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The Effect of Polyunsaturated and Saturated Fatty Acids on Seizure Presence and Severity in a Cohort of Subjects in an Epilepsy Monitoring Unit

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THE EFFECT OF POLYUNSATURATED AND SATURATED FATTY ACIDS ON SEIZURE PRESENCE AND SEVERITY IN A COHORT OF PATIENTS IN AN EPILEPSY MONITORING UNIT

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University of Nebraska, 2021

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Ketogenic diet therapies (KDTs) have been used to treat epilepsy for nearly 100 years. Although effective, restrictive diet patterns and unknown impacts on long-term health outcomes often prevent their use as first-line therapy. To date, a distinct mechanism of action for KDTs has not been determined and evidence suggests fatty acids (FAs) may play a role in eliciting anti-seizure effects. This dissertation aimed to provide insights into the effect of polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs) on seizure presence and severity through the analysis of dietary intake in a cohort of patients admitted to an epilepsy monitoring unit at Nebraska Medicine. Seizure activity was monitored throughout the inpatient stay via continuous video electrocephalogram monitoring (EEG) and dietary intake was determined by administering a food frequency questionnaire. PUFAs were evaluated separately while SFAs were analyzed by carbon length groupings: short chain (SCFA), medium chain (MCFA) and long chain (LCFA). EEG reports were reviewed and assessed for seizure presence (yes/no) and seizure severity (score of 1-4). Differences in median FA intakes across seizure presence and severity groups were evaluated and regression models assessed the ability of PUFAs and SFAs to act as predictors of seizure presence and severity, respectively. Seizure presence was observed in 31 of 82 (37.8%) subjects included in the final analysis. While no statistically significant (p <0.05) differences of PUFA or SFA intake were observed across seizure presence or severity groups, clinical observations suggest the relationship between FA intake and seizure severity. Even with dietary intakes far below what may be considered a therapeutic dose, the influence of FA intake appeared possible in

subjects with seizure presence. Furthermore, a trend toward significance was seen in the multivariate linear regression model with increased MCFA intake having an inverse linear relationship with severity scores ($\beta = -0.11$, $p = 0.16$). Results from this dissertation suggest specific dietary FAs have potential anti-seizure properties that warrant exploration as epilepsy treatment. A minimally invasive, targeted dietary treatment could decrease the devastating side effects of epilepsy, reduce the staggering cost of treatment and improve quality of life for patients and their families.

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LIST OF ABBREVIATIONS

ILAE	International League Against Epilepsy
ASMs	Anti-seizure Medications
KDT	Ketogenic Diet Therapy
RCTs	Randomized Controlled Trials
KD	Ketogenic Diet
BHB	Beta-hydroxybutyrate
cKD	Classic Ketogenic Diet
MCTKD	Medium Chain Triglyceride Diet
MAD	Modified Atkins Diet
LGIT	Low Glycemic Index Treatment
KBs	Ketone Bodies
MCTs	Medium Chain Triglycerides
MCFA	Medium-Chain Saturated Fatty Acid
BBB	Blood Brain Barrier
Gms	Grams
GI	Glycemic Index
ATP	Adenosine Triphosphate
FAs	Fatty Acids
FAO	Fatty Acid Oxidation
PUFA	Polyunsaturated Fatty Acid

SFA	Saturated Fatty Acid
n	Omega
AIs	Adequate Intakes
IOM	Institute of Medicine
ALA	Alpha-Linolenic Acid
LA	Linoleic Acid
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
DRIs	Dietary Reference Intakes
SPM	Specialized Pro-Resolving Mediators
RvD-	Resolvin D
MaR	Maresin
RvE	Resolvin E
AA	Arachidonic Acid
COX	Cyclooxygenase
LOX	Lipoxygenase
SCFA	Short-Chain Saturated Fatty Acids
HDACis	Histone Deacetylase Inhibitors
LCFAs	Long-Chain Saturated Fatty Acids
ROS	Reactive Oxygen Species
TLR	Toll-Like Receptor

VPA	Valproic Acid
NaB	Sodium Butyrate
GPR	G-Protein Coupled Receptors
PPARs	Peroxisome Proliferator-Activated Receptors
HDAC	Histone Deacetylase
GABA	Gamma-Aminobutyric Acid
AMPA	Amino-Hydroxyl-5-Methyl-4-Isoxazoleproprionic acid
IL-	Interleukin
TNF-α	TNF-α
NDP1	Neuroprotectin D1
NF-kB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
KO	Knockout
EEG	Electroencephalogram
IRB	Internal Review Board
EMU	Epilepsy Monitoring Unit
FDA	Food and Drug Administration
WHO	World Health Organization
OR	Odds Ratio

INTRODUCTION

Epilepsy is a devastating condition that impacts 3.4 million people worldwide with 150,000 new cases of epilepsy per year. In 2013, annual direct cost estimates ranged from $10,000 to a staggering $47,000 per year [1]. The multifaceted impact of the condition on those affected is extensive. Not only is seizure occurrence unpredictable and potentially fatal, but may also result in cognitive impairment, social exclusion, psychiatric comorbidities and a decreased quality of life [2]. According to the Epilepsy Foundation, the official definition of a seizure is "a transient occurrence of signs and/or symptoms due to an abnormal or excessive synchronous neuronal activity in the brain." Epilepsy is characterized by abnormal brain activity thought to be due to factors including genetics, developmental disorders or neurologic damage such as a traumatic brain injury, hypoxia or fevers [3]. These factors, in turn, may lead to hyperexcitability and seizure activity by negatively impacting neurotransmitters, ion channels, neuroinflammation, oxidative stress or the microbiome.

Seizures are classified by where they originated in the brain. Determining location of onset is important because it effects treatment. In 2017, the International League Against Epilepsy (ILAE) revised its classification of seizures to make diagnoses accurate. The organization divided seizures into two broad groups: focal and generalized. Focal (previously described as partial) seizures start in an area or network of cells on one side of the brain, while generalized seizures involve networks on both sides of the brain at onset. Seizures are then sub-categorized by describing awareness and motor onset (Table 1). If awareness remains intact, even if the person were unable to talk or respond during a seizure, the seizure would be classified a focal aware seizure. Focal impaired awareness (previously described as complex partial) seizures can make a person confused or dazed and are unable to respond to questions or direction for up to a few minutes. A generalized tonic-clinic or grand mal seizure describes seizures with stiffening and jerking.

Table 1: ILAE Seizure Classifications with Possible Motor and Non-Motor Characteristics			
Focal (Partial) Onset		Generalized Onset	
Aware	Impaired Awareness (Complex Partial)	Impaired Awareness	
Motor	Non-Motor	Motor	Non-Motor
Automatisms Atonic Clonic Epileptic Spasms Hyperkinetic Myoclonic Tonic	Autonomic Behavioral arrest Cognitive Emotional Sensory	Tonic-clonic Clonic Tonic Myoclonic Myoclonic-tonic-clonic Myoclonic-atonic Atonic Epileptic Spasms	Typical Atypical Myoclonic Eyelid myoclonia
Previously accepted seizure classification nomenclature noted in parentheses *ILAE: International League Against Epilepsy*			

Current drug treatments target seizure symptoms and do not directly affect or prevent the underlying cause contributing to the epileptic state or epileptogenesis [4-7]. Epileptogenesis is a continuous and chronic process by which a brain network, that was previously normal, is functionally altered toward increased seizure susceptibility, thus having an enhanced probability to generate spontaneous recurrent seizures [8]. Although treatments may improve seizure symptoms and provide a better quality of life, the underlying cause of the seizure needs to be identified in order to inhibit epileptogenesis and prevent brain insult, microglial cell activation and neuronal cell death.

Up to 65% of individuals with epilepsy will have seizures controlled with anti-seizure medications (ASMs) or enter spontaneous remission in their lifetime [9]. However, this leaves a large percentage of patients that are refractory to drug therapy. Some of the current methods for treating refractory epilepsy include surgery or vagus nerve stimulation. Nutritional approaches to managing epilepsy also exist with one of the primary treatments being Ketogenic Diet Therapy (KDT).

The term ketogenic diet therapy (KDT) refers to any diet therapy in which dietary composition results in a ketogenic state of human metabolism [10]. Since the 1930s, observational trials, randomized controlled trials (RCTs) and meta-analyses have consistently shown KDTs are able to significantly reduce seizure frequency in children and adolescents with refractory epilepsy [11-16]. The diet generally refers to a high fat, low carbohydrate and moderate protein diet. Macronutrient distribution is described by a ketogenic diet (KD) ratio, or the ratio of fat to carbohydrate and protein. KDTs imitate the biochemical effects of fasting and induces a shift away from glycolic energy production toward energy generation through oxidative phosphorylation, leading to fatty acid β-oxidation and ketone production [17]. Beta-hydroxybutyrate (BHB) is the predominate ketone measured in plasma or urine and has been used as a clinical measure of KD implementation, effectiveness and adherence.

Currently, there are four major KDTs (Table 2): the classic ketogenic diet (cKD), the medium chain triglyceride ketogenic diet (MCTKD), the modified Atkins diet (MAD), and the low glycemic index treatment (LGIT) [18].

First proposed by Wilder et al. in 1921, the cKD is considered the most restrictive of the described KDTs. Food is weighed on a gram scale and urine ketone bodies (KBs) are measured to ensure the patient remains in ketosis. The cKD is generally based upon consumption of a 3:1 to 4:1 KD ratio and is often characterized by high intakes of long chain fatty acids from butter and cream [17, 19]. Following the development of the cKD, new diets have been developed in an attempt to increase retention and palatability while mimicking the effects produced by the original diet.

Table 2: KDT Diet Patterns and Macronutrient Distributions				
KDT	**Diet Pattern**	**Percent Total Daily Energy Intake**		
		Fat	Carbohydrate	Protein
cKD	KD Ratio of 3:1–4:1	90	4	6
Traditional MCTKD	60% total energy intake from MCT	70–75	15–18	10
Modified MCTKD	30% total energy intake from MCT	70–75	15–18	10
MAD	KD ratio of 1:1–2:1	60–65	5–10	30
LGIT	40–60 g carbohydrate per day Restricts carbohydrate sources to a GI < 50	60	10	30
KDT: Ketogenic Diet or Ketogenic Diet Therapy; cKD: classic ketogenic diet; MAD: Modified Atkins diet; MCTKD: medium chain triglyceride ketogenic diet; LGIT: low glycemic index treamtent				

The MCTKD was developed in 1971 and encourages medium chain triglycerides (MCTs) as a large percentage of daily fat intake [18, 20]. Historically, the traditional MCTKD provided 60% of total energy from MCTs. However, due to the tendency of MCTs to cause gastrointestinal side effects, a modified MCTKD, which recommends 30% of total energy intake from MCTs, was developed. Fats are often introduced into the diet via an oral supplement that contains only MCTs. The medium-chain saturated fatty acid (MCFA) octanic acid (C8:0) is the most abundant FA in the diet (50-75% content), followed by the 10-carbon decanoic acid (23-45% content) with minimal 12-carbon lauric acids (1-5%) [21]. After ingestion, MCFAs are carried directly from the digestive system to the liver for KB production via the portal vein [11]. After crossing the blood brain barrier (BBB), KBs are oxidized by the brain to provide a direct fuel source [22]. With an increased ketogenic potential, less total fat is needed in the MCTKD, which allows for increased carbohydrate and protein intake, likely resulting in more food choices.

Later, the MAD and LGIT were proposed as alternatives to the cKD and MCTKD. The MAD, created at Johns Hopkins Hospital in 2003, is considered a less restrictive alternative to the cKD with a KD ratio of 1:1 to 2:1 and an unrestricted calorie intake [23]. Upon implementation, carbohydrates are limited to 10 grams (gms) per day in children and are increased after one month to 15 gms with a final increase to 20 to 30 gms per day depending on seizure severity. Finally,

The LGIT, initially introduced in 2005, allows 40 to 60 gms carbohydrate per day but restricts sources of carbohydrates to a glycemic index (GI) of less than 50 to prevent postprandial increases in blood glucose [24]. Foods with a high GI produce substantial increases in blood glucose and insulin, whereas those with a low GI (e.g. vegetables and unprocessed whole grains) induce lower postprandial plasma glucose and insulin profiles [25]. Fats and proteins are unrestricted. With liberalized KD ratios and lack of MCT supplementation, the MAD and LGIT do not produce the amount of ketones seen in the cKD or MCTKD. However, both have proven to be effective in the treatment of epilepsy against the more restrictive diets [13, 26, 27].

