseline (-1.61) than six months (-3.52), nine months (-1.99), or 12 months (-2.79) after Ketogenic Diet initiation. This patient's height z-score was also higher at baseline (-0.63) than 12 months after diet initiation (-4.06).

Patient Eleven experienced a steady decrease in weight z-score throughout dietary treatment from -1.82 at one month to -3.62 at eight months and -3.61 at 12 months. This patient's dietary prescription was modified ten months after dietary intervention to address this decline in weight z-score. This patient's height z-score remained relatively stable between -1.19 and -1.68 throughout dietary treatment.

Growth in children two years of age and older

Figures 10-12 illustrate the individual change in weight, height, and BMI z-scores, respectively, among patients two years of age and older at Ketogenic Diet initiation (n=11). Patients who experienced a substantial increase or decrease in weight, height, and/or BMI z-scores are identified numerically (12-17) in Figures 10, 11, and 12, respectively, and are described in more detail below.

Of the 11 patients included in this analysis, weight z-scores increased in five patients, decreased in three patients and remained relatively stable in three patients. The average weight z-score among this subset of patients was -0.24, 95% CI [-0.81, 0.32] at baseline (Time 0 months) which increased significantly by an average of 0.04 units per month, 95% CI [0.01, 0.08] (p=0.02) over 12 months. The average height z-score at baseline among this subset of patients was -0.82, 95% CI [-1.54, -0.10] which decreased by an average of -0.003 units per month, 95% CI [-0.04, 0.04] following Ketogenic Diet initiation. The downward trend for height z-score was not significant (p=0.90). The average BMI z-score among this subset of patients was 0.33, 95% CI [-0.44, 1.10] at baseline, which increased by an average of 0.06 units per month, 95% CI [-0.01, 0.12] following Ketogenic Diet initiation. The upward trend for BMI z-score was not significant (p=0.08).

Patient Twelve's weight z-score was lower two months prior to Ketogenic Diet initiation (-0.23) than at one month (0.17), three months (0.86), six months (1.27), or 12 months (0.73) after diet initiation. This patient's height z-score was 1.41 two months

prior to diet initiation, 1.41 at three months, 0.94 at six months, and 1.92 at 12 months after diet initiation. BMI z-score was -2.69 two months prior to Ketogenic Diet initiation, -0.33 at three months, 0.49 at six months, and -1.15 at 12 months after Ketogenic Diet initiation. The decrease in weight and BMI z-score from six to 12 months was likely due to a modification to this patient's dietary prescription to address excess weight gain.

Patient Thirteen was hospitalized five months after initiating the Ketogenic Diet for possible small bowel obstruction, which was ruled out. This patient did, however, experience ongoing feeding difficulties due to formula intolerance throughout Ketogenic Diet therapy. Weight z-score was higher at baseline (1.14) than six months after diet initiation (0.51). Height z-score was slightly lower at baseline (-0.09) than six (0.06) months after diet initiation. BMI z-score was higher at baseline (1.84) than six months (0.69) after dietary treatment.

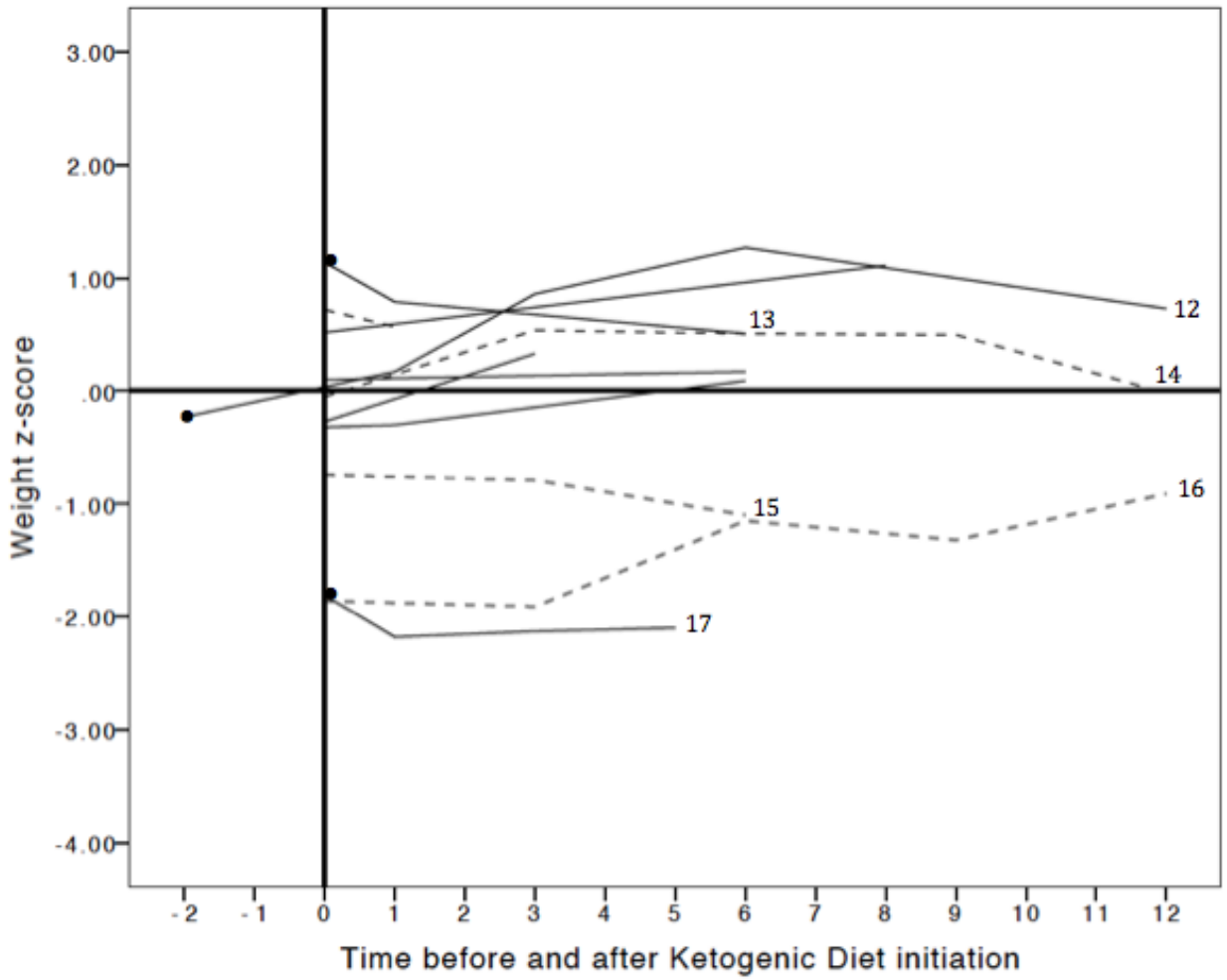

Figure 10. Individual change in weight z-scores after Ketogenic Diet initiation in patients two years of age and older with intractable epilepsy (n=11). _____ Enteral nutrition support - - - - - - - - - - Orally fed • Gastrostomy, jejunostomy or gastrojejunostomy feeding tube o Nasogastric or nasojejunal feeding tube

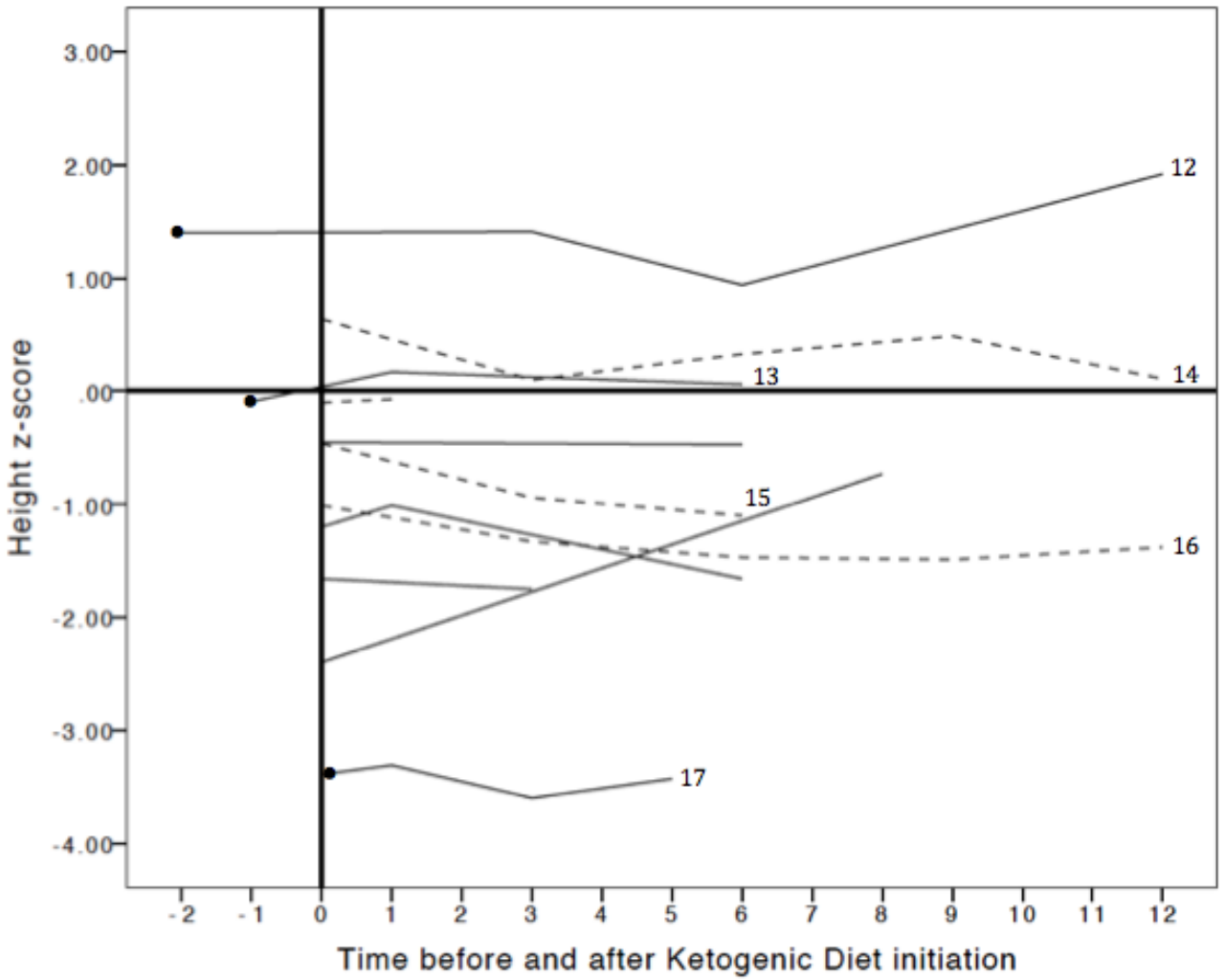

Figure 11. Individual change in height z-scores after Ketogenic Diet initiation in patients two years of age and older with intractable epilepsy (n=11). _____ Enteral nutrition support - - - - - - - - - - Orally fed • Gastrostomy, jejunostomy or gastrojejunostomy feeding tube o Nasogastric or nasojejunal feeding tube

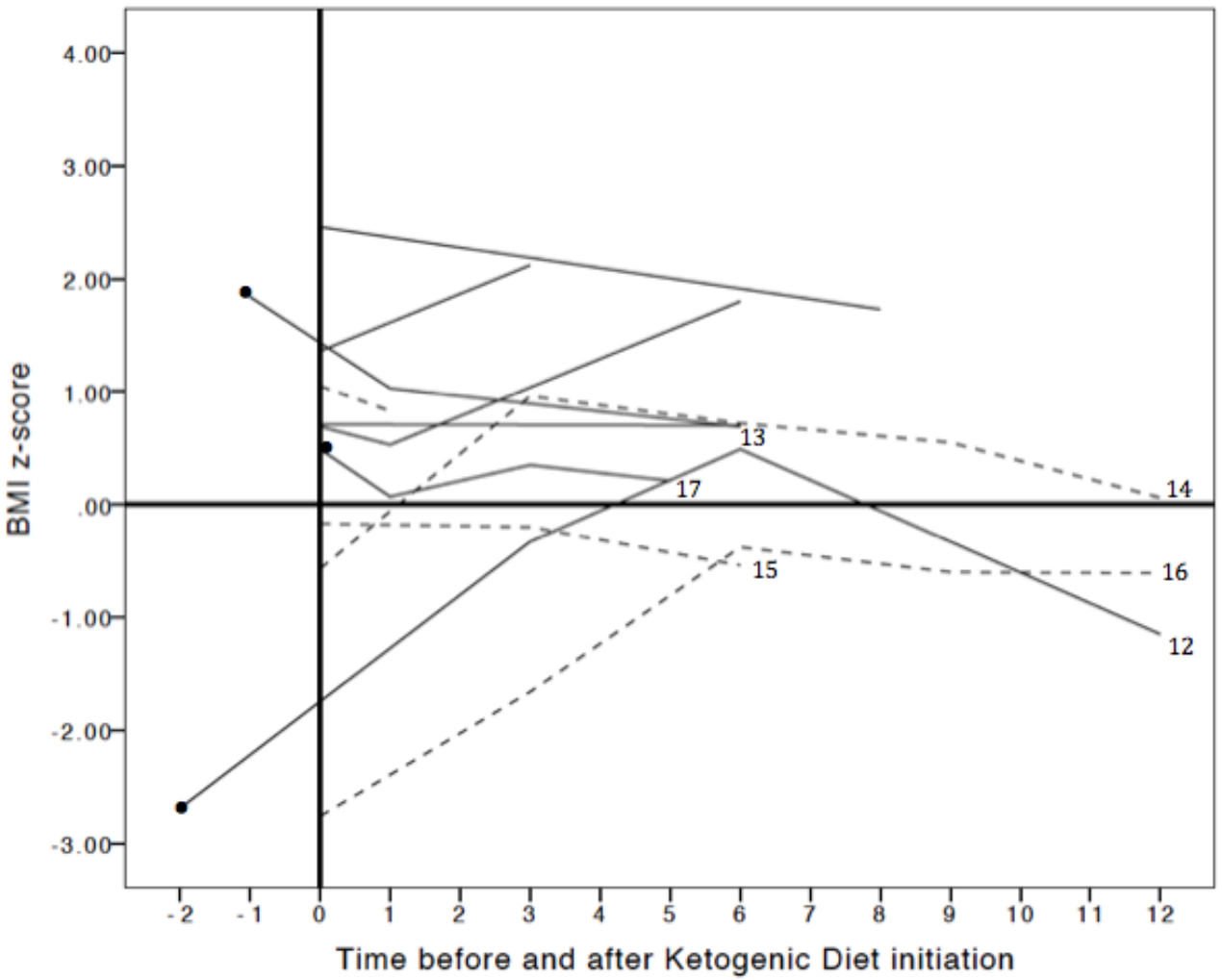

Figure 12. Individual change in BMI z-scores after Ketogenic Diet initiation in patients two years of age and older with intractable epilepsy (n=11). _____ Enteral nutrition support - - - - - - - - - Orally fed • Gastrostomy, jejunostomy or gastrojejunostomy feeding tube o Nasogastric or nasojejunal feeding tube

Patient Fourteen's weight z-score was lower at baseline (-0.05) than one (-0.04), three (0.54), six (0.51), nine (0.5), and 12 months (-0.02) after diet initiation. The initial increase in weight z-score was likely due to a change in dietary prescription made to address this patient's extreme hunger. No concern was documented regarding the decrease in weight z-score from nine to 12 months. Height z-score was higher at baseline (0.64) than three (0.10), six (0.33), nine (0.49), and twelve months (0.11) after initiation. BMI z-score was lower at baseline (-0.57) than three (0.96), six (0.72), nine (0.55) or 12 months (0.06).

Patient Fifteen's weight, height and BMI z-score were all higher at baseline (-0.74, -0.46, -0.17) than at three months (-0.79, -0.94, -0.2) and six months (-1.1, -1.1, -0.54) after Ketogenic Diet initiation. The decrease in z-scores is likely due to reported feeding difficulties.

Patient Sixteen experienced difficulty eating one month after starting the Ketogenic Diet. This patient had their dietary prescription modified two months after initiation, and their energy intake improved at three months. Weight z-score was -1.86 at baseline, -1.91 at three months, -1.15 at six months, and -0.91 at 12 months. Height z-score remained relatively stable throughout dietary treatment; it fluctuated between -1.01 at baseline, -1.47 at six months and -1.38 at 12 months. BMI z-score was lower at baseline (-2.76) than six (-0.38) or 12 months (-0.61).

Patient Seventeen's weight z-score was higher at baseline (-1.81) than at one month (-2.18) or five months (-2.10) after Ketogenic Diet initiation. This patient's height and BMI z-scores remained relatively stable from baseline (-3.39, 0.49) to five months (-3.43, -0.21) after initiation.

Correlation between weight, height, and BMI z-scores at baseline and change in plasma carnitine concentrations after Ketogenic Diet initiation

The second aim of this study was to determine whether weight, height or BMI z-scores at initiation of the Ketogenic Diet are correlated with change in plasma carnitine concentrations and seizure frequency one, three, six, nine, and twelve months after diet initiation among children with intractable epilepsy.

Figures 13-15 describe the relationships between weight z-score at baseline and change in carnitine concentrations one (n=8), three (n=6), and six (n=5) months after Ketogenic Diet initiation, respectively. Correlational analyses were not performed at nine and 12 months due to insufficient sample size (n<5).

No significant correlations were observed between baseline weight z-score and change in carnitine concentrations after one, three, or six months of treatment with the Ketogenic Diet, however, a number of trends were observed. As shown in Figure 13, seven of eight patients had a baseline weight z-score at or below 0. Five of these patients demonstrated a reduction in free carnitine concentration (Figure 13A), six demonstrated an increase in acylcarnitine concentration (Figure 13B), five

demonstrated an increase in total carnitine concentration (Figure 13C), and six patients experienced an increase in acyl/free carnitine concentration ratio (Figure 13D).

Figures 14 illustrates the correlations between baseline weight z-score and changes in plasma carnitine concentrations three months after Ketogenic Diet initiation. As shown in Figure 14A, all six patients had baseline weight z-scores at or below 0. Free carnitine was lower at three months than baseline in all but one patient (Figure 14A), while acylcarnitine concentrations were higher at three months than baseline in all patients (Figure 14B). Total carnitine concentrations were higher in three patients, and three patients also experienced lower carnitine concentrations (Figure 14C). All patients experienced an increase in acyl/free carnitine concentration ratio after three months of treatment with the Ketogenic Diet (Figure 14D).

Figure 15 illustrates the correlations between baseline weight z-score and change in plasma carnitine concentrations six months after Ketogenic Diet initiation among five patients. All patients had baseline weight z-scores at or below 0. One patient had a higher free carnitine concentration (Figure 15A), all patients had higher acylcarnitine (Figure 15B) and total carnitine (Figure 15C) concentrations and higher acyl/free carnitine concentration ratios (Figure 15D) at six months than baseline.