Although KDTs have proven to be effective, they are generally not considered a first-line treatment due to adverse effects of therapy and retention. In a systematic review of KDT prospective studies, more than 40 categories of adverse effects were identified [28]. These included direct negative effects on gastrointestinal function, lipid profiles, bones and ultimately growth. Adverse effects likely also have an enormous impact on retention rate of KDTs, which have been reported at years one and two to be 45.7% and 29.2%, respectively. Furthermore, attrition is also largely affected by poor educational and support systems in place for patients and families. Therefore, the ability to identify a mechanism of action which may be targeted through precision nutrition may be paramount in a novel line of treatment for individuals with epilepsy.

After 100 years of use, the exact mechanism of KDTs remains unknown and no KDT has clearly shown superiority in an RCT of patients over the age of two [29]. Previous assumptions related the effectiveness of a KDT to the amount of BHB produced and measured. However, although significant elevations of BHB are seen during KDT, there are currently no significant correlations between levels and seizure activity [30]. In fact, the evolution from the most restrictive KDT, the cKD with a 4:1 KD ratio, to more liberal MAD and LGIT options with increased carbohydrate and protein provision, did not appear to impact efficacy. Therefore, a question of whether presence of BHB or ketone metabolism is strictly necessary for the clinical

effectiveness of KDTs is raised. Many hypothesize the KDT mechanism of action includes halting or reversing one or more of the hallmark consequences of seizures including neuroinflammation, oxidative stress or dysregulation of neurotransmitters and ion channels. [3].

KDT was first developed to mimic the effects of starvation. When carbohydrates are present in human metabolism, glucose is metabolized to produce adenosine triphosphate (ATP) while fatty acids (FAs) are stored in the form of triglycerides. However, when intake is limited, triglycerides are β-oxidized and used to produce energy. As all KDTs provide greater than 50% of total daily energy intake from fat, the drastic elevation from the recognized norm would also lead to higher fat metabolism and β-oxidation (Figure 1). Therefore, another possibility, besides ketones directly inhibiting seizures, is that a very high fat diet elicits anti-seizure characteristics through the accumulation and metabolism of FAs [31, 32].

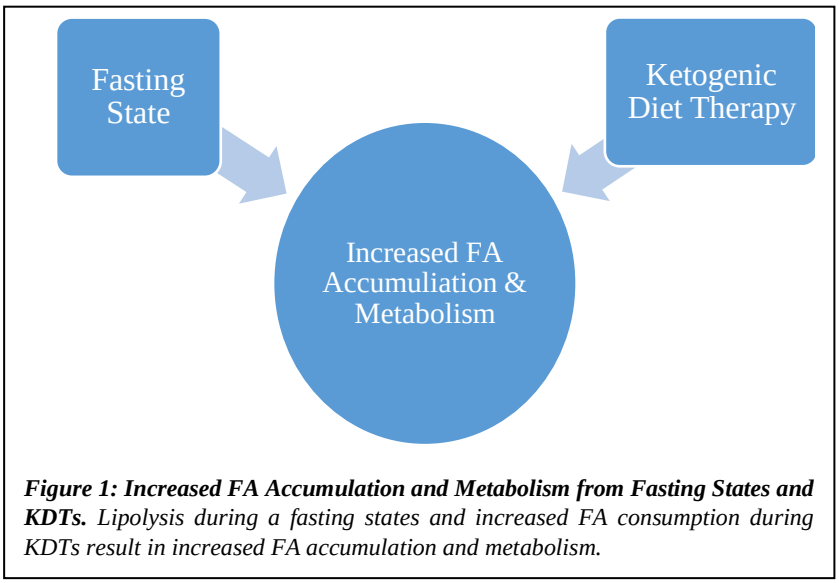

Figure 1: Increased FA Accumulation and Metabolism from Fasting States and KDTs. *Lipolysis during a fasting states and increased FA consumption during KDTs result in increased FA accumulation and metabolism.*

FAs are carboxylic acids with a long aliphatic chain that is either saturated, monounsaturated or polyunsaturated. These nutrients are unique in the fact that they provide energy but are also metabolic regulators [33]. In the body, metabolism of fatty acids results in oxidation for energy, storage as triglycerides and use for phospholipid synthesis that form the structures of cell

membranes [34]. Interestingly, the human brain mobilizes FAs for oxidation, irrespective of diet or metabolic state and astrocytes and neural stem cells express all the enzymes necessary for fatty acid oxidation (FAO) [35-37]. Statistically significant elevations in serum polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA) concentrations have been recognized in animal and human subjects on KDT, likely due to the aforementioned increase in FAO [17, 38-43].

Research suggests that modification of FA intake, under KDT or with a modified intake, can provide neuroprotection, reduce seizure frequency and increase seizure thresholds in pre-clinical, clinical and epidemiologic reports [44-48]. However, a gap in knowledge exists regarding the impact of individual FAs on seizure presence and severity in individuals with epilepsy.

CHAPTER 1: REVIEW OF THE LITERATURE

Polyunsaturated Fatty Acids (PUFAs)

PUFAs are long-chain fat molecules that have more than one unsaturated carbon bond. According to the position of the carbon atom linked by the first unsaturated double bond in the methyl end of the carbon chain, PUFAs are divided into two major classes: omega (n)-3 and n-6 FAs (Figure 2). Both are important cell-membrane components, essential for optimal brain function and potent mediators of inflammation [49]. Humans are unable to synthesize these long chain fatty acids and are required in the diet to prevent clinical deficiency.

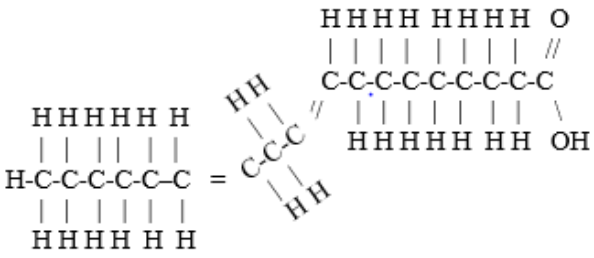

Figure 2: Chemical Structure of Polyunsaturated Fatty Acids (PUFAs). *Depicted: Linoleic Acid (C18:2)*

Adequate Intakes (AIs) are set at levels assumed to ensure nutritional adequacy when there is insufficient evidence to develop a Recommended Dietary Allowance (RDA). In 2002, the United States Institute of Medicine (IOM) established AIs for n-3 and n-6 FAs (Table 3) [50]. Recommendations for n-3 and n-6 apply only to alpha-linolenic acid (ALA), and linoleic acid (LA), respectively. According to data from the 2011-2012 National Health and Nutrition Examination Survey, most children and adults in the United States consume recommended amounts of n-3s as ALA.

ALA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are the primary n-3 FAs. ALA (18:3) is present in plant oils such as flaxseed, soybean and canola oils and can be converted to EPA and DHA in the liver [51, 52]. However, with a very low conversion rate of 0-4%, ingesting EPA and DHA directly from foods is the best way to increase levels in the body [53]. EPA (20:5) and DHA (22:6) are found in fish but originate in marine algae and phytoplankton. When fish consume phytoplankton that consumes algae, these n-3s accumulate in

their tissues [54]. Proposed dietary reference intakes (DRIs) for combined EPA and DHA are 250-500 mg per day in healthy individuals [55]. N-3 FAs have long been recognized for their anti-inflammatory effects, but mechanisms underlying various protective effects are incompletely understood [56]. Interestingly, n-3 FAs have recently been found to serve as substrates for the biosynthesis of specialized pro-resolving lipid mediators (SPM) that promote the endogenous resolution of inflammation [57, 58]. SPM derived from the n-3 FAs DHA and EPA include the resolvin D (RvD)-, maresin (MaR)-, and protectin- series SPM, and the resolvin E (RvE)- series SPM, respectively [59]. Preclinical investigations indicate that these SPM are key in the active regulation of inflammation resolution [57, 58, 60-63].

Table 3: Adequate Intakes (AIs) for n-3 as ALA and n-6 as LA as proposed by the IOM [50]

	ALA (gm/d)		LA (gm/d)	
Age	**Male**	**Female**	**Male**	**Female**
Birth to 6 months	0.5	0.5	4.4	4.4
7-12 months	0.5	0.5	4.6	4.6
1-3 years	0.7	0.7	7	7
4-8 years	0.9	0.9	10	10
9 -13 years	1.2	1.0	12	10
14-18 years	1.6	1.1	16	11
19-50 years	1.6	1.1	17	12
51+ years	1.6	1.1	14	11

AIs: Adequate Intakes; n-3: Omega 3; ALA: Alpha-Linolenic Acid; LA: Linoleic Acid

Major forms of n-6 FAs include LA (18:2) and arachidonic acid (AA) (C20:4). LA is derived from vegetable and meat sources while AA is metabolized from LA. N-6 FAs have beneficial effects such as lowering LDL cholesterol, increasing HDL cholesterol and improving the body's sensitivity to insulin. However, consuming large amounts of n-6 FAs increases the plasma concentrations of eicosanoids derived from AA metabolism. In contrast to n-3 FAs, AA generally exerts pro-inflammatory properties via its conversion by the cyclooxygenase (COX) and lipoxygenase (LOX) pathways to bioactive mediators called eicosanoids (i.e. prostaglandins,

thromboxanes and leukotrienes) [64]. Yet, during the acute phase of an inflammatory response, AA metabolism can also change to the production of other eicosanoid classes such as lipoxins, prostaglandin E2 and leukotriene B4 that have the ability to limit the extent and duration of the inflammatory process and promote the resolution of inflammation [59].

Low n-3 intake is a common characteristic of the Western diet due to low intakes of foods such as fatty fish and nuts [65]. N-6 FAs are consumed at a disproportionately high level in a standard western diet compared to n-3 due to high intakes of meat, processed foods, soy and corn oils [52]. Although both FAs are considered essential, it is important to maintain a reasonable ratio between n-6 and n-3 FAs in the diet because they compete for the same enzyme complex (PLA2) for conversion to their respective long chain derivatives [66]. The bioavailability of PUFAs and their bioactive derivatives depends on diet composition with an ideal ratio of 4 (n-6):1 (n-3) to 5:1 [67]. However, a typical Western diet pattern tends to contain 14 to 25 times more n-6 FAs than n-3. This imbalance can result in inappropriately elevated or sustained inflammatory conditions.

PUFAs in the Brain

The brain is the second-most fatty organ next to adipose tissue. Due to high phospholipid content, approximately 60% of the brain's dry weight consists of lipids with about 30% of that lipid consisting of PUFAs and 5-10% as AA [68]. Brain phospholipids form the membrane lipid bilayers of neurons, glia and cerebrovascular cells. Normal physiological functioning of the neuronal membrane is highly dependent on its structure. Membrane lipids indirectly control a variety of biological functions through their regulation of enzymes, receptors and neurotransmitters. Therefore, any alteration in lipid content has a pivotal role on membrane properties and can induce functional changes [69]. As opposed to triglycerides, PUFAs are preferentially incorporated by phospholipids with the majority of the PUFAs in cell membranes

synthesized from LA and ALA [70, 71]. In fact, up to 60% of lipids incorporated in neural membranes consist of DHA, as it can easily pass through the BBB [72-74]. The neuronal cell membrane is in a constant state of flux as FAs are released from the membrane by phospholipases. Types of FAs consumed in the diet determines the FAs that are available for the composition of cell membranes. Increased consumption of n-3 PUFAs results in a partial replacement of AA by DHA in the neuronal cell membrane [75]. Conversely, lower n-3 intake leads to lower brain levels of DHA with increased AA levels.

N-3 FAs in the Brain

Incorporation of n-3 FAs into the phospholipid bilayer has a prominent role in maintaining membrane fluidity, which is important in regulating many membrane functions such as signal transduction, solute transport and enzyme activity [76, 77]. Inadequate n-3 intake can result in neuronal cell membranes with increased straight-chain SFAs, which decrease membrane fluidity and consequently alter these functions. N-3 FAs can also directly modify neurotransmitter production, accumulation, release and re-uptake. For example, rats with n-3 deficiency had significantly decreased dopamine content and storage in the frontal cortex, suggesting that alterations in n-3 intake may have an impact on dopamine synaptic neurotransmission and plasticity [78-80]. Interestingly, n-3 supplementation was able to increase dopamine levels in those same areas, hinting at a possible therapeutic effect [81].