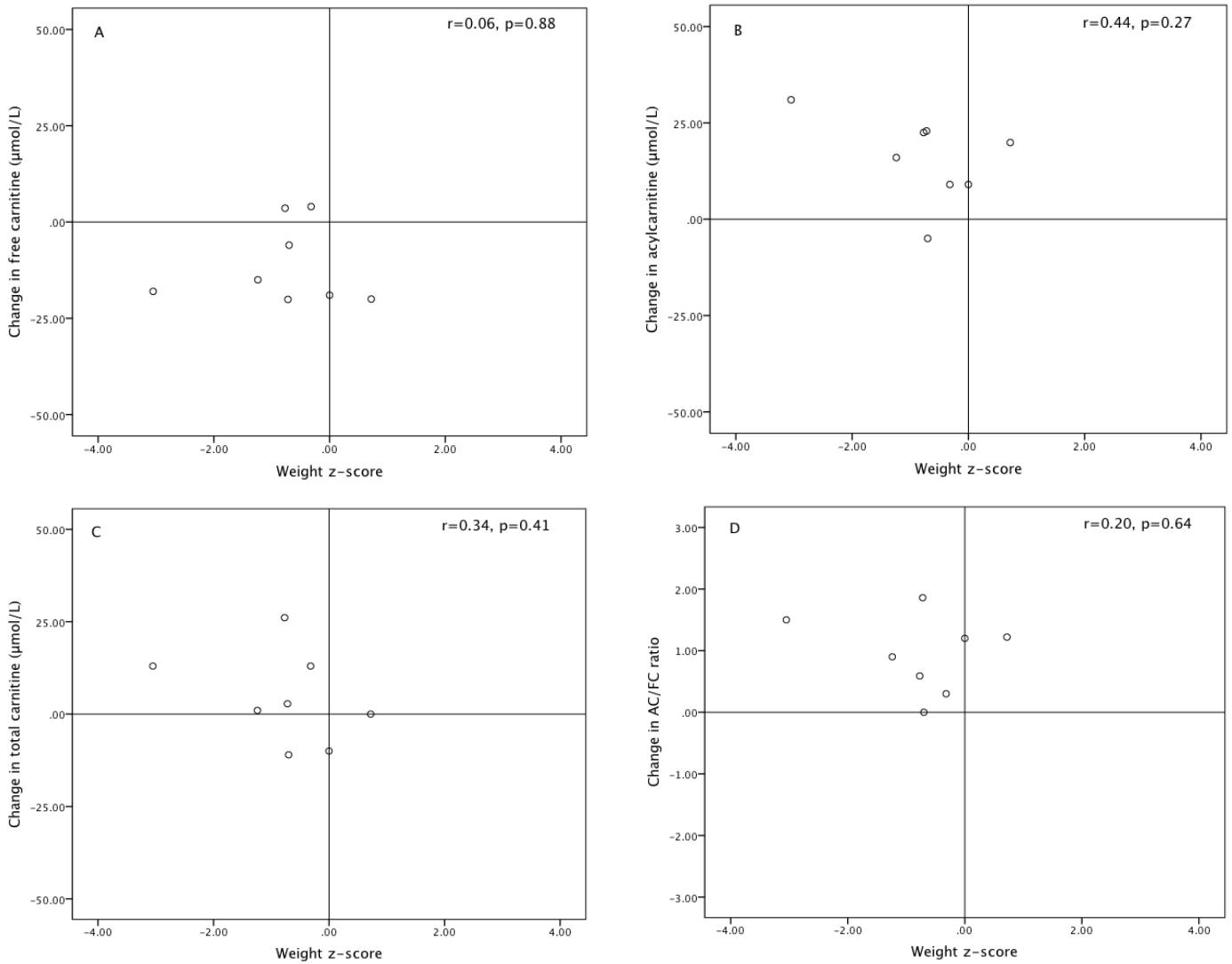

Figure 13. Correlation between baseline weight z-score and change in plasma free, acyl-, and total carnitine concentrations, and acyl/free carnitine concentration ratio 1 month after Ketogenic Diet initiation (n=8)

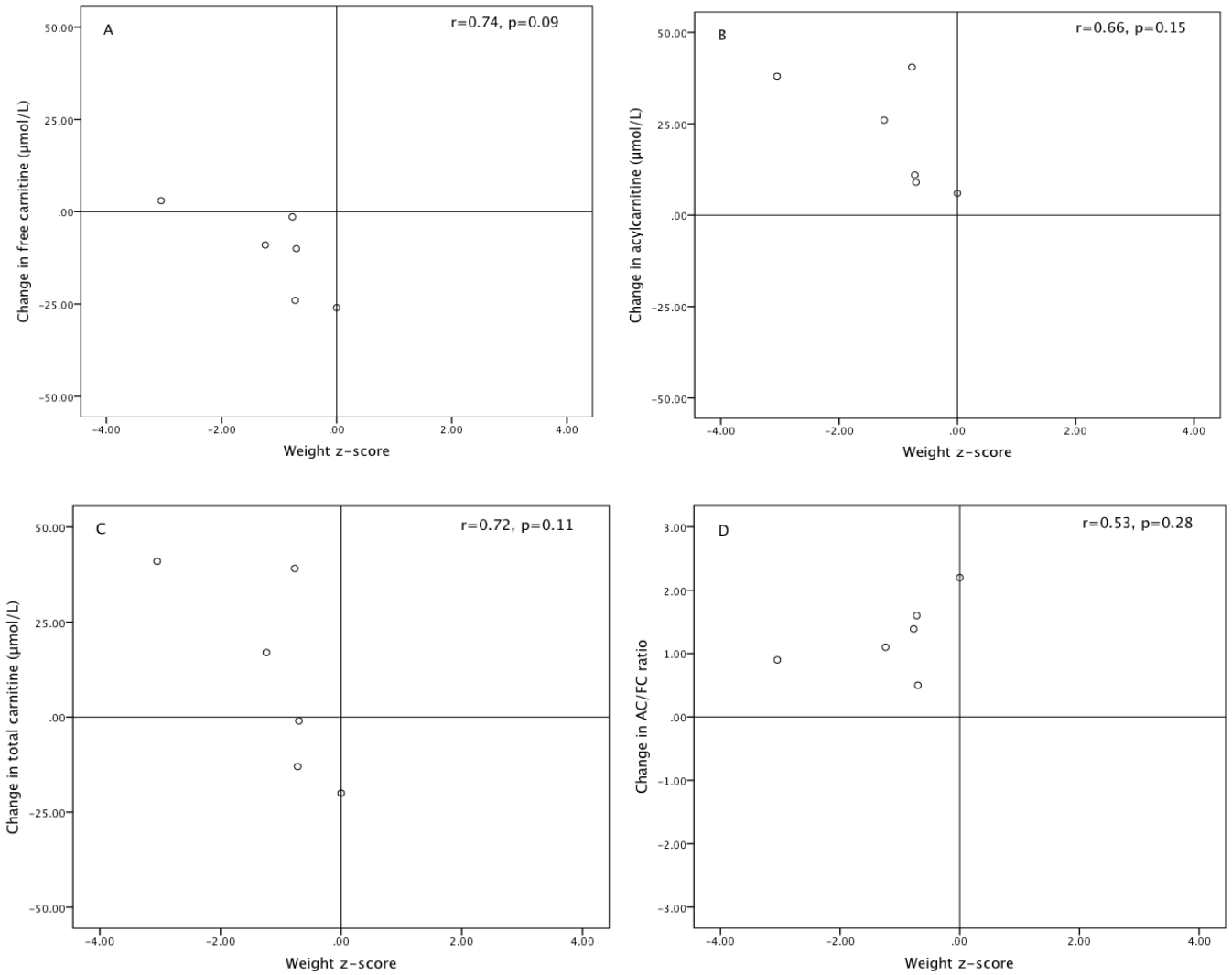

Figure 14. Correlation between baseline weight z-score and change in plasma free, acyl-, and total carnitine concentrations, and acyl/free carnitine concentration ratio 3 months after Ketogenic Diet initiation (n=6)