DHA is especially essential during pre- and post-natal human brain development. Being prevalent in grey matter and synapses, DHA deficiency can include consequences such as cognitive impairment and diminished visual acuity [82]. Relationships between n-3 FAs and neurological disorders and diseases have been extensively researched. Severe restriction of n-3s usually correlates with anxiety-like behavior and a deficit in cognitive functions, including memory and learning. Diets low in n-3s have been linked to neuroinflammation and the development of neurological disorders such as depression, bipolar disorder, schizophrenia and

Parkinson Disease [83-86]. These findings have led to the investigation of the neuroprotective effect of n-3.

N-6 FAs in the Brain

Necessary for cell membrane integrity, AA is the second most predominate PUFA in the brain. In addition to DHA, AA likely enters the brain by free diffusion across the cell membranes of the BBB. AA has a much shorter half-life (five months) compared to DHA (two years) [87]. Therefore, it has been estimated that the brain needs 18 mg of AA per day. Animal models have suggested AA may be beneficial in neuroinflammatory diseases by attenuating amyloid β deposition and improving cognition [88, 89]. However, results have not been consistently replicated.

Conversely, AA may also have detrimental effects in the brain. These FAs in the neuronal cell membrane are metabolized to different eicosanoids by COX/LOX pathways which are involved in the neuroinflammatory process. In neurological diseases such as Parkinson Disease, organisms are not able to buffer PUFA concentrations. Therefore, with higher consumption of LA, higher amounts of AA may be incorporated into neuronal cell membranes and ultimately result in increased neuroinflammation and neurological diseases.

Saturated Fatty Acids (SFAs)

SFAs are straight-chain organic acids that make up 10-40% of the total fatty acids in most natural lipids and can also be synthesized by the body (Figure 3). Not only are they a source of fuel and structural components of cell membranes, but various SFAs are also associated with proteins and are necessary for their normal

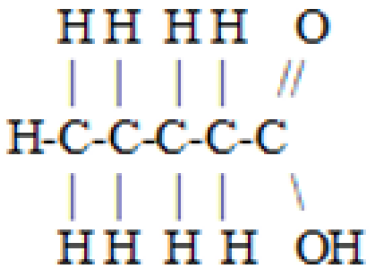

Figure 3: Chemical Structure of Saturated Fatty Acids (SFAs). *Depicted: Butyrate (C4:0)*

function. SFAs do not contain double bonds and are named and abbreviated systematically from the saturated hydrocarbon with the same number of carbon atoms. In addition to systematic names and abbreviations, each SFA may also be referred to by its trivial or common name (Table 4). Currently, there are no recommendations for daily intake as the IOM notes no evidence of SFAs being essential in the diet or having beneficial roles in the prevention of chronic diseases. However, prominent organizations like the American Heart Association recommend less than 10% of total fat calories per day as saturated fat, or less than 4.4 to 7.7 grams per day based on a 2000 calorie per day diet, irrespective of chain length.

Table 4: SFA Trivial Names, Systemic Names and Abbreviations by Chain Length Subcategory

SFA Subcategory	Trivial Name	Systematic Name	Abbreviation
SCFA	Acetic	Acetate	C2:0
	Propionic	Proprionate	C3:0
	Butanoic	Butyrate	C4:0
	Caproic	Hexanoic	C6:0
MCFA	Caprylic	Octanoic	C8:0
	Capric	Decanoic	C10:0
	Lauric	Dodecanoic	C12:0
LCFA	Myristic	Tetradecanoic	C14:0
	Palmitic	Hexadecanoic	C16:0
	Stearic	Octadecanoic	C18:0
	Arachidic	Eicosanoid	C20:0
	Behenic	Docosanoic	C22:0
	Linoceric	Tetracosanoic	C24:0

SFA: Saturated fatty acid; SCFA: Short-chain saturated fatty acid; MCFA: Medium-chain saturated fatty acid; LCFA: Long-chain saturated fatty acid

Although the compounds are historically thought of as one group, evidence shows SFAs with different physical and chemical structures have different metabolic and health effects [90, 91]**.** In appreciation of their differences, SFAs are often comprised into three sub-groups: short chain (C2:0-C6:0), medium chain (C8:0-C12:0) and long chain (C14:0-C24:0) FAs.

Short-chain saturated fatty acids (SCFAs) are small, monocarboxylic acids with a chain length of up to six carbon atoms and are the main products of anaerobic fermentation of indigestible dietary fibers, proteins and resistant starch in the large intestine [92]. The SFA group

is comprised of acetate (C2:0), proprionate (C3:0), butyrate (C4:0) and hexanoic (C6:0) acids [93]. Approximately 500-600 mmol of SCFAs are produced in the gut per day depending on the fiber content in the diet, intestinal bacteria and intestinal transit time [94]. Aside from fermentation, sources of butyrate include milk fat [95] and acetate produced from acetyl-CoA derived from glycolysis [96, 97]. The highest levels of SCFAs are found in the proximal colon, where they are utilized locally by enterocytes or transported across the gut epithelium into the bloodstream [98].

SCFAs are mobilized to improve intestinal health through a number of local effects, ranging from maintenance of intestinal barrier integrity, reduction of oxidative stress, protection against inflammation and modulation of the mucosal immune response to reduction of the risk of colorectal cancer [99-102]. The production of SCFAs through increased intake of dietary fiber to enhance these beneficial characteristics has been an attractive intervention in the field of gastrointestinal diseases. However, recent research also indicates that increased SCFAs intake and/or fermentation is advantageous in the brain by playing a pivotal role in the gut-brain axis [103-105]. The gut-brain axis describes the close connection and tight bidirectional communication between the intestinal tract and brain and the importance of their cross-talk for brain functions in health and disease [106].

SCFAs bind to several G protein-coupled receptors (GPR) which results in secretion of glucagon-like peptide 1 and signals the pancreas to increase insulin secretion. Dietary butyrate (C4:0) supplementation enhances FAO in mice and both butyrate and proprionate (C2:0) have been shown to inhibit high fat diet-induced weight gain [107, 108]. SCFAs are also histone deacetylase inhibitors (HDACis) which promote the acetylation of lysine residues present in histones in various cell populations, including neurons.

MCFAs comprise SFAs with 6 to 12 carbons: hexanoic (C6:0) octanoic (C8:0), decanoic (C10:0) and dodecanoic (C12:0) acids. Major suppliers of MCFAs are coconut, palm kernel and *Cuphea* species oils and dairy fats. Besides natural sources, MCT oils, produced by hydrolysis of

one of the aforementioned oils, contains almost exclusively octanoic (C8:0) and decanoic (C10:0) acids at a ratio of 50:50 to 80:20 [21].

Triglycerides containing MCFAs are broken down almost immediately by enzymes in the saliva and gastric juices without using pancreatic fat-digesting enzymes. As MCFAs does not depend on proteins for binding, transport and transmembrane location, the FAs are absorbed quickly and efficiently in the intestinal lumen [21]. Their ability to be absorbed outside of the lymphatic system allows MCFAs to be utilized as a calorie and fat source in many digestive abnormalities such as fat malabsorption. Studies have demonstrated that MCFAs modulate carbohydrate and lipid metabolism, as well as mitochondrial energy production [109]. MCFAs are more rapidly β-oxidized compared to LCFAs and do not appear affected by the carbohydrate content in the diet [110]. St-Pierre et al. showed oral MCFA supplementation was able to raise plasma MCFA and KB concentration in humans eating a carbohydrate-rich diet. [111]

Long-chain saturated fatty acids (LCFAs) contain over 12 carbons and constitute the greatest bulk of FAs in animal diets. Palmitic acid (C16:0) and stearic acid (C18:0) are considered the most abundant SFAs in nature comprising 20-30% and 10-20% of lipids in animal tissues and lipid classes, respectively. Additional LCFAs present in lower amounts of a human diet include Myristic acid (C14:0), Arachidic acid (C22:0), Behenic acid (C22:0) and Linoceric acid (C24:0). LCFAs are an important source of energy in tissues and are essential to the structure and function of lipid membranes. In contrast to SCFAs and MCFAs, LCFAs require fatty acid-binding proteins for their cellular uptake, intracellular transport, regulatory functions and metabolism [112]. LCFAs are esterified to triglycerides in enterocytes, incorporated into chylomicrons and then enter the lymphatic system.

LCFAs are often associated with negative health effects such as insulin resistance, generation of reactive oxygen species (ROS) and inflammatory responses in the body [113, 114]. Palmitic acid (C16:0) can activate inflammatory pathways directly by stimulating macrophages to produce cytokines via toll-like receptors (TLR) 2 and 4 and by stimulating signaling molecules as protein

kinase R. Excessive LCFAs levels are believed to contribute towards lipotoxicity and neuroinflammation [115].

SCFAs in the Brain

The gut microbiota has been shown to significantly influence central nervous system physiology and neurochemistry [116]. Intestinal dysbiosis has been identified in many neurological diseases, including Parkinson's disease and Alzheimer's disease [117, 118]. As stated previously, SCFAs are the main energy source for endothelial cells and have a role in preventing or ameliorating microbiome dysbiosis [107]. Therefore, relationships between butyrate, the gut-brain axis and neurological outcomes are being explored.

SCFAs appear to influence neuronal function by modulating levels of neurotransmitters and neurotrophic factors which support the growth, survival and differentiation of developing and maturing neurons [119]. SCFAs are also able to cross the BBB and play an important role in maintaining its integrity and even ensuring permeability [120, 121].

Butyrate (C4:0) is part of a class of epigenetic modulators known as HDACis which have been identified as a possible treatment option for neurodegenerative diseases [122, 123]. The branched chain SCFA, valproic acid (VPA), is another HDACi and a commonly used broad-spectrum ASM [124]. Although effective, VPA therapy has been considered sub-optimal due to numerous side effects [125]. Oral sodium butyrate (NaB) supplementation has been shown to protect neurons from cell death in models of Parkinson's disease and decrease the infarct size in models of ischemic stroke, which limits the damage to the brain and improves behavioral outcomes [122, 126]. *In vitro* and *in vivo* data suggest butyrate can induce resistance to oxidative stress and increase histone acetylation [127]. Together, these observations are consistent with the theory that butyrate can modulate the expression of a large number of genes which may affect neurological function.

MCFAs in the Brain

In addition to DHA and SCFAs, MCFAs are also able to cross the BBB and may have brain health benefits by modulating brain metabolism [128]. KBs derived from MCFAs have been shown to improve cognition in patients with Alzheimer's disease and attenuate neurodegeneration in an animal model of amyotrophic lateral sclerosis [129].

Although a majority of dietary MCFAs are converted to KBs, studies have shown that they also have diverse effects on cognitive and neuronal function [130]. For example, MCFAs had differential effects on synaptic stability, protein synthesis and cognitive behavior in Wistar rats that may be independent of ketone levels [131]. The ability of MCFAs to improve cognitive function has also been reported with improvement seen in patients with Alzheimer's Disease [132]. MCFAs reduce amyloid β-production and deposition in the brain and activate G-protein coupled receptors (GPR), which are highly expressed in the brain and have roles in glucose regulation and inflammation [133, 134].

Recent research proposes MCFAs have anti-seizure effects at clinically relevant concentrations in vitro and in vivo, suggesting they directly contribute to the therapeutic effect of the MCTKD [135, 136]. Preliminary evidence suggests MCFAs may be able to impact seizure activity independent of a carbohydrate restricted diet.

LCFAs in the Brain

As previously mentioned, excessive LCFAs have a significant impact on inflammation in the human body. Specifically, LCFAs increase proinflammatory cytokine expression in astrocytes and the microglia [137]. The brain is highly sensitive to inflammatory mediators and there is ample data to indicate that cytokines can have clinically significant adverse effects on cognition and neuronal homeostasis [138]. In fact, LCFAs have been explicitly implicated in the development of neuroinflammatory diseases [137, 139, 140].

In addition to neuroinflammation, LCFAs have been shown to modulate amyloid processing in neurons and astrocytes, increase α-synuclein and tyrosine hydroxylase levels and reduce the expression of insulin-degrading enzyme which is implicated in the pathogenesis of Alzheimer's Disease [139, 140]. Together, these data indicate excess intake and production of LCFAs appear to have a detrimental effect and brain function and likely perpetuate neurological disease.