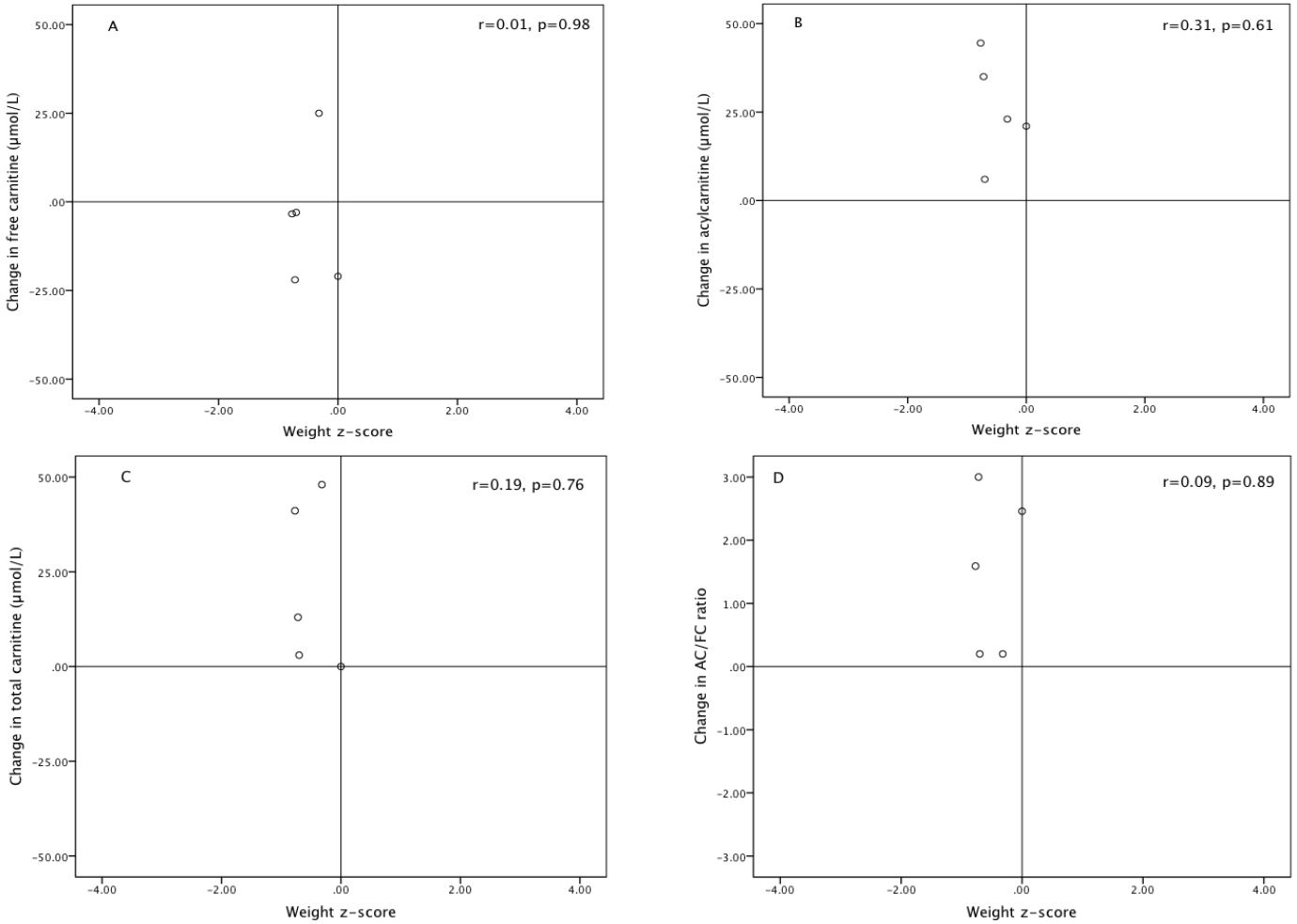

Figure 15. Correlation between baseline weight z-score and change in plasma free, acyl-, and total carnitine concentrations, and acyl/free carnitine concentration ratio 6 months after Ketogenic Diet initiation (n=5)

Figures 16 and 17 illustrate the relationships between baseline height z-score and changes in carnitine concentrations one (n=7) and three months (n=5) after Ketogenic Diet initiation, respectively. Correlational analyses were not performed at six, nine and 12 months due to insufficient sample size (n<5). No significant correlations between baseline height z-score and change in carnitine concentrations were observed; however, several trends were identified and are described in more detail below.

As shown in Figure 16, all but two patients had height z-scores less than 0 at baseline. Among these patients, all but one had lower free carnitine concentrations (Figure 16A), all but one had higher acylcarnitine concentrations (Figure 16B), all but two had higher total carnitine concentrations (Figure 16C), and all but one patient experienced an increase in acyl/free carnitine concentration ratios at three months than baseline (Figure 16D).

As shown in Figure 17, all but two patients had height z-scores less than 0 at baseline. Four of the five patients had lower free carnitine concentrations (Figure 17A), all patients had higher acylcarnitine concentrations (Figure 17B), half had higher total carnitine concentrations (Figure 17C), and all patients had higher acyl/free carnitine ratio concentrations three months after dietary intervention than before.

Figure 18 illustrates the relationship between baseline BMI z-score and change in carnitine concentrations one month after Ketogenic Diet initiation (n=6). Correlational analyses were not performed at three, six, nine, and 12 months due to insufficient sample size (n<5). Three patients had a BMI z-score at or below 0 at baseline. Four patients had lower free carnitine concentrations (Figure 18A), five had higher

acylcarnitine concentrations (Figure 18B), four had higher total carnitine concentrations (Figure 18C), and all patients had equal or higher acyl/free carnitine ratio concentrations at one month than baseline (Figure 18D).

In general, these results suggest that treatment with the Ketogenic Diet, dietary formula adjustments, tolerance issues, and frequent hospitalizations, results in lower weight, height and BMI z-scores. These results also suggest that treatment with the Ketogenic Diet results in lower free-, increased acyl- and increased total carnitine concentrations, as well as increased acyl/free carnitine concentration ratios in most, but not all, patients with intractable epilepsy. Finally, these results, although based on analyses with small sample sizes, suggest that no relationship exists between baseline growth parameters and change in carnitine concentration.

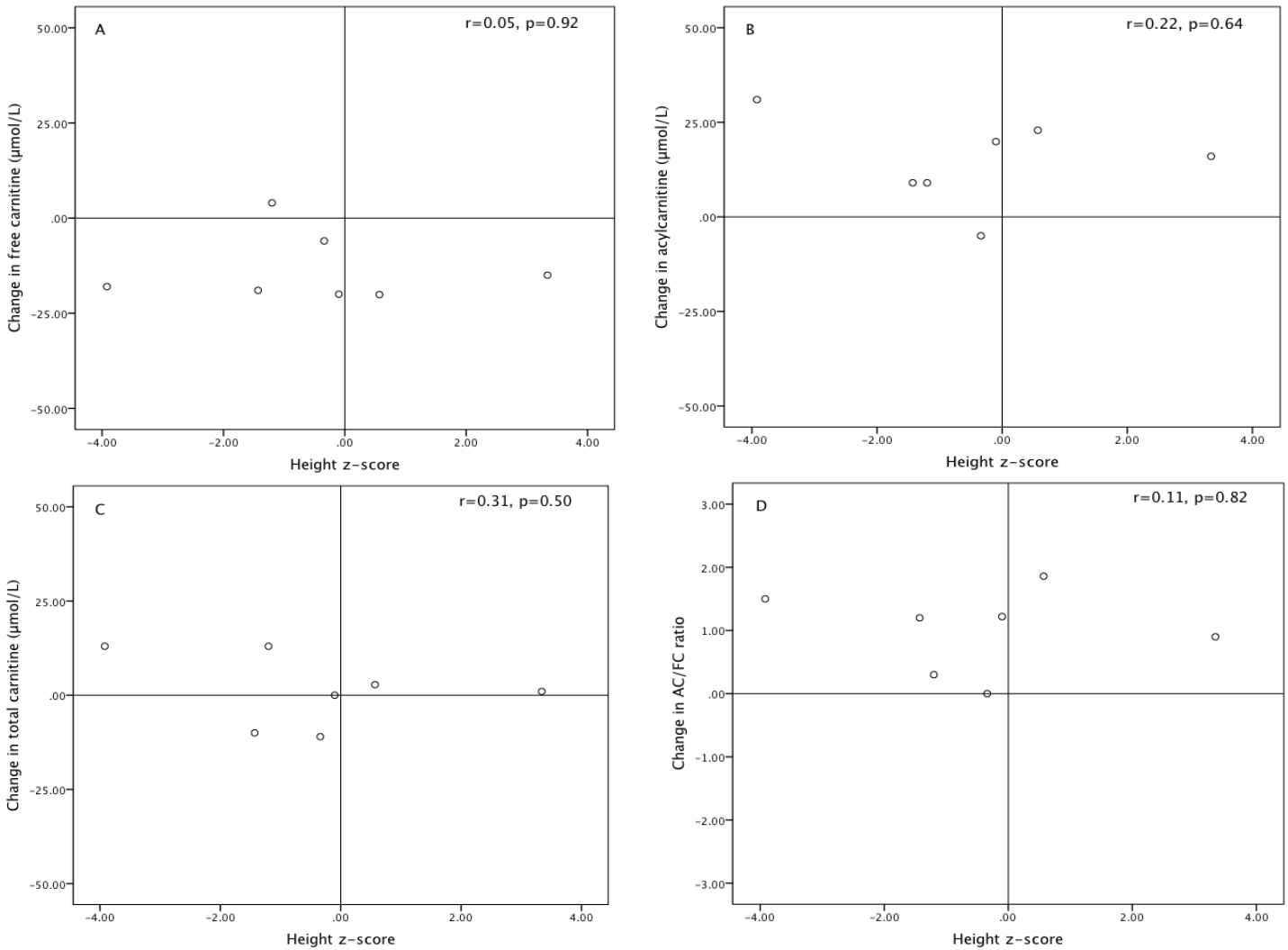

Figure 16. Correlation between baseline height z-score and change in plasma free, acyl-, and total carnitine concentrations, and the acyl/free carnitine concentration ratio 1 month after Ketogenic Diet initiation (n=7)