Theorized Anticonvulsant Properties of FAs

Current literature suggests PUFAs and SFAs may elicit anticonvulsant properties through one or more theorized mechanisms of action: modification of neuronal cell signaling and hyperexcitability, mediation of neuroinflammation, activation of peroxisome proliferator-activated receptors (PPARs) and inhibition of histone deacetylase (HDAC) dysregulation (Figure 4).

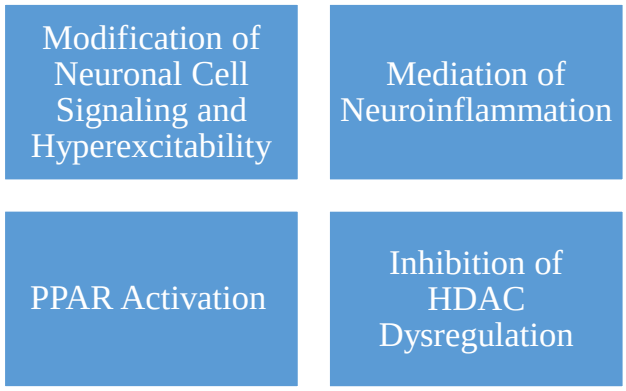

Figure 4: Theorized Anticonvulsant Properties of Fatty Acids (FAs)*: Mediation of Neuronal Cell Signaling and Hyperexciatbility, Mediation of Neuroinflammation, Activation of Peroxisome Proliferator-Activated Receptors (PPAR) and Inhibition of Histone Deacetylase (HDAC) Dysregulation*

Modification of Neuronal Cell Signaling and Hyperexcitability

Seizures often occur when a disruption in the normal balance of excitation and inhibition occurs. Normally, there are controls that keep neurons from excessive action potential discharge, but there are also mechanisms that facilitate neuronal firing so the nervous system can function properly. Disrupting the mechanisms that inhibit firing or promoting the mechanisms that facilitate excitation can lead to seizures [141]. However, seizure activity may be prevented by disrupting the mechanisms that bring neurons close to their firing threshold, or enhancing the ways neurons are inhibited. The incorporation of select PUFAs and SFAs into the neuronal cell membrane may have a significant impact on action potentials through modulation of voltage gated channels and conductance of currents [142-145]. Here, n-3 PUFAs, specifically DHA, along with SCFAs and MCFAs show an ability to modify neuronal cell signaling and hyperexcitability, potentially inhibiting seizure onset or potentiation.

Landmark studies in the 1990's by Xiao et al. and Vreugdenhil et al. showed PUFAs are able to stabilize the neuronal membrane by suppressing and causing a significant shift in steady state inactivation for voltage-gated Ca^{2+} currents and Na^{+} channels [142, 146, 147]. ASMs including carbamazepine, phenytoin and valproate have very similar actions on Na+ channels which provides evidence that these actions significantly contribute to anti-seizure functions [124, 148]. A later study by Xiao et al. also found that DHA, specifically, could raise the action potential depolarization threshold and decrease the frequency of stimulus-evoked action potentials [143]. This may be explained by the ability of DHA to suppress conductance of currents in neuronal cells and reduce neuronal hyperexcitability [143, 147, 149-152].

In further support of FAs inhibiting seizure activity via this theorized mechanism of action, SCFAs and MCFAs appear to modify cell signaling and neuronal hyperexcitability [145]. Acetate (C2:0) has previously been shown to alter the levels of the neurotransmitters glutamate, glutamine and gamma-aminobutyric acid (GABA) in the hypothalamus [153]. Furthermore, propionate

(C3:0) and butyrate (C4:0) exert an influence on intracellular potassium levels, implying their involvement in the operation of cell signaling systems [154]. MCFAs have been shown to act on phosphoinositide signaling in seizure control in a similar, but more potent mechanism to VPA [155]. Furthermore, MCFAs have shown anticonvulsant activity via direct inhibition of the α-amino-hydroxyl-5-methyl-4-isoxazoleproprionic acid (AMPA) receptor [144]. Inhibition of this receptor is the mechanism behind the currently available and widely used ASM, perampanel. AMPA receptors play a key role in generating and propagating epileptic activity. In addition to mediating synchronous epileptic discharges from the seizure origin, AMPA receptors are critically important in the spread of seizure activity locally and to distant brain regions. Data indicate that 100 μmol/L decanoic acid (C10:0) results in a 3-fold increase in the inhibition of the AMPA receptor.

Mediation of Neuroinflammation

Neuroinflammation is characterized by an increased inflammatory response, centralized in the brain and spinal cord. Harmful downstream effects of sustained neuroinflammation can lead to the development of dementia and a number of neurological diseases, including Parkinson Disease and Amyotrophic Lateral Sclerosis [156, 157]. Neuronal damage and the unhalted progression of neuroinflammation may also lead to epilepsy onset or potentiate recurrent seizure development (epileptogenesis) through extra-hippocampal neuronal cell death and microglial cell activation [151, 152, 158].

Cytokine levels have been suggested as predictors of the beginning of spontaneous seizures as both Interleukin (IL-) 6 and Tumor Necrosis Factor alpha (TNF-α) levels are increased in microglial cells after epileptic seizures [159]. An overexpression of these cytokines leads to a decrease in seizure threshold and an increase in spontaneous seizure frequency by participating in neuronal hyperexcitability through alterations in ion channels and glutamate production [160-

163]. PUFAs and SFA are potent regulators of neuroinflammation with n-3 FAs, SCFAs and MCFAs often thought of as having anti-inflammatory properties.

Deprivation of n-3 FAs has been shown to decrease levels of DHA and increase markers of brain AA, and neuroinflammation [86, 164]. SPM from DHA and EPA are produced in a dose-dependent manner in the brain to counteract and resolve neuroinflammation by exerting pro-survival, neuroprotective capabilities and preventing apoptosis induced by oxidative stress [165-167]. In pre-clinical studies, animal models treated with SPM, including MaR1, RvD1, RvD2 and Neuroprotetin D1 (NDP1), have decreased cytokine (TNF-α, IL-6, IL-1β) levels, decreased nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) protein and decreased COX-2 expression [168-173]. Additionally, separate studies have reported treatment of brain-injured animal models with RvD2 and MaR1 inhibit microglial activation, therefore decreasing neuroinflammation [171, 172]. In 2018, Frigerio et al. showed that injecting mice with DHA-derived protectin D1 during epileptogenesis, reduced both neuroinflammation and the frequency of spontaneous seizures by half and the time spent in seizures by three-fold [174]. A significant upregulation in both inflammation and SPM at 72 hours supports the hypothesis that neuroinflammation in epileptogenesis may progress without adequate SPM substrate. Specifically, cumulative levels showed a downregulation of DHA-derived resolvins and AA-derived lipoxins and an upregulation in DHA-derived protectins and EPA-derived resolvins [174].

SCFAs also appear to have a significant impact on regulation of neuroinflammation. Several studies have reported that oral NaB is able to decrease microglial activation and pro-inflammatory cytokine secretion [123, 175, 176]. Furthermore, there is some evidence that suggests butyrate's (C4:0) antioxidant activity decreases the level of reactive oxygen species (ROS) while increasing glutathione [177]. Butyrate treatment *in vivo* and *in vitro* induces changes in the microglia and inhibits pro-inflammatory modifications [175]. Similarly, *in vivo* SCFA

treatment and supplementation has been shown to decrease microglial activation in astrocytes and reduce inflammatory signaling through reduced IL-1β, IL-6 and TNF-α expression and NF-kB phosphorylation [178-180]. The role of intestinal dysbiosis and intestinal inflammation in reducing seizure threshold or aggravating seizures has also been previously reported and confirmed in a colitis animal model [181, 182]. Consequently, SCFA may also provide anti-inflammatory properties by preventing or attenuating intestinal dysbiosis and intestinal inflammation.

Activation of Peroxisome Proliferator-Activated Receptors (PPARs)

PPARs are members of the nuclear receptor family and are critically involved in the regulation of numerous genes that regulate energy homeostasis. When activated, PPAR isoforms (PPARα, PPARβ and PPARγ) act as agonist-activated transcription factors that regulate specific target gene transcription. PPARγ, in particular, has garnered significant interest in the potential neuroprotection in diverse neurodegenerative diseases, including epilepsy [183]. PPARγ is a master regulator of adipogenesis, lipid and glucose metabolism, and insulin sensitivity [184]. PPARγ also regulates anti-inflammatory, anti-oxidant and mitochondrial genes [185, 186]. It is primarily located in the nucleus of cells in the hippocampus and regulates the activation of microglial cells, suggesting that PPARγ agonists may impact neuroinflammation. PPARγ activation is initiated by ligand binding from a wide variety of naturally occurring monounsaturated FA, SFA, PUFAs, eicosanoids, and oxidized lipid agonists [187]. In humans, PPARγ exists in multiple isoforms (PPARγ1, PPARγ2 and PPAR-γ4) [188]. PPARγ1 is found universally, while PPARγ2 is restricted to adipose tissue. PPARγ2 is induced during high fat diets and has been shown to be differentially regulated between genotypes [189-191]. It is the only PPARγ isoform regulated at the transcription level by nutrition and activation stimulates the transcription of genes involved in fatty acid oxidation [192, 193].

Hung et al. found activation of PPARγ in mice significantly reduced sustained time of stage 4-5 seizures, percentage of status epilepticus, and mortality due to seizures in mice [194]. Authors concluded PPARγ deficiency aggravated neuronal excitability and excitotoxicity. Conversely, the presence of PPARγ attenuated seizure severity, neuronal loss, BBB impairment and sodium currents in hippocampal neurons. Simeone et al. reported that administering PPARγ to epileptic knockout (KO) mice increased PPARγ2 expression by 2-fold ($P < 0.01$) and reduced seizures by 80% ($P < 0.01$) [195]. Indeed, pretreatment with a PPARγ agonist prior to induced status epilepticus can inhibit neuronal cell death, which is a principal cause of acute and chronic neurodegenerative disease [196]. PPARγ antagonism has also been found to directly interfere with the anti-seizure mechanism of the cKD. KDT of normal mice increases PPARγ in the brain [195, 197]. However, a cKD was unable to raise the seizure threshold of PPARγ2 KO mice and conditional neuron-specific PPARγ KO mice, indicating the loss of PPARγ may exacerbate markers of injury and possibly seizures.

N-3 PUFAs have features that allows optimal binding to PPARs. Previous studies have shown n-3 supplementation significantly upregulated gene expression of PPARγ specifically in humans with neurological disease [198-200]. While SFAs are PPARγ agonists, they generally considered poor ligands in comparison to long chain PUFAS [201, 202]. However, the MCFA decanoic (C10:0) acid is a recognized PPARγ agonist with a binding pocket which is drastically different from that of PUFAs [130]. A dramatic increase in MCFAs through dietary intervention or supplementation may be able to increase binding, resulting in anticonvulsant effects.

Inhibition of Histone Deacetylase (HDAC) Dysregulation

Previous studies have demonstrated that epigenetic signaling is essential in the progression of epileptogenesis and chronic epilepsy [203]. Histone acetylation has been widely linked to transcriptional activity and is regulated by opposing actions of two classes of enzymes: histone

acetyltransferases, which facilitate histone acetylation and activate gene transcription and HDACs, which suppress gene transcription and gene expression [204]. HDAC inhibition upregulates the expression of brain-derived neurotrophic factor, which is responsible for the activation of multiple proteins involved with neuronal biogenesis and cognition [205, 206]. Conversely, HDAC dysregulation may lead to impaired acetylation and deacetylation resulting in the development of neurological diseases, including epilepsy. Specific histone modifications, H3 and H4 phosphorylation, have been reported in several animal seizure models [207-209]. Therefore, inhibiting epigenetic changes in histone acetylation may be a key step in preventing or halting epileptogenesis.

The SCFAs butyrate (C4:0) and VPA are able to inhibit HDACs, subsequently offering neuroprotective benefits to the brain by enhancing acetylation of histones, restoring the balance of histone acetylation and adjusting transcriptional dysfunction [210, 211]. NaB behaves similarly to VPA, but inhibits a wider range of HDAC classes (1, 2 and 7) compared to VPA (classes 1 and 2 only) [212]. Supplementation has previously been shown to inhibit HDACs and increase the acetylation of H3 and H4 histones in the brain [213, 214]. In fact, NaB significantly increased H3 and H4 acetylation in comparison to VPA [215]. Thus, increasing butyrate (C4:0) intake through dietary intake, supplementation or endogenous production may be beneficial in individuals with epilepsy.

Anticonvulsant Effects of PUFAs and SFAs in Animals and Humans

Data suggests that FAs, specifically n-3, SCFAs and MCFAs, have the potential to increase seizure thresholds and prevent or halt epileptogenesis. Proposed mechanisms of action have substantiated the need for research to assess the impact of FAs on animal and human epilepsy models.

Anticonvulsant Effects of PUFAs in Animal Models

Pre-clinical trials have regularly shown PUFAs may have anticonvulsant properties, but results have not been consistently replicated in clinical trials [68, 216, 217]. Early animal models reported on the anticonvulsant effects of ALA and LA and their ability to increase latency to seizure onset [218-221]. However, when unsuccessful attempts were made to replicate these studies, researchers hypothesized that increased doses of PUFA's may be needed to elicit anti-seizure effects [222, 223]. In support of this hypothesis, investigations by Taha et al. and Porta et al. found higher-dose PUFA administration (200 mg/kg intraperitoneal and 60000 mg/kg gavage, respectively) increased seizure thresholds in animal models with PTZ induced seizures [39, 224].

When assessed separately and dose-dependently, DHA (100-300 mg/kg), but not EPA, raised seizure thresholds [225, 226]. In mice, acute administrations of DHA at dosages of 65 mg/kg or higher raised seizure thresholds with levels, but effects were not sustained after one hour [68, 221, 227]. In another positive trial, Trepanier et al. treated Wistar rats with 50 mg/kg DHA or saline for 14 days followed by seizure induction [228]. Here, a lower DHA dose significantly increased seizure latency by approximately three-fold as compared to controls when provided over an extended period of time.

Animal studies indicate that single or insufficient dosing may explain some variability in pre-clinical trials. In addition, researchers suggest a minimum of three months of dietary n-3 supplementation is required to raise seizure thresholds [226]. Chronic consumption has been shown to increased concentrations of unesterified EPA and DHA in the brain by two-fold, suggesting time is an important factor in n-3 dosing. Increased concentrations of EPA and DHA in the brain may provide adequate substrate to elicit anticonvulsant properties or result in a shift metabolism thereby increasing seizure thresholds.

Anticonvulsant Effects of PUFAs in Clinical Trials

Early clinical trials of n-3 supplementation in subjects with epilepsy were largely positive. In a 2002 study, Schlanger et al. saw a significant reduction in seizure frequency and strength after six months of 3.25 g n-3 (0.9 gm EPA and 2.3 gms DHA) per day [229]. Unfortunately, only five patients completed the study due to the taste of the oral fish oil spread. Nonetheless, the study provides optimism in the ability of high dose, long-term n-3 supplementation to provide therapeutic benefit to epileptic patients.

A 12-week placebo-controlled parallel group study published in 2005 provided 1 gm EPA and 0.7 gm DHA per day to the intervention group. Here, Yuen et al. reported 17% of subjects receiving supplementation had at least a 50% reduction in seizures during the first six weeks [230]. Supplementation also resulted in significant increases of serum EPA and DHA concentrations and associated decreases in LA and AA concentrations, mirroring results from KDT and animal studies. The same group followed up in 2011 with a non-randomized open assessment of 10 subjects with refractory focal seizures receiving 1000 mg EPA per day for three months. Compared to baseline, six subjects had fewer seizures while receiving supplementation with one subject reporting markedly reduced seizure severity. Mean reduction in seizure frequency was 16% ($p = 0.26$). Although results from these two groups are encouraging, small sample sizes and subjective seizure reporting limit the validity and reproducibility of the data.

In disagreement, two studies published in 2008 reported no beneficial effect of PUFA supplementation in reducing seizure frequency or severity. A double-blind, placebo controlled RCT by Bromfield et al. supplemented adult refractory epilepsy patients with 2.2 mg EPA plus DHA in a 3:2 ratio (1.32 g EPA and 0.88 g DHA) for 12 weeks [231]. Negative results from this study include two subjects on placebo versus none on n-3 supplementation experiencing a 50% decrease in seizure frequency. Furthermore, median seizure frequency increased by 6% in the intervention group (n=12) and decreased by 12% in the placebo group ($p = 0.21$). In a prospective, randomized, 3-period crossover trial of two doses of fish oil, DeGiorgio et al.

exposed subjects with partial-onset seizures to each PUFA treatment group (high-dose, low-dose and placebo) for 10 weeks [232]. The low-dose group received a total n-3 dose of 1080 mg/day (648 mg EPA, 432 mg DHA) and the high dose group received 2160 mg/day (1296 mg EPA, 865 mg DHA). For those receiving low-dose fish oil, 5/20 experienced a 50% seizure reduction as compared with placebo with two subjects becoming seizure free. No differences were observed when comparing high-dose fish oil to placebo. Authors surmised that patients receiving lower doses of n-3 FAs have improved efficacy over high-doses. This and previous studies have suggested that high doses of n-3 FAs do not exhibit anticonvulsant effects due to the suppression of AA levels [230]. However, significant limitations of this trial, to include the crossover design and short exposure period, minimize the ability to conclude low-dose fish oil supplementation is superior to high-dose supplementation.

Three studies published in the past decade have not only report positive findings, but also begin to elucidate the n-3 mechanism of action in human subjects. First, a clinical trial published in 2010 by Al Khayat et al. studied serum levels of PUFAs and therapeutic response in 20 Egyptian children with idiopathic intractable epilepsy [233]. Prior to supplementation, subjects had lower levels of DHA and higher levels of ALA, EPA and LA compared to healthy controls as assessed by gas liquid chromatography. After six months of oral PUFA supplementation, levels of DHA were increased while ALA, LA, EPA and AA were decreased. Furthermore, there was an improvement in n-6:n-3 and increased DHA:EPA ratios. Changes in serum levels of PUFAs and their ratios were accompanied by a decrease in seizure frequency, duration and severity. A significant negative correlation between serum DHA and seizure duration and seizure severity was reported. Next in 2015, Reda et al. conducted a case control study investigating the effect of 1200 mg (720 mg EPA and 480 mg DHA) n-3 as fish oil per day to Egyptian children with epilepsy [234]. Subjects (n=70) had a median number of four seizures per month prior to supplementation. The median seizure occurrence decreased in the intervention group (n=35) to two seizures after one month. The median again decreased to zero seizures in both months two

and three of supplementation ($p < 0.05$). No significant difference in the medians of seizure severity was observed. Finally, a recently published triple-blind, placebo-controlled parallel group trial by Omrani et al. supplemented n-3 PUFAs to 50 subjects with refractory epilepsy [235]. Those in the intervention group received 180 mg EPA and 120 mg DHA twice per day (360 mg EPA and 240 mg DHA total) for 16 weeks. Seizure frequency and duration in addition to TNF-α and IL-6 were significantly lower in the supplement group with corresponding improved electroencephalogram (EEG) patterns.

Conflicting results from clinical trials, not unlike animal models, may be explained by variations in n-3 dosing and duration of supplementation. Although total PUFA levels increase in plasma following oral administration, initially the majority of the elevated PUFA subtypes are probably trapped in the chylomicron/LDL pools. Auvin et al. claims PUFA subtypes probably do not appear in its unesterified form in plasma until the chylomicron/LDL pool is eventually saturated [216]. Depending on the age and baseline diet of the patient, supplemental doses of n-3 may need to be higher than the 1.1 to 3.2 gms per day that have been used in previous clinical trials. Specifically, it has been widely demonstrated that the effective dose in other neurological disease is 1 g/day of DHA from animal or algal sources [83, 236]. This higher dosage likely needs to be continued over an extended period of time to ensure unesterified n-3 is reaching the brain.

Aside from discrepancies in dose and duration, another factor to consider in these trials may be the relationship of long-term diet exposure on seizure outcomes. As previously discussed, n-6 intake significantly outweighs n-3 intake in a typical Western diet. Recently published data show prolonged intake of a pro-inflammatory diet can alter gene expression and metabolism [237, 238]. Currently, there is a paucity of data describing the impact of typical dietary intake on seizure presence and severity.

Anticonvulsant Effects of SFAs Animal Models

The majority of research on the impact of SCFAs in epilepsy is centered on the epigenetic mechanism of butyrate (C4:0) as an HDACi. In an animal epilepsy model in 2017, Reddy et al. showed treatment of SCFAs as NaB (600 mg/kg, i.p.) twice daily for two weeks markedly decreased the development of spontaneous seizures with a corresponding increase in H3 and H4 acetylation and reduced HDAC activity in the hippocampus [239]. Citraro et al. provided mice oral treatment of NaB (30 mg/kg/day), VPA (600 mg/kg/day) or a combination to rats for 17 weeks prior to seizure onset [240]. Researchers showed long-term oral supplementation was able to significantly reduce seizure development and inhibit epileptogenesis. Here, H3 acetylation was significantly increased by both treatments while H4 acetylation was only significantly increased by NaB. Finally, Li et al. evaluated the effect of smaller doses of NaB (5, 10, and 20 mg/kg, intraperitoneally) for 40 days prior to seizure induction [241]. Again, a significant reduction in seizure score and significantly longer latencies in seizure development were seen in all three groups. Interestingly, the 10 mg/kg dose appeared most effective. Here, NaB exhibited neuroprotective qualities by rescuing seizure-induced neuronal loss and apoptosis in the hippocampus. Supplementation also led to an increase in antioxidant enzyme activity and reduced ROS levels, hinting at the potential of an anti-inflammatory effect of SCFAs.

Although data suggest intestinal inflammation may perpetuate seizure activity and SCFAs may mediate this inflammation, studies directly relating SCFA to seizure activity are rare. Recently, De Caro et al. studied the intestinal inflammation on PTZ-induced seizures in mice mice fed NaB at 100 mg/kg per day. Not only was the SCFA significantly effective in increasing seizure thresholds, it also elicited significant protective effects against intestinal inflammation. This study substantiated the hypothesized link between SCFAs and epilepsy.

The efficacy of the MCTKD in subjects with epilepsy has launched investigations into the anticonvulsant properties of MCFAs. The first study to assess the impact of MCFAs *in vivo* was

published in 1990 by Nakamura et al. [242]. The group administered MCFAs i.p. at two doses (0.5 and 1.0 mmol/kg) in mice 15 minutes before seizure induction and found decanoic acid (C10:0) at 1.0 mmol/kg delayed the onset to clonic convulsions and increased the survival to lethality induced by induced seizures. Since 1990, studies have consistently shown the MCFAs octanoic (C8:0) and decanoic (C10:0) acids have anticonvulsant effects and are effective during seizure induction [135, 243]. In 2015, Wlaz et al. administered oral doses of MCFAs to albino Swiss mice prior to seizure induction [136]. Here, decanoic acid (C10:0) dose-dependently (1.7-8.6 g/kg) increased seizure thresholds. Both octanoic (C8:0) and decanoic (C10:0) acids at 1.4-4.3 g/kg, respectively produced comparable increases in seizure thresholds. When administered together, seizure thresholds increased even further, suggesting an additive interaction or symbiotic relationship between the MCFAs.

In another 2015 study, Chang et al. assessed if acute anti-seizure effects of the MCTKD were related to a direct effect of KBs or MCFAs [144]. Here two *in vitro* models showed KBs did not have any effect on epileptiform activity while an MCFA (decanoic acid) completely blocked epileptiform activity in both models. This supports an anti-seizure effect of MCFAs in the MCTKD, rather than diet-derived KBs. This study also aimed to find a mechanism of action behind the anti-seizure effects of MCFAs and identified their ability to block AMPA receptors.

A recent study by Tan et al. fed mice 35% of calories as MCFAs for 10 days prior to acute seizure tests [244]. Feeding MCFAs increased seizure thresholds in independent experiments compared to a control diet (both $p < 0.05$). After 20 days of dietary treatment, blood and forebrain samples were collected from mice that had undergone seizure induction 10 days earlier to assess levels of glucose, BHB and MCFA levels. Plasma glucose levels were similar in all diet groups and neither plasma nor brain BHB were elevated in any diet group. However, oral MCFA intake significantly increased plasma as well as brain MCFA levels.

Anticonvulsant Effects of SFAs in Clinical Trials

To the best of our knowledge, no studies specifically investigating the impact of SFAs on epilepsy have been published. Although research evaluating probiotics and MCTs in humans are available, we are unable to verify if an outcome is due to a confounding factor such as GABA production or KB metabolism.