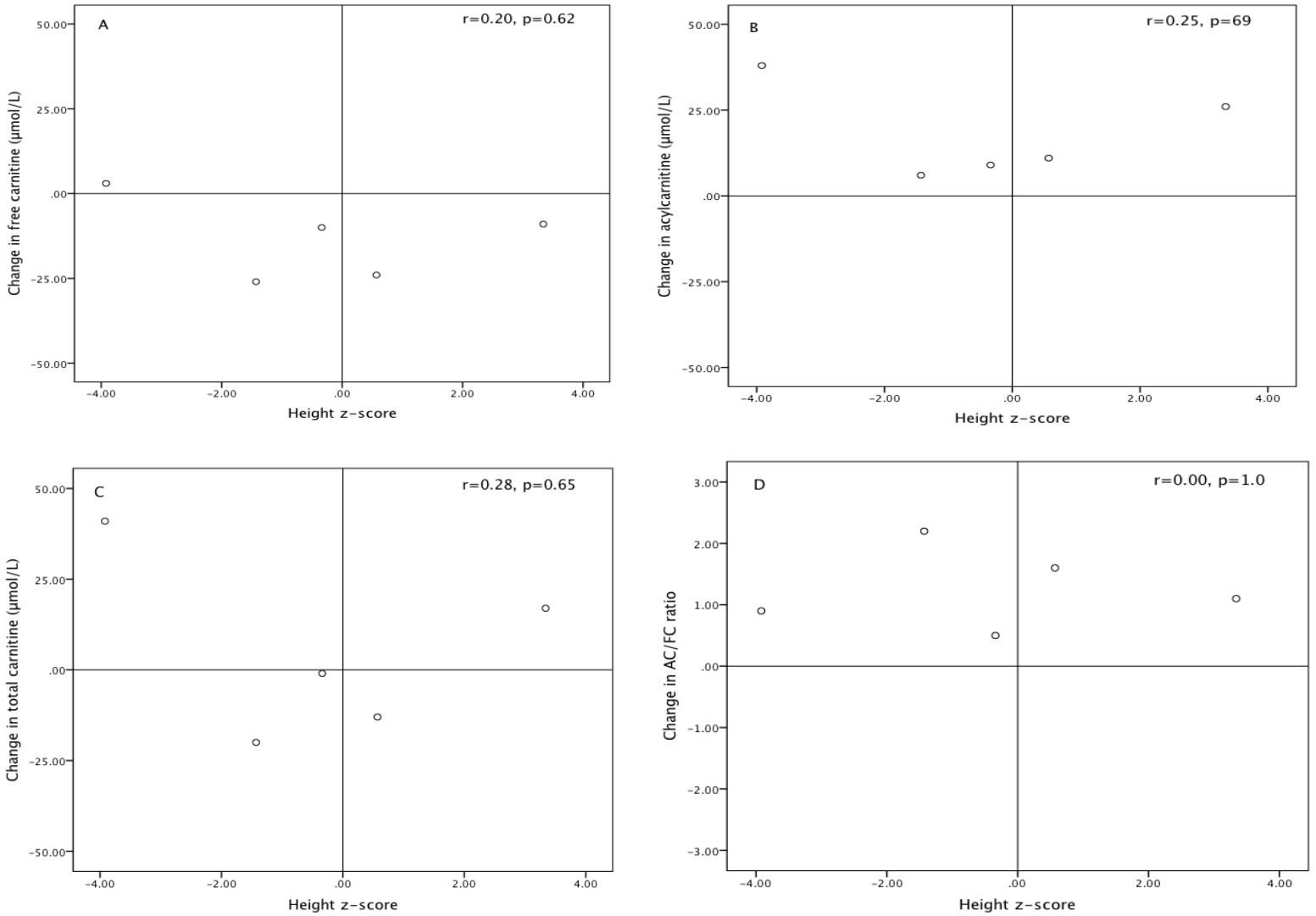

Figure 17. Correlation between baseline height z-score and change in plasma free, acyl- and total carnitine concentrations, and the acyl/free carnitine concentration ratio 3 months after Ketogenic Diet initiation (n=5)

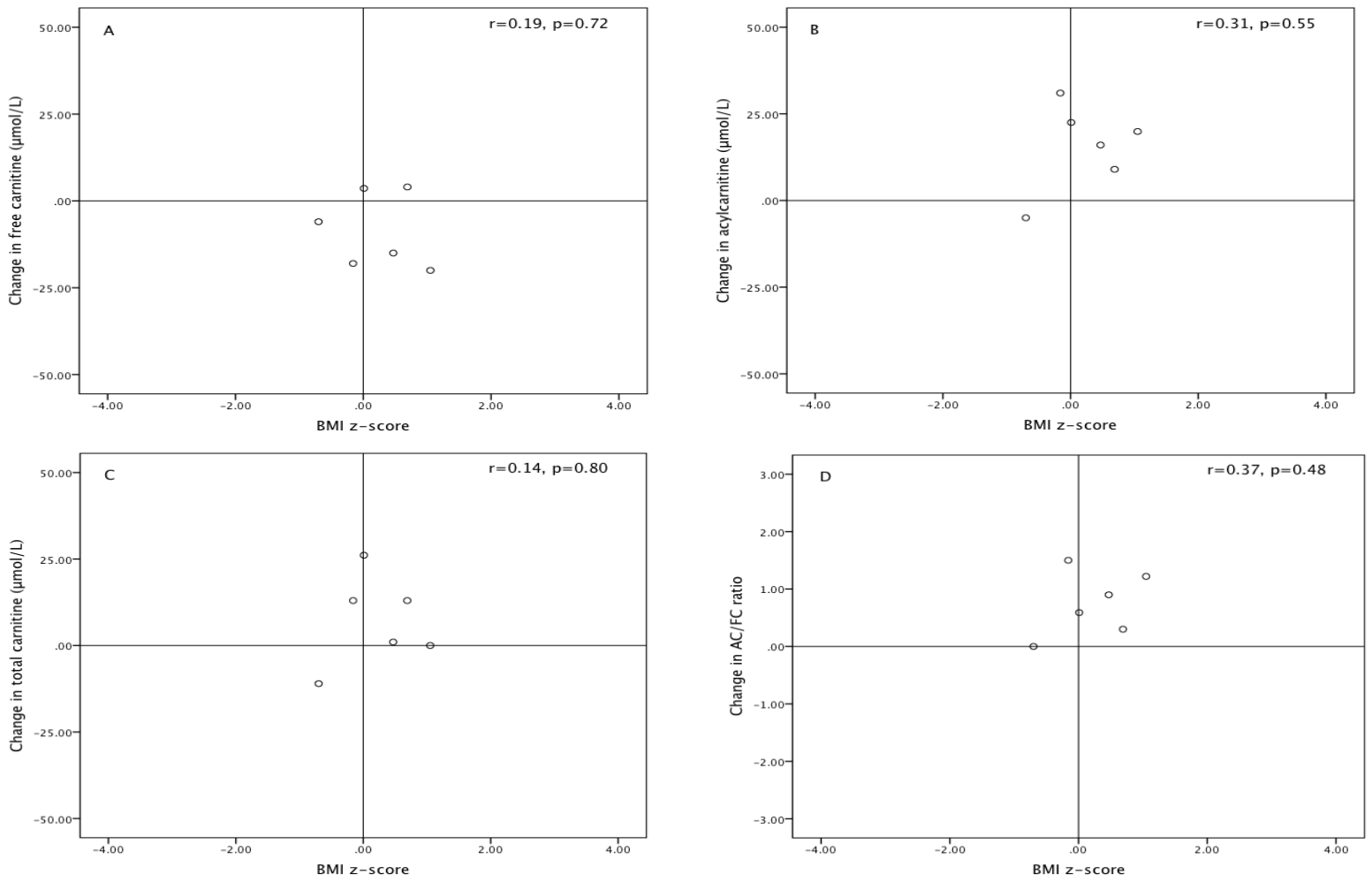

Figure 18. Correlation between baseline BMI z-score and change in plasma free, acyl-, and total carnitine concentrations, and the acyl/free carnitine concentration ratio 1 month after Ketogenic Diet initiation (n=7)

Correlation between change in weight, height and BMI z-score and change in plasma carnitine concentrations at one, three, six, nine and twelve months after Ketogenic Diet initiation

The third aim of this study was to determine whether changes in weight, height or BMI z-scores are correlated with changes in plasma carnitine concentrations one, three, six, nine and twelve months after Ketogenic Diet initiation among children with intractable epilepsy. The relationships between changes in weight z-score and change in carnitine concentration one and three months after Ketogenic Diet initiation are shown in Figures 19 and 20.

As illustrated in Figure 19 four patients experienced an increase in their weight z-scores from baseline to one month after Ketogenic Diet initiation. Among the six patients included in this analysis, five had lower free carnitine concentrations (Figure 19A), five had higher acylcarnitine concentrations (Figure 19B), and four had higher or the same total carnitine concentrations (Figure 19C). All patients had the same or higher acyl/free carnitine concentration ratios at one month than baseline (Figure 19D).

As illustrated in Figure 20, four of the six patients experienced an increase in their weight z-scores. Among these six patients, all but one had lower free carnitine (Figure 20A), all had higher acylcarnitine concentrations (Figure 20B), and three had higher total carnitine concentrations at three months than baseline (Figure 20C). A more positive or greater increase in weight z-score was significantly associated with less of an increase in acyl/free carnitine concentration ratio ($p=0.003$).

Analysis of changes in weight z-score and change in carnitine concentrations were not performed at six, nine, and 12 months due to insufficient sample sizes ($n<5$). Likewise, change in height and BMI z-scores were also not analyzed at any of the time points due to insufficient sample size ($n<5$).