Objective

Animal and clinical studies appear to confirm proposed mechanisms of action suggesting therapeutic benefit of FAs in epilepsy. However, the ability of these FAs to elicit anti-seizure effects in individuals following a general diet has not been extensively studied. In this analysis, we aimed to provide insights into the effect of dietary PUFA and SFA intake on seizure presence and severity in a cohort of subjects admitted to an EMU. Our long-term goal is to identify targets for nutrition manipulation through metabolic profiling as a possible treatment for epilepsy.

CHAPTER 1: METHODS

Study Design and Population

The present study aims to explore the impact of dietary PUFA and SFA intake on seizure presence and severity in subjects with suspected or confirmed epilepsy. After obtaining approval by the Institutional Review Board (IRB) at the University of Nebraska Medical Center (Omaha, NE, IRB protocol #571-18-FB), prospective enrollment of a cohort of epileptic patients admitted to an epilepsy monitoring unit (EMU) at Nebraska Medicine in Omaha, Nebraska was completed.

All patients admitted for management of refractory epilepsy in the EMU between May 2019 and December 2019 were considered for enrollment. Inclusion criteria included patients of all ages deemed medically stable. Medical stability, determined by the admitting provider, was characterized as admission to the EMU under voluntary and non-emergent conditions.

Those without capacity to provide informed consent, as determined by an assessment of cognitive status by Dr. Arun Swaminathan MBBS, patients who were not medically stable, patients consuming greater than 6000 calories per day, pregnant women, and prospective subjects/guardians who could not read or write English were excluded as the validated tool administered to subjects is only available in English.

Procedure

Medical staff/study personnel approached prospective subjects and informed consent on adults older than 19, parental consent on children less than 19, and child assent on children 7 to 18 years of age was obtained. After enrollment, baseline and clinical data were collected from the electronic medical record (EMR) and a study packet containing the Harvard Food Frequency Questionnaire (FFQ), self-contained blood sampling devices (NoviPlex™ cards), fingerstick supplies and instructional materials was allocated to the subject. The duration of the study lasted for the period of patient's hospitalization without scheduled follow-up.

Dietary Intake

The Harvard FFQ was utilized to determine dietary intake. This tool has been validated in adults of all ages and sexes and among a variety of socio-economic groups [245-249]. The FFQ consists of a list of foods and beverages that represent major contributors to the diet of the participants and frequency of how often each food is consumed. Specific information on the use of supplements and their contribution to fatty acid intake is part of the FFQ. From responses to the questionnaire, individualized nutrient intake can be calculated based on the known nutrient content of foods.

Verbal education was provided on the approach to completing the FFQ and assistance was offered to those unable to answer the form independently. Completed FFQs were collected by study personnel and sent to the Harvard School of Public Health for nutrient analysis.

Seizure Monitoring

Seizure activity was assessed via continuous video EEG monitoring throughout the inpatient stay. Video EEG is considered one of the most important diagnostic tests for the evaluation of seizures. It allows a provider to visualize an event or symptom that occurs while conducting normal daily activities and sleep. The procedure requires hospital admission with a 3 to 5 day average length of stay. After admission, providers often lower or discontinue ASMs and may introduce stimuli in order to induce seizure activity. The patient wears an EEG transmitter and video cameras provide continuous behavioral observation. Both EEG and video signals are transmitted to a control room where the EEG is reformatted and conducted to a video monitor. The EEG signal and video are displayed simultaneously for on-line observation by EEG technologists and both are recorded on videotape. The goal of testing is to record typical seizures in a safe environment, which provides important information for diagnosis and treatment.

Metabolic Profiling

Metabolic profiling was accomplished using recently developed NoviPlex™ cards. These, Food and Drug Administration (FDA) approved devices produce plasma aliquot discs from a fingerstick. NoviPlex Technology uses advanced membrane systems to pull the plasma from whole blood while leaving the blood cells behind. After application, rapid air-drying stabilizes the sample and the dried plasma discs remain stable at room temperature until analysis is completed.

Study personnel trained subjects to conduct fingersticks for NoviPlex card blood samples utilizing a single-use, contact-activated lancet. Each subject performed one fingerstick daily for blood collection purposes during the course of his or her hospitalization. Pediatric patients performed the fingerstick themselves (if able) or received assistance for a parent or guardian. Careful attention was paid to instruct subjects on use of sterile alcohol wipes to clean the puncture site before the fingerstick and correct lancet positioning in order to minimize risk of infection and pain. Once the lancet was deployed, blood from the single puncture site was applied to two separate NoviPlex cards (NoviPlex Card 1 and NoviPlex Card 2).

As less than 1 mL of blood is required in order to form each plasma aliquot disc, one fingerstick produced enough blood for the separate cards. NoviPlex Card 2 served to confirm the metabolic profiling completed on NoviPlex Card 1 or as a back-up in the event that NoviPlex Card 1 was deemed defective. If the subject did not produce enough blood to collect a second sample, the procedure was aborted and metabolic profiling was completed only on NoviPlex Card 1. A second fingerstick was not pursued.

On the day of admission (NoviPlex Day 0), a non-fasting fingerstick was performed prior to reduction or discontinuation of ASMs to serve as a control sample. Each subsequent day of admission (NoviPlex Day 1-5), subjects were instructed to perform the fingerstick in the morning before eating breakfast. Study personnel rounded on subjects once to twice daily to ensure samples were collected in a fasting state. Fingersticks not performed or peformed following oral

intake were documented in the data collection form. Completed NoviPlex cards were each labeled with the subject ID, date, and NoviPlex Card day (Table 5).

Table 5: NoviPlex™ Card Labeling System		
Day of Admission	**NoviPlex Card Labels**	
Hospital Day 1	Card 1 Day 0	Card 2 Day 0
Hospital Day 2	Card 1 Day 1	Card 2 Day 1
Hospital Day 3	Card 1 Day 2	Card 2 Day 2
Hospital Day 4	Card 1 Day 3	Card 2 Day 3
Hospital Day 5	Card 1 Day 4	Card 2 Day 4
Hospital Day 6	Card 1 Day 5	Card 2 Day 5

Prior to discharge from the hospital, study personnel collected NoviPlex cards and any remaining fingerstick supplies. Completed NoviPlex cards were placed in a sealed envelope and transported to the biochemistry lab by study personnel for metabolomic profiling. Alliquot samples and NoviPlex cards were destroyed after analysis. This data will be used in a subsequent analysis of a larger study looking at metabolomics profiling with a goal of identifying targets for nutrition manipulation.

Variables

Dietary FAs

Results from the Harvard FFQ were reviewed and analyzed for total dietary intake of kilocalories (kcal), fat, PUFAs and SFAs per day. Independent variables for the primary outcome of the study included the following PUFA and SFA intakes in grams per day: total n-3, ALA, EPA, DHA, total n-6, LA, AA, and total SFA, Butyric (C4:0), Caproic (C6:0), Octanoic (C8:0), Decanoic C10:0), Dodecanoic (C12:0), Myristic (C14:0), Palmitic (C16:0) and Stearic (C18:0) acids. SFAs were sub-categorized into groups based on carbon chain length. In our analysis, Butyric (C4:0) and Caproic (C6:0) acids were classified as SCFAs, Octanoic (C8:0), Decanoic C10:0) and Dodecanoic (C12:0) acids as MCFAs and Myristic (C14:0), Palmitic (C16:0) and

Stearic (C18:0) acids as LCFAs. Total daily intake of n-6 and n-3 FAs was also expressed as a ratio of n-6:n-3.

Seizure Presence and Severity

Following discharge, board-certified neurologist and epileptologist, Dr. Arun Swaminathan, reviewed completed EEG reports for each subject and assessed seizure presence and severity. Seizure presence and severity served as dependent variables of the primary and secondary outcomes of the study, respectively. Classification of seizure severity was accomplished by assigning a score to each subject based on seizure activity interpreted from the continuous video EEG (Table 6). A score of 0 indicated no seizure activity was captured during the stay, while a score of 1-4 indicated seizure presence. Single or few brief seizures with minimal impairment of awareness or preserved awareness received a score of 1 while single or multiple complex partial seizures received a 2. A 3 indicated one Tonic Clonic or one Grand Mal Seizure with a score of 4 representing more than one Tonic Clonic or Grand Mal seizure.

Table 6: Seizure Severity Score Key

Score	Seizure Severity
0	No seizure
1	Single or few brief seizures with minimal impairment of awareness or preserved awareness
2	Single or multiple complex partial seizures
3	1 Tonic Clonic or 1 Grand Mal
4	> 1 Tonic Clonic or >1 Grand Mal

Other Covariates

Baseline and clinical data including sex, age, ethnicity, height, weight, and BMI were collected from the electronic medical record during each subject's inpatient stay.

Statistical Analysis

Descriptive statistics were calculated and are provided as means, standard deviations, medians and ranges for continuous variables, and frequencies and percentages for categorical variables. To determine if dietary FA intake (PUFA, SCFA, MCFA and LCFA) could impact presence of seizures, FA intake in subjects with documented seizure presence were compared with FA intake of those without using a Mann Whitney U-test. Univariate and multivariate logistic regression, adjusted for age, sex, BMI and total calorie intake, were leveraged to assess the impact of dietary FA intake on seizure presence. The confounders were chosen a priori based on clinical knowledge of relationships.

Next, the relationship between dietary FA intakes and seizure severity were examined in subjects with documented seizure presence (seizure severity groups 1-4). Mean ranks of dietary PUFA, SCFA, MCFA and LCFA intake, were compared between seizure severity groups using a Kruskal-Wallis test. Any significant findings were corrected by using a Bonferoni post-hoc analysis. Univariate and multivariate linear regression were utilized to evaluate if PUFA, SCFA, MCFA and LCFA were predictors of seizure severity. In an effort to avoid collinearity, individual FAs were evaluated separately in all regression models. Given the nature of the data, univariate and multivariate ordinal logistic regression models for PUFA, SCFA, MCFA and LCFA intake were also explored to determine if dietary FA intake increased or decreased the proportional odds of falling at a higher seizure severity score. Assumptions of regression models were tested to ensure valid statistical tests.

All tests were two-sided and a P-value < 0.05 was considered statistically significant. A power analysis was not calculated as the goal of this study was to collect pilot data. Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS™) software version 26.

CHAPTER 3: RESULTS

Participants

Eighty-five subjects were successfully enrolled into the study. Three subjects were excluded from the final analysis for the following reasons: not completing a FFQ, leaving prior to completion of EEG monitoring, and a FFQ reporting a total daily intake of over 6000 calories per day. Therefore, a total of 82 subjects were included in the analysis (Figure 5). Subject demographics and descriptive statistic are outlined in Table 7.

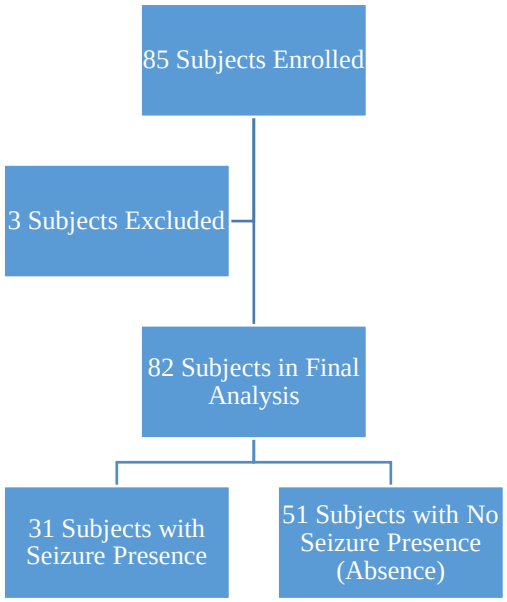

Figure 5: Subject Enrollment, Exclusion and Inclusion in the Final Analysis*. Informed consent was obtained from 85 Subjects. Three subjects were excluded for the following reasons: not completing a FFQ, leaving prior to completion of EEG monitoring, and a FFQ reporting a total daily intake of over 6000 calories per day, providing A total of 82 subjects were included in the final analysis. Of 82 subjects in the final analysis, seizure presence was identified in 31 subjects, while the remaining 51 did not experience a seizure (seizure absence).*

Descriptive Data

Of the 82 subjects included in the analysis, 26 (31.7%) were male with 74 (90.2%) identifying as Caucasian, 4 (4.2%) as African American, 3 (3.1%) as Hispanic and 1 (1.0%) as Asian American. The cohort's median age was 36.5 years with a BMI of 30.5 kg/m^2. During admission, seizure presence was observed in 31 (37.8%) of subjects. The distribution of seizure severity scores among those with seizures include 4 (4.2%) with a score of one, 18 (22.0%) with a score of two, 5 (6.1%) with a score of three and 4 (4.9%) with a score of four.