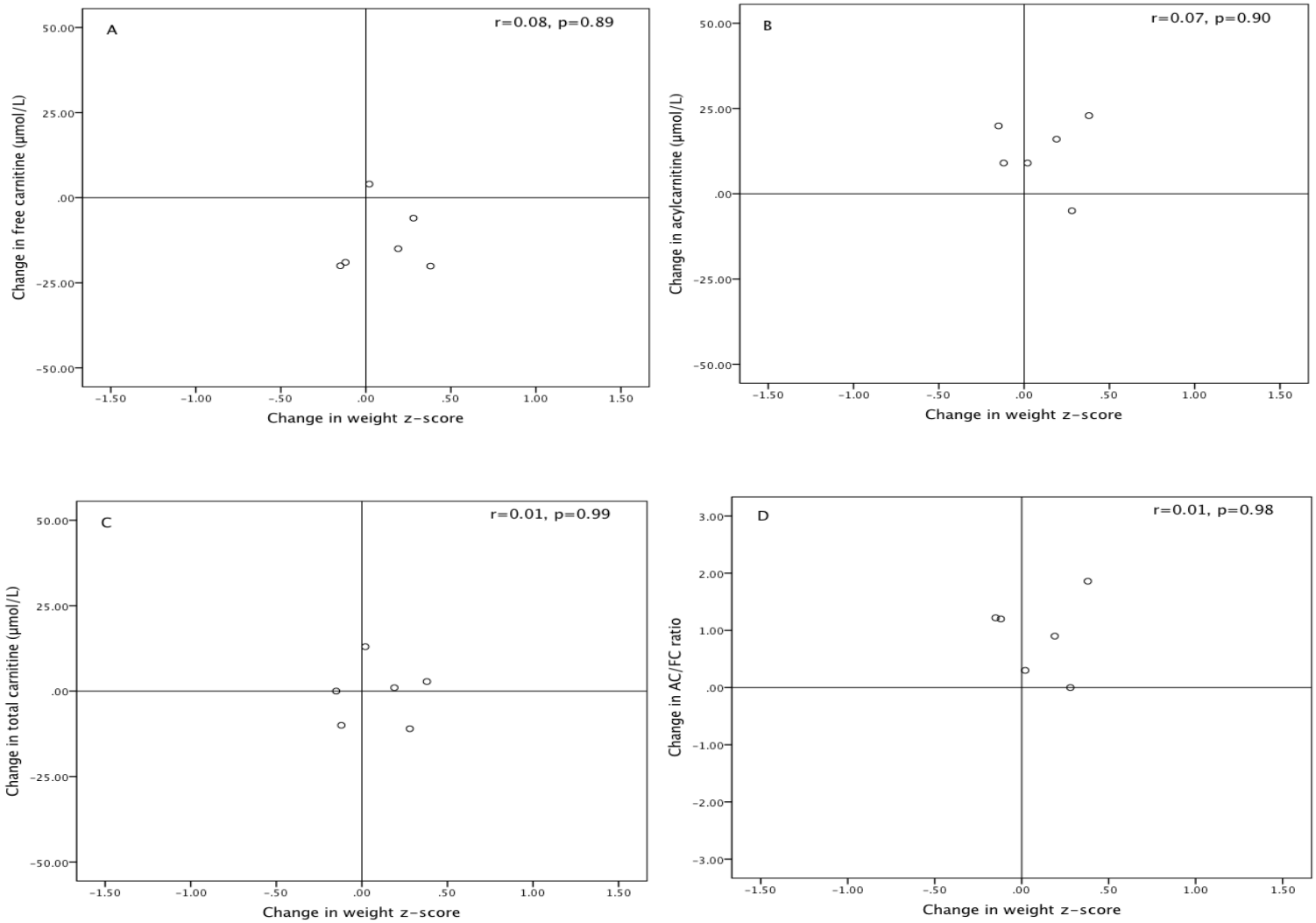

Figure 19: Correlation between change in weight-for-age z-score and change in plasma free-, acyl- and total carnitine concentrations, and acyl/free carnitine concentration ratio 1 month after Ketogenic Diet initiation (n=6)

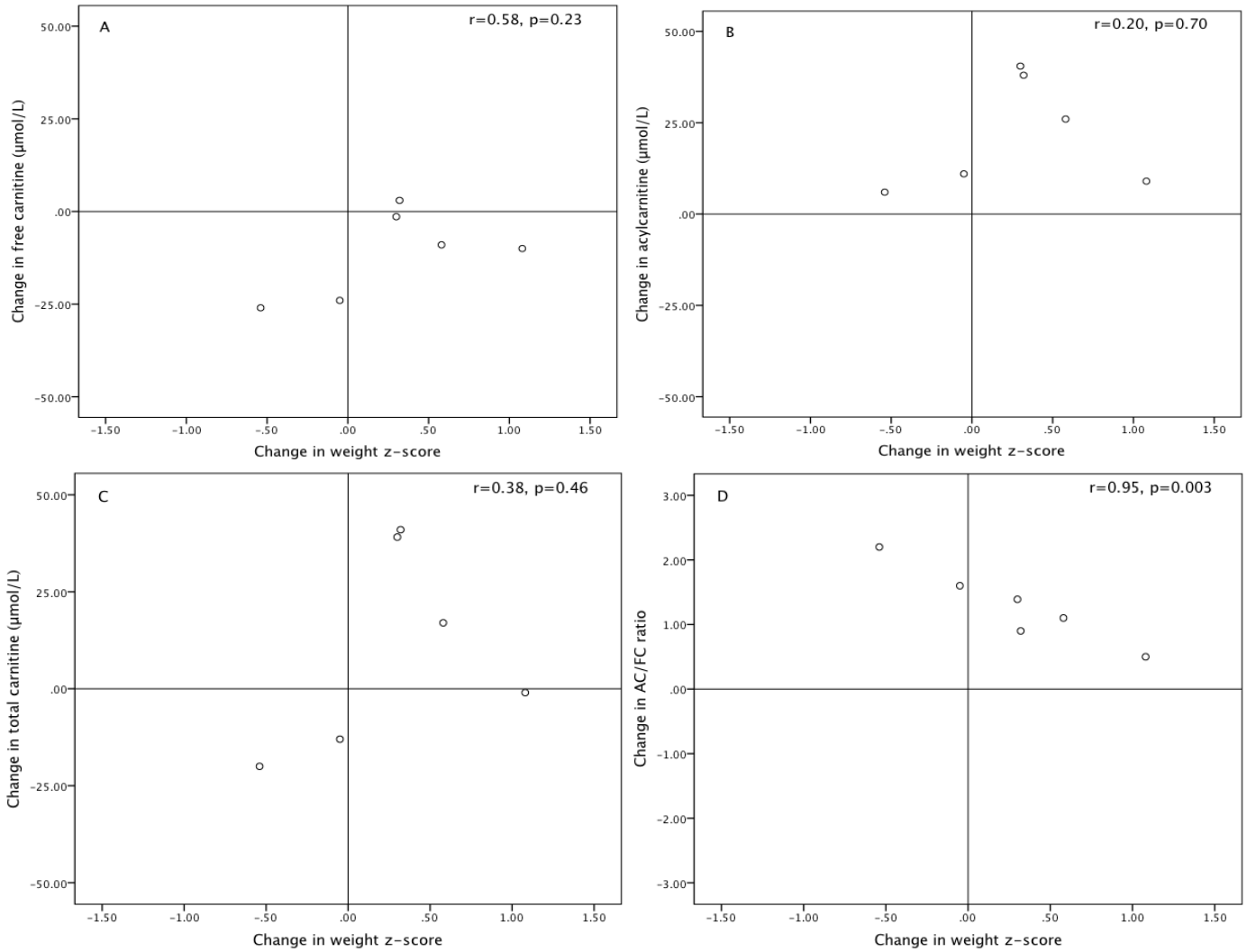

Figure 20. Correlation between change in weight-for-age z-score and change in plasma-, acyl-, and total carnitine concentrations, and acyl/carnitine concentration ratio 3 months after Ketogenic Diet initiation (n=6)

Relationship between weight, height, and BMI z-scores and time to respond to Ketogenic Diet therapy

A Kaplan-Meyer survival analysis was conducted to determine time to respond to the Ketogenic Diet among 34 patients with intractable epilepsy. Response to the Ketogenic Diet was defined as at least a 50% reduction in seizure frequency based on quantitative and qualitative data obtained by medical record review. Qualitative data included report of the caregivers' perception of reduced seizure frequency and severity captured by statements such as, “is like a new baby” after diet initiation, when quantitative data was not provided.

Figure 21 shows the time from initiation of the Ketogenic Diet to achievement of at least a 50% reduction in seizure frequency of the 34 patients included in this analysis. More than half (56%) (n=17) responded to the Ketogenic Diet within one month of diet initiation. Fifty-nine percent of patients responded within two months, 65% responded within three months, 69% responded within four months, 72% responded within five months, 77% responded within 11 months, and 82% of patients responded within 12 months of initiating the Ketogenic Diet. Eight patients (18%) did not respond to the Ketogenic Diet during the 12-month follow-up period. Of these patients, two discontinued the diet due to intolerance, one did not respond by six months but remained on the diet, and five did not respond by 12 months but continued dietary treatment.

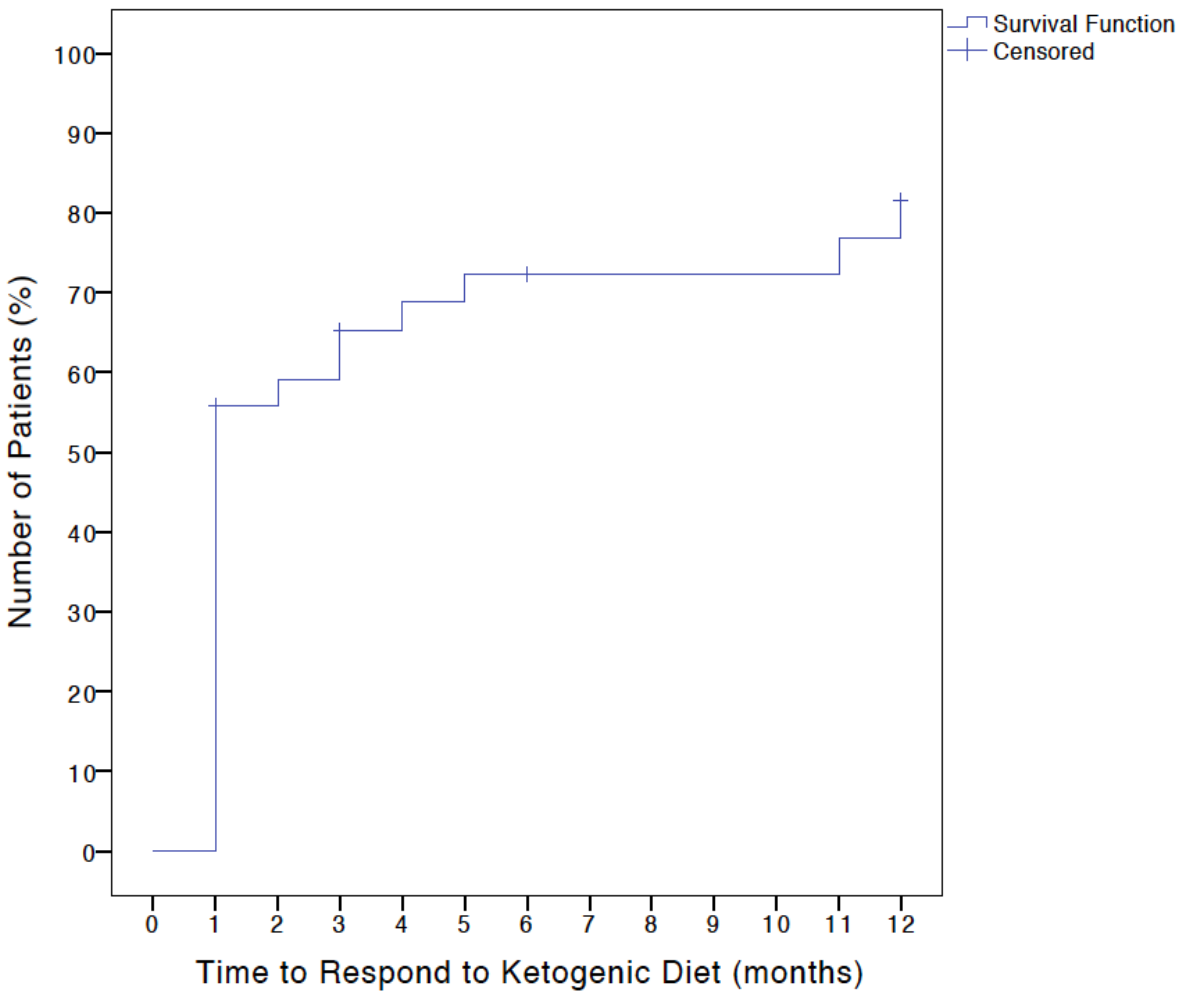

Figure 21. Time to response after Ketogenic Diet initiation (n=34)

Cox regression analysis was performed to determine whether an individual's response to the Ketogenic Diet was associated with their height, weight, or BMI z-scores at baseline. Results from this model found no significant (χ^2 2.80 (3), p=0.42) association among these three growth parameters and the time from initiation of the Ketogenic Diet to at least a 50% reduction in seizure frequency. Based on this analysis, weight,

height and BMI z-scores at baseline do not influence an individual's response to treatment with the Ketogenic Diet.

Chapter 5: Discussion

Epilepsy affects a large number of children nationwide, with 2,500 children affected in Oregon alone. In addition to the financial burden associated with epilepsy, children who experience recurrent seizures may also have a reduced quality of life. Patients are often prescribed multiple antiepileptic drugs, which are typically associated with adverse side effects. The Ketogenic Diet has been proven to be an effective treatment for the management of children with epilepsy who do not respond to medication.

Although the exact mechanism of the Ketogenic Diet remains unknown, it is believed to work through the restriction of carbohydrates and increased consumption of fatty acids, mostly in the form of long-chain triglycerides, in order to induce ketosis through various potential mechanisms previously described in this thesis. Due to the increased intake of fatty acids during dietary treatment, children on the Ketogenic Diet may have greater carnitine requirements to meet the increased rate of fatty acid metabolism. Children receiving dietary treatment may also experience a decrease in linear growth, as previously described in the background of this thesis.

This pilot study aimed to describe growth of children on the Ketogenic Diet, as well as the correlation between growth and carnitine concentrations. As a majority of carnitine is stored in the muscle, our study aimed to determine whether a correlation exists between weight, length/height and BMI Z-scores as surrogate markers of muscle mass and changes in carnitine in children on the Ketogenic Diet. More specifically, we

aimed to determine whether increased muscle mass could potentially have a protective effect against patients developing hypocarnitinemia.

Growth in children treated with the Ketogenic Diet

In order to describe growth patterns, we categorized children treated with the Ketogenic Diet by age (less than two years old (n=17) and two years and older (n=11)), as BMI is unavailable in children less than two years of age. Growth patterns were described via height, weight, and BMI z-scores.

In patients less than two years of age, we observed a downward trend in weight and height z-scores during treatment with the Ketogenic Diet. We compared these findings to those of Peterson et al. and Groleau et al. (36, 37). The mean weight z-score $\pm$ SD observed after 12 months of dietary treatment in our study (n=17) was -0.94 $\pm$ 1.69 compared to -0.61 $\pm$ 1.44 in the study conducted by Peterson et al. (n=33) and 0.04 $\pm$ 0.5 observed after 15 months of dietary treatment in the study conducted by Groleau et al. (n=15). The mean height z-score $\pm$ SD observed after 12 months of dietary treatment in our study was -1.46 $\pm$ 1.80 compared to -0.99 $\pm$ 1.13 observed after 12 months of treatment in the study conducted by Peterson et al, and -0.6 $\pm$ 0.6 observed after 15 months of treatment in the study conducted by Groleau et al. It's important to note that while children included in our study were categorized according to age, the two other studies described included children less than and greater than two years of age in the same analysis.

In patients two years of age and older, we observed an upward trend in weight and BMI z-scores and a downward trend in height z-score. In our study (n=3), the mean weight z-score ± SD was -0.07 ± 0.82 compared to -0.61 ± 1.44 in the study conducted by Peterson et al. (n=33) and -0.9 ± 1.4 observed after 15 months of dietary treatment in the study conducted by Groleau et al. (n=15) The mean height z-score in our study (n=3) was 0.22 ± 1.65 compared to -0.99 ± 1.13 observed after 12 months of treatment in the study conducted by Peterson et al, and -0.6 ± 0.6 observed after 15 months of treatment in the study conducted by Groleau et al. The mean BMI z-score in our study (n=3) was 0.23 ± 1.18 compared to 0.8 ± 1.1 observed after 15 months of treatment in the study conducted by Groleau et al. BMI z-score data was unavailable in the study conducted by Peterson et al.

Significance between baseline weight, height and BMI z-scores and change in plasma carnitine concentrations after Ketogenic Diet initiation

We observed a non-significant correlation between lower baseline weight z-scores and a greater increase in acyl- and total carnitine concentrations one (n=8) and three (n=6) months after Ketogenic Diet initiation. We also observed a non-significant correlation between lower baseline weight z-scores and greater acyl/free carnitine concentration ratios after one and three months of treatment. Plasma free carnitine concentrations appeared to be lower in patients with lower weight z-scores one and three months after diet initiation. Changes in plasma carnitine concentrations were more variable six months after Ketogenic Diet initiation (n=5). Our results are somewhat

similar to findings from the study conducted by Morita et al, who aimed to determine the significance of a variety of factors, including muscle mass, with the development of hypocarnitinemia (25).

In the study conducted by Morita et al, free and total carnitine concentrations were lower in participants receiving anti-epileptic drugs than free carnitine concentration in the control group, however study duration was not stated. It must also be noted that the participants in the study conducted by Morita et al were described as either the control group or were described as "institutionalized" and receiving antiepileptic medication, however the medical condition(s) of the participants included in this study were not disclosed. In Morita et al's study, z-scores were unavailable, and muscle mass was estimated using mid-arm muscle circumference in order to estimate lean body mass. Investigators in the study conducted by Morita et al. found a significant correlation between a decrease in total and free carnitine concentrations and the values of mid-arm muscle circumference.

Our study aimed to describe the relationship between baseline weight z-score and change in plasma carnitine concentrations. We observed a greater non-significant increase and decrease in total carnitine and free carnitine concentrations, respectively, along with a lower baseline weight z-score after one month of treatment with the Ketogenic Diet (n=8). A greater increase in acyl/free carnitine concentration ratio along with a lower weight z-score following one month of treatment with the Ketogenic Diet was also observed (n=7). As a majority of carnitine is stored in muscle tissue, the lower free carnitine concentration seen in patients with a weight z-score <0 after one and

three months of dietary treatment may theoretically be due to lower amounts of carnitine initially stored in the muscle, therefore contributing to lower plasma free carnitine concentrations. This potential relationship should be considered with caution, as muscle carnitine concentrations were not measured in our study.

In addition to the study conducted by Morita et al, our study is the only other study that we are aware of that describes the existence of a potential relationship between muscle mass prior to starting treatment with the Ketogenic Diet and changes in carnitine concentration after diet initiation. However, this conclusion should also be considered with caution due to the small sample size analyzed and because of the difficulty in taking accurate growth measurements of these medically fragile patients in a clinical setting.

Significance between changes in weight, height and BMI z-scores and change in plasma carnitine concentrations after Ketogenic Diet initiation

We observed a greater increase in free, acyl-, and total carnitine concentrations along with a decrease in weight z-score at one month (n=6) compared to changes seen after three months (n=6) of treatment with the Ketogenic Diet. We also observed a significantly greater increase in acyl/free carnitine concentration ratio with a greater decrease in weight z-score after three months than after one month of dietary treatment.

The associations between changes in skeletal muscle mass and plasma carnitine concentrations have been described in few studies, including those previously discussed

in the background of this thesis. To our knowledge, no studies to date describe a correlation between changes in plasma carnitine concentrations and change in weight, height and BMI z-scores in this patient population. As a majority of carnitine is stored in muscle tissue, the greater decrease in weight z-score along with a greater increase in plasma acyl/free carnitine concentration ratio seen at three months may be indicative of carnitine being released from the muscle; this may contribute to the increased plasma acyl/free carnitine concentration ratio observed. Again, this possible explanation should be considered with caution due to the small sample size analyzed, growth measurements taken by multiple clinicians in various settings, and because muscle carnitine concentrations were not measured in our study.