Table 7: Baseline Characteristics of 82 Subjects Included in the Final Analysis

Categorical Demographics (n=82)		**n (%)**		
Sex				
	Male	26 (31.7)		
	Female	56 (68.3)		
Race				
	Caucasian	74 (90.2)		
	African American	4 (4.2)		
	Hispanic	3 (3.1)		
	Asian American	1 (1.0)		
Seizure Presence				
	No	51 (62.2)		
	Yes	31 (37.8)		
Seizure Severity				
	1	4 (4.2)		
	2	18 (22.0)		
	3	5 (6.1)		
	4	4 (4.9)		
Continuous Demographics (n=82)		**Mean ± SD**	**Median**	**Range**
Age (years)		38.1 ± 14.0	36.5	67
BMI (kg/m2)		31.4 ± 8.6	30.5	38.2
Dietary Intake				
	Calorie, kcals/d	2112 ± 1045.4	1813.5	5412.6
	Protein, gm/d	93.4 ± 57.1	76.5	365.6
	Carbohydrate, gm/d	253.1 ± 120.0	228.0	572.4
	Fat, gm/d	82.3 ± 46.7	72.2	214.5
	Total Omega-3, gm/d	1.8 ± 1.0	1.5	5.1
	Alpha Linolenic Acid, gm/d	1.5 ± 0.8	1.3	4.4
	Eicosapentaenoic Acid, gm/d	0.07 ± 0.1	0.02	0.7
	Docosahexaenoic Acid, gm/d	0.14 ± 0.2	0.07	0.2
	Total Omega-6, gm/d	15.5 ± 8.6	13.7	45.9
	Linoleic Acid, gm/d	14.01 ± 7.7	12.7	41.0
	Arachadonic Acid, gm/d	0.21 ± 0.2	0.16	1.3
	Omega-6:Omega-3, gm/d	8.9 ± 2.2	8.8	15.1
	Total Saturated Fatty Acid, gm/d	29.0 ± 16.9	25.3	82.5
	Short Chain Fatty Acid, gm/d	1.0 ± 0.8	0.8	4.4
	Medium Chain Fatty Acid, gm/d	3.8 ± 2.6	3.2	14.1
	Long Chain Fatty Acid, gm/d	22.7 ± 13.1	19.3	64.7

Median dietary intakes of macronutrients in the total population consisted of 1813.5 calories, 76.5 grams (gm) protein, 228.0 gm carbohydrate and 72.2 gm fat per day. Protein, carbohydrate and fat comprised approximately 15%, 50% and 35% of total daily caloric intake, respectively. Median PUFA intake for the cohort was 1.5 gm of n-3 with 1.32 gm ALA, 0.02 gm EPA, 0.07 gm DHA (Figure 6) and 13.7 gm n-6 with 12.7 gm LA, 0.16 gm AA per day (Figure 7). The overall n-6 to n-3 ratio for the cohort was 8.8:1. Finally, median daily intakes of total SFAs was 25.3 gm, including 0.76 gm as SCFA, 3.2 gm as MCFA and 19.3 gms as LCFA (Figure 8).

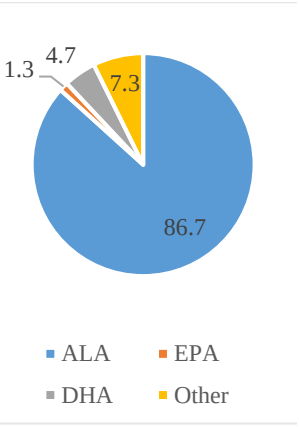

Figure 6: ALA, DHA and EPA as Proportions of N-3 FA intake. *ALA comprised 86.7% with EPA and DHA comprising 1.3% and 4.7%, respectively.*

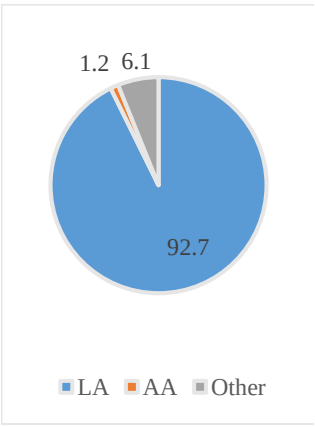

Figure 7: LA and AA as Proportions of the N-6 FA intake*. LA comprised 92.7% with AA comprising 1.5%*

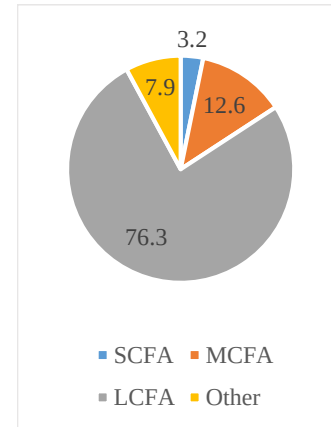

Figure 8: SCFA, MCFA and LCFA as Proportions of SFA Intake*. SCFA comprised 3.2% with MCFA and LCFA comprising 12.6% and 76.3%, respectively.*

Dietary FA Intake and Seizure Presence

Table 8: Baseline Characteristics of Seizure Absence vs. Seizure Presence Groups

		Seizure Absence (n=51)	Seizure Presence (n=31)	
Categorical Demographics		**n (%)**		***p*[a]**
Sex				0.04*
	Male	12 (23.5)	14 (45.2)	
	Female	31 (60.8)	17 (54.8)	
Ethnicity				0.36
	Caucasian	48 (92.3)	26 (83.9)	
	African American	2 (3.9)	2 (6.5)	
	Hispanic	1 (1.9)	2 (6.5)	
	Asian American	0 (0.0)	1 (3.2)	
Continuous Demographics		**Median**		***p*[b]**
Age (years)		36	40	0.61
BMI (kg/m2)		30.67	30.24	0.56
Calorie Intake (kcals/d)		1760.5	1954.5	0.18

[a]Chi Square Test to compare seizure absence and seizure presence groups
[b]Mann-Whitney U Test to compare seizure absence and seizure presence groups
**Statistically significant p-value <0.05*

A Mann-Whitney U test comparing median dietary FA intakes between seizure presence groups (seizure absence vs seizure presence [Table 8]) showed marginally significant differences in median SCFAs (0.65 gm vs 1.01 gm for no vs. yes seizure presence, $p = 0.07$) and MCFAs (2.92 gm vs 3.82 gm for no vs. yes seizure presence, $p = 0.07$) (Table 9). No statistically significant results were observed in the remaining variables (Figure 9).

Table 9: Differenced in Median Dietary FA Intakes Between Seizure Absence and Seizure Presence Groups (n=82)			
Fatty Acid	**Seizure Absence (n=51)**	**Seizure Presence (n=31)**	***p*[a]**
Total Fat, gm/d	71.8	76.0	0.3
Total N-3, gm/d	1.4	1.6	0.3
ALA, gm/d	1.2	1.4	0.5
EPA, gm/d	0.02	0.02	0.3
DHA, gm/d	0.07	0.08	0.28
Total N-6, gm/d	13.7	13.2	0.4
LA, gm/d	12.7	12.4	0.4
AA, gm/d	0.2	0.2	0.5
N-6:N-3, gm/d	8.8	8.9	0.9
Total SFA, gm/d	22.1	26.8	0.2
SCFA, gm/d	0.65	1.01	0.07[+]
MCFA, gm/d	2.92	3.83	0.07[+]
LCFA, gm/d	17.9	20.01	0.2
[a]*Mann-Whitney U Test to compare seizure absence and seizure presence groups*			
[+]*Marginally significant p-value*			

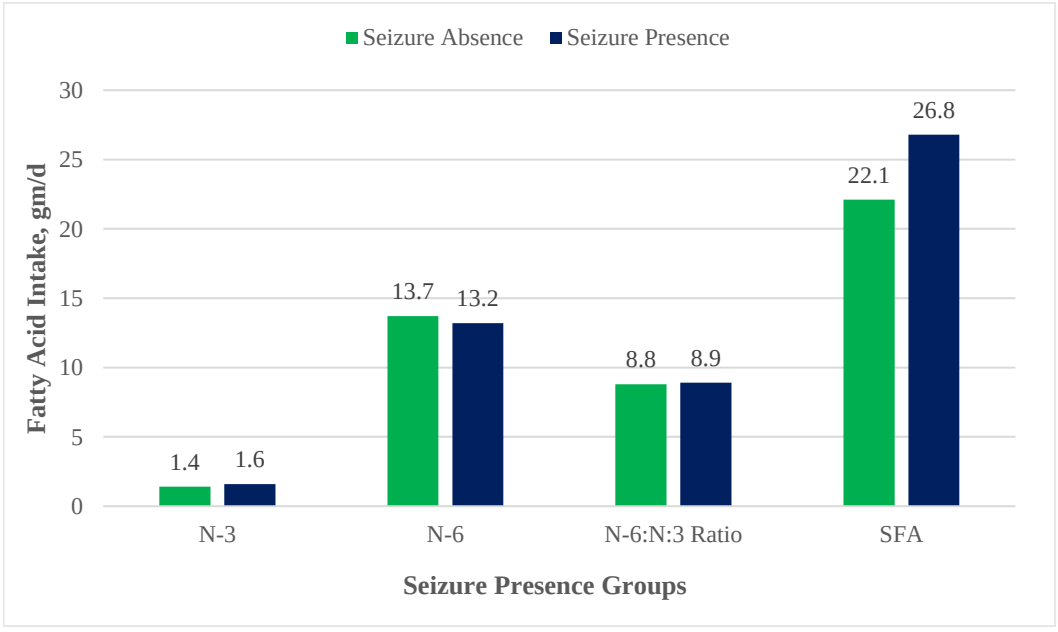

Figure 9: Median Dietary Total, N-3, N-6, N-6:N-3 Ratio and SFA Intakes in Seizure Absence and Seizure Presence Groups. *FA intake (gm/d) represented above corresponding seizure absence and seizure presence group columns (FA: Mann-Whitney-U p-value; n-3: 0.3; n-6: 0.4; n-6:n-3 ratio: 0.9; SFA: 0.2).*

Logistic Regression modeling assumptions were verified and goodness of fit was confirmed using Hosmer Lemeshow ($p = 0.09$). Results of both univariate and multivariate models were unable to show dietary FA intake increased or decreased risk of seizure presence (Table 10).

Table 10: Results of Univariate and Multivariate Logistic Regression between FA Intake and Seizure Presence

Fatty Acid Intake (gm/d)	*Univariate*			*Multivariate*		
	OR	**95% CI**	***p*[a]**	**OR**	**95% CI**	***p*[b]**
Total Fat	1.0	1.0, 1.0	0.26	1.0	1.0, 1.02	0.42
Total n-3	1.2	0.8, 1.8	0.45	0.61	0.2, 1.6	0.33
ALA	1.2	0.7, 2.0	0.54	0.43	0.1, 1.6	0.22
EPA	6.4	0.2, 199.4	0.29	2.77	0.1, 117.4	0.59
DHA	1.7	0.2, 14.3	0.63	0.58	0.5, 7.3	0.67
Total n-6	1.0	1.0, 1.1	0.35	0.96	0.8, 1.1	0.61
LA	1.0	1.0, 1.1	0.37	-0.04	0.8, 1.1	0.62
AA	2.5	0.3, 19.2	0.38	0.46	0.02, 12.1	0.64
N-6:N-3	1.0	0.8, 1.2	0.83	0.99	0.8, 1.2	0.92
Total SFA	1.0	1.0, 1.0	0.13	1.02	1.0, 1.1	0.54
SCFA	1.5	0.9, 2.7	0.15	1.27	0.6, 2.7	0.53
MCFA	1.2	1.0, 1.4	0.1	1.12	0.9, 1.5	0.41
LCFA	1.0	1.0, 1.1	0.17	1.02	0.9, 1.1	0.68

[a]*Univariate Logistic Regression of FA intake and seizure presence risk*

[b]*Multivariate Logistic Regression of FA intake and seizure presence risk, adjusted for age, sex, BMI and total calorie intake*

Dietary FA Intake and Seizure Presence

Results of the Kruskal-Wallis test showed there were no significant differences in FA intake between seizure severity groups (Table 11, Figure 10). A post-hoc analysis was not performed due to lack of significant results.