Study Strengths and Limitations

Strengths

This pilot project has several strengths. First, this was a single center, retrospective and prospective longitudinal study. Second, there are only a few published studies that describe changes in body composition or carnitine status of pediatric patients on the Ketogenic Diet. Third, we assessed plasma free carnitine, acylcarnitine, and total carnitine concentrations and plasma acyl/free carnitine concentration ratios, which helped us determine if changes in body composition during Ketogenic Diet therapy are associated with changes in plasma carnitine concentrations, ketosis and seizure frequency/severity following initiation of the Ketogenic Diet.

Limitations

This pilot project has the potential to yield meaningful information about the relationship between the Ketogenic Diet, body composition, carnitine concentration, and changes in seizure frequency and severity; however, the small sample size limits our ability to draw conclusions and apply our findings clinically.

Because of the small sample size there is an increased risk of committing a type II statistical error, otherwise known as drawing a "false negative" conclusion. This occurs when the alternative hypothesis fails to be accepted when it actually should be accepted. Second, anthropometric data was collected in a clinical setting by multiple clinicians and in multiple settings, which may have contributed to measurement error and affected the accuracy of results. Third, a variety of epilepsy etiologies were included in each analysis. Some patients may have a reduced growth potential due to their medical diagnosis and would not be appropriate to compare to standard reference data. Therefore, it may be beneficial to further divide patients with similar diagnoses into separate cohorts that would include children with the same or similar diagnoses.

Chapter 6: Summary and conclusions

Summary

The individual growth patterns described illustrate the challenges associated with measuring and tracking growth in this medically complex patient population. Children with intractable epilepsy have multiple factors that contribute to atypical weight gain and linear growth patterns compared to standard reference data derived from healthy, unaffected children. Patients with intractable epilepsy are often treated with multiple antiepileptic drugs that may affect hunger and appetite, and subsequently weight gain and linear growth. In addition, children with intractable epilepsy are frequently hospitalized for issues such as placement of enteral feeding tubes, respiratory distress, and/or pneumonia, which may influence food intake and growth rates. Additionally, accurate growth measurements are difficult to take in clinical settings under the best conditions and are even harder to take among children who are hypertonic and have contractures, which are often experienced by children with epilepsy.

Future Directions

This is the first study that we know of that evaluates the relationship between growth, body composition and carnitine concentrations in pediatric patients with intractable epilepsy treated with the Ketogenic Diet.

In order for findings from future studies to be clinically applicable, it may be beneficial for those studies to be conducted in a prospective, longitudinal manner. In

addition, it may be beneficial for the collection of anthropometric measurements to be standardized, and for them to be taken by one clinician to help reduce the risk of error and improve accuracy. For increased sample size and greater clinical application, a multicenter study may also be required.

We hope for this study to serve as a foundation for future studies describing the relationship between growth and/or body composition and carnitine concentrations.

References

1. National Survey of Children's Health. Data query from the Child and Adolescent Health Measurement Initiative, Data Resource Center for Child and Adolescent Health website. Retrieved [8/20/2014] from www.childhealthdata.org.
2. Available at: http://www.cdc.gov/epilepsy/basics/fast-facts.htm. Accessed December 13, 2015.
3. Moshé SL, Perucca E, Ryvlin P, Tomson T. Epilepsy: new advances. Lancet. 2015;385(9971):884-98.
4. Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014;55(4):475-82.
5. Kossof EH, Zupec-Kania BA, Amark PE, et al. Optimal clinical management of children receiving the ketogenic diet: recommendations of the international ketogenic diet study group. Epilepsia 2009;50:304-17.
6. Berg AT, Vickrey BG, Testa FM, Levy SR, Shinnar S, DiMario F, Smith S. How long does it take for epilepsy to become intractable? A prospective investigation. Ann Neurol 2006;60:73-9.
7. Available at: http://neurosurgery.mgh.harvard.edu. Accessed December 12, 2015.
8. Groves DA, Brown VJ. Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. Neurosci Biobehav Rev. 2005;29(3):493-500.
9. Neal EG, Chaffe H, Schwartz RH, et al. The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. Lancet Neurol 2008;7:500-6.
10. Freeman J, Veggiotti P, Lanzi G, Tagliabue A, Perucca E. The ketogenic diet: from molecular mechanisms to clinical effects. Epilepsy Res. 2006;68(2):145-80.
11. Hartman AL, Gasior M, Vining EPG. The Neuropharmacology of the Ketogenic Diet. Pediatr Neurol 2007;36(5):281-92.
12. Lutas A, Yellen G. The ketogenic diet: metabolic influences on brain excitability and epilepsy. Trends Neurosci. 2013;36(1):32-40.
13. Neal EG, Zupec-kania B, Pfeifer HH. Carnitine, nutritional supplementation and discontinuation of ketogenic diet therapies. Epilepsy Res. 2012;100(3):267-71.
14. Kang HC, Chung DE, Kim DW, Kim HD. Early- and late-onset complications of the ketogenic diet for intractable epilepsy. Epilepsia. 2004;45(9):1116-23.
15. Kossoff EH, Turner Z, Bluml RM, Pyzik PL, Vining EP. A randomized, crossover comparison of daily carbohydrate limits using the modified Atkins diet. Epilepsy Behav. 2007;10(3):432-6
16. Stadler DD, Chenard CA, Rebouche CJ. Effect of dietary macronutrient content on carnitine excretion and efficiency of carnitine reabsorption. Am J Clin Nutr 1993;58(6):868-72.
17. Ringseis R, Keller J, Eder K. Role of carnitine in the regulation of glucose homeostasis and insulin sensitivity: evidence from in vivo and in vitro studies with carnitine supplementation and carnitine deficiency. Eur J Nutr 2012;51:1-18.

18. Rebouche CJ, Seim H. Carnitine metabolism and its regulation in microorganisms and mammals. Annu Rev Nutr. 1998;18:39-61.
19. Coppola G, Epifanio G, Auricchio G, Federico RR, Resicato G, Pascotto A. Plasma free carnitine in epilepsy children, adolescents and young adults treated with old and new antiepileptic drugs with or without ketogenic diet. Brain Dev. 2006;28(6):358-65.
20. Houten SM, Wanders RJ. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. J Inherit Metab Dis. 2010;33(5):469-77.
21. De Vivo DC, Tein I. Primary and secondary disorders of carnitine metabolism. Int Pediatr 1990;5:134-41.
22. De vivo DC, Bohan TP, Coulter DL, et al. L-carnitine supplementation in childhood epilepsy: current perspectives. Epilepsia. 1998;39(11):1216-25.
23. Cederblad G, Lindstedt S, Lundholm K. Concentration of carnitine in human muscle tissue. Clin Chim Acta. 1974;53(3):311-21.
24. Hiatt WR, Regensteiner JG, Wolfel EE, Ruff L, Brass EP. Carnitine and acylcarnitine metabolism during exercise in humans. Dependence on skeletal muscle metabolic state. J Clin Invest. 1989;84(4):1167-73.
25. Morita J, Yuge K, Yoshino M. Hypocarnitinemia in the handicapped individuals who receive a polypharmacy of antiepileptic drugs. Neuropediatrics. 1986;17(4):203-5.
26. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev. 1999;15(6):412-26.
27. Yudkoff M, Daikhin Y, Melø TM, Nissim I, Sonnewald U, Nissim I. The ketogenic diet and brain metabolism of amino acids: relationship to the anticonvulsant effect. Annu Rev Nutr. 2007;27:415-30.
28. Juge N, et al. Metabolic control of vesicular glutamate transport and release. Neuron. 2010;68:99–211.
29. Berry-kravis E, Booth G, Sanchez AC, Woodbury-kolb J. Carnitine levels and the ketogenic diet. Epilepsia. 2001;42(11):1445-51.
30. Riebold J. 2015. *Carnitine status of children treated with the ketogenic diet for intractable epilepsy.*
31. Houtkooper LB, Lohman TG, Going SB, Howell WH. Why bioelectrical impedance analysis should be used for estimating adiposity. Am J Clin Nutr. 1996;64(3 Suppl):436S-448S.
32. Kyle UG, Bosaeus I, De lorenzo AD, et al. Bioelectrical impedance analysis--part I: review of principles and methods. Clin Nutr. 2004;23(5):1226-43.
33. Green corkins K. Nutrition-focused physical examination in pediatric patients. Nutr Clin Pract. 2015;30(2):203-9.
34. Frisancho AR. New norms of upper limb fat and muscle areas for assessment of nutritional status. Am J Clin Nutr. 1981;34(11):2540-5.
35. Brook CG. Determination of body composition of children from skinfold measurements. Arch Dis Child. 1971;46(246):182-4.
36. Groleau V, Schall J, Stallings V, Bergqvist C. Long-term impact of the ketogenic diet on growth and resting energy expenditure in children with intractable epilepsy. *Dev Med Child Neurol*. 2014;56(9):898-904. doi:10.1111/dmcn.12462.

37. Peterson S, Tangney C, Pimentel-Zablah E, Hjelmgren B, Booth G, Berry-Kravis E. Changes in Growth and Seizure Reduction in Children on the Ketogenic Diet as a Treatment for Intractable Epilepsy. *Journal of the American Dietetic Association*. 2005;105(5):718-724. doi:10.1016/j.jada.2005.02.009.
38. Schooneman MG, Napolitano A, Houten SM, et al. Assessment of plasma acylcarnitines before and after weight loss in obese subjects. Arch Biochem Biophys. 2016;606:73-80.
39. Laurson KR, Eisenmann JC, Welk GJ. Body fat percentile curves for U.S. children and adolescents. Am J Prev Med. 2011;41(4 Suppl 2):S87-92.
40. Available at: https://www.cdc.gov/growthcharts/zscore.htm. Accessed May 25, 2017.