Table 11: Differences in Median Dietary FA Intakes among Seizure Severity Groups

Fatty Acid Intake (gm/d)	Seizure Severity Score Groups				p^a
	1 (n=4)	2 (n=18)	3 (n=5)	4 (n=4)	
Total N-3	1.11	1.61	1.59	1.61	0.6
ALA	0.99	1.39	1.34	1.3	0.68
EPA	0.00	0.03	0.03	0.02	0.3
DHA	0.04	0.09	0.15	0.1	0.51
Total N-6	9.8	13.73	19.15	16.15	0.43
LA	8.61	12.62	18.45	14.56	0.43
AA	0.16	0.16	0.22	0.15	0.84
N-6:N-3	8.36	8.87	8.34	10.08	0.52
Total SFA	23.79	26.32	27.73	26.44	0.9
SCFA	0.95	1.03	1.08	0.58	0.9
MCFA	3.64	4.11	4.08	2.74	0.88
LCFA	18.98	19.57	23.82	22.21	0.89

[a]Kruskal-Wallis test to compare across all seizure severity categories
Group 1: Single or few brief seizures with minimal impairment of awareness or preserved awareness; Group 2: Single or multiple complex partial seizures; Group 3: 1 Tonic Clonic or 1 Grand Mal seizure; Group 4: >1 Tonic Clonic or >1 Grand Mal seizure.

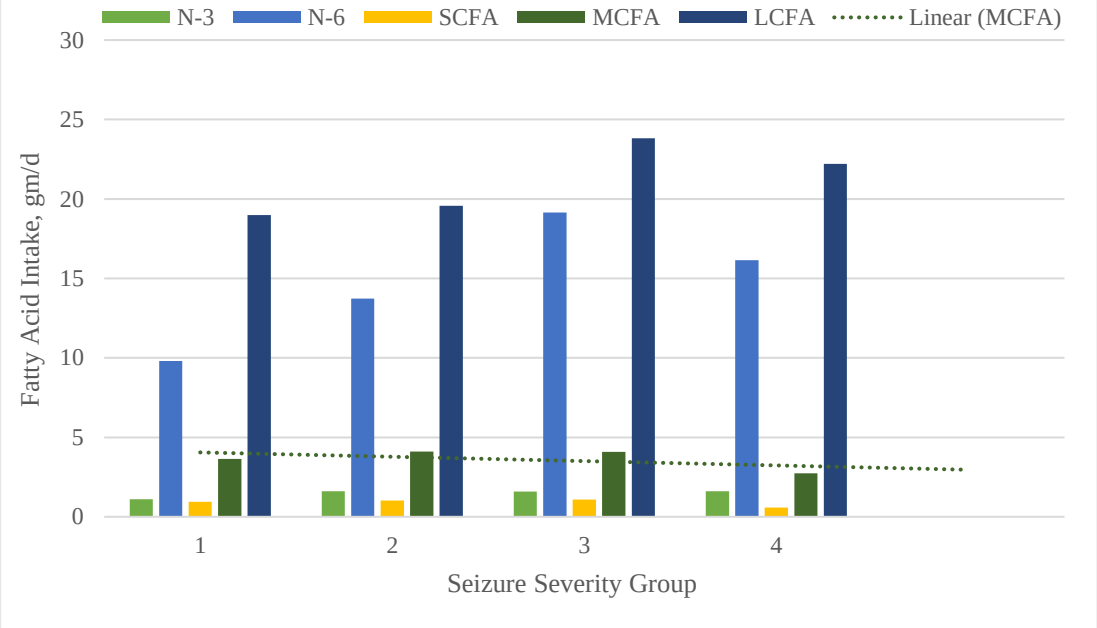

Figure 10: Median Daily FA Intake (gm/d) by Seizure Severity Score Group. *Group 1: Single or few brief seizures with minimal impairment of awareness or preserved awareness; Group 2: Single or multiple complex partial seizures; Group 3: 1 Tonic Clonic or 1 Grand Mal seizure; Group 4: >1 Tonic Clonic or >1 Grand Mal seizure. Dotted line represents the trend toward significance of the inverse linear relationship between MCFA intake and seizure severity score (β = -0.11, p = 0.16).*

Tests of assumptions for linear regression showed that this method was an appropriate fit for modeling the data (Figures 11, 12). No significant associations between dietary FA intake and seizure severity scores were observed using univariate linear regression (Table 12). After adjusting for the relevant confounders of age, sex, BMI and total calorie intake, results remain insignificant. However, a trend toward significance was seen with the relationship between MCFA intake and seizure severity ($p = 0.16$) (Table 12). Univariate and multivariate ordinal logistic regression models violated statistical assumptions. Therefore, data were unable to provide insights on the impact of dietary FAs on the proportional odds of falling into a seizure severity group.

Table 12: Results of Univariate and Multivariate Linear Regression between FA Intake (gm/d) and Seizure Severity Score (n=31)

Fatty Acid Intake (gm/d)	*Univariate*			*Multivariate*	
	R	**β**	***p*[a]**	**β**	***p*[b]**
Total N-3	0.21	0.00	0.91	-0.23	0.55
ALA	0.00	0.00	0.98	-0.38	0.41
EPA	-0.02	-0.02	0.9	0.64	0.58
DHA	0.13	0.74	0.49	-0.2	0.83
Total N-6	0.02	0.09	0.91	0.03	0.52
LA	0.01	0.08	0.68	0.04	0.4
AA	0.1	0.01	0.6	-1.12	0.38
N-6:N-3	-0.04	-0.13	0.83	0.1	0.22
Total SFA	0.19	0.07	0.31	-0.02	0.35
SCFA	-0.04	-0.00	0.81	-0.29	0.2
MCFA	-0.17	-0.16	0.36	-0.11	0.16[§]
LCFA	-0.14	-0.04	0.45	-0.02	0.5

[a] *Univariate Linear Regression of FA intake predicting seizure severity*

[b] *Multivariate Linear Regression of FA intake predicting seizure severity, adjusted for age, sex, BMI and total calorie intake*

[§] *Trend toward significance*

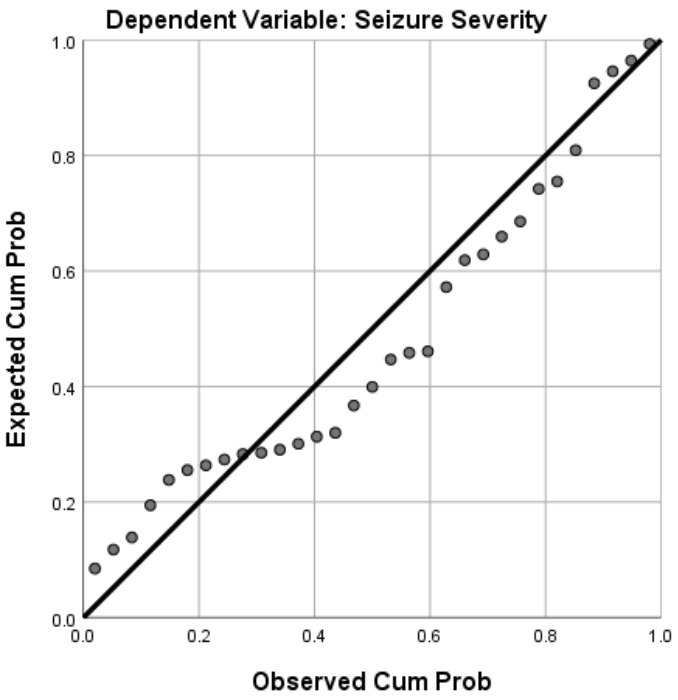

Figure 11: Normal P-P Plot (observed vs. expected cumulative distribution of the standardized residual) of multivariate linear regression for MCFA

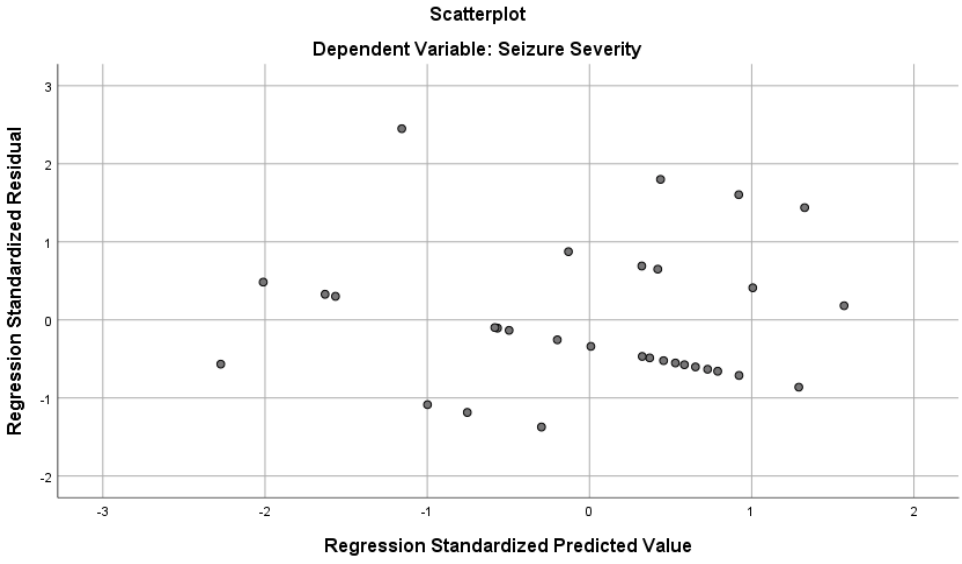

Figure 12: Homoscedasticity Represented by a Scatterplot of Predicted vs. Residual Values from Multivariate Linear Regression for MCFA

CHAPTER 4: DISCUSSION

Key Results

Published research suggests increased dietary intake or supplementation of n-3 FA, SCFA and/or MCFA may elicit anticonvulsant properties through one or more of the previously described theorized mechanisms. Further, data indicate increased LCFA intake, n-6 intake and/or a high n-6:n-3 ratio could result in an overexpression of cytokines, leading to a decrease in seizure threshold and an increase in spontaneous seizure frequency.

To the best of our knowledge, this is the first study to examine the impact of dietary PUFA and SFA intakes on seizure presence and severity in a cohort of subjects admitted to an EMU. In this analysis of a prospective cohort, we show increased dietary MCFA may be associated with decreased seizure severity in individuals with epilepsy. Additionally, a FFQ provides unique insights into the long-term effects of dietary intake on seizure outcomes.

Dietary Intake of the Cohort

In our final analysis, the cohort consumed adequate median intakes of calories and protein, equating 20.7 kcal/kg and 0.9 g/kg, respectfully with a median cohort BMI of 30.5 kg/m^2.

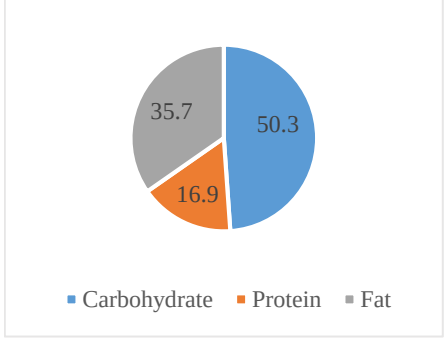

Figure 13: Carbohydrate, Protein and Fat as Proportions of the Total Median Daily Calorie Intake. *Carbohydrates comprised approximately 50% with protein and fat comprising 15% and 35%, respectively.*

Macronutrient distributions indicate dietary intake is representative of a typical Western diet pattern. Here, fat provided approximately 35% of calories with 50% from carbohydrates (Figure 13). In comparison, KDTs consistently provide greater than 50% of total calories per day from fat with less than 20% from carbohydrate. Therefore, we can assume that results in this study are reflective of the influence of individual FAs rather than a KDT. Although KBs

may have been produced from MCFA consumption, we do not anticipate high circulating levels in the plasma.

Daily median total n-3 intake of our sample was 1.5 gm/d, with 1.3 gm/d (87%) of that total as ALA. Although a low n-3 intake is common in a Western diet pattern, cohort median intakes of ALA were above the AI as set by the IOM for adult females between the ages of 19 and 50. (Figure 14). Median intakes of EPA and DHA in this study were 0.02 and 0.07 gm/d, respectively with a combined intake of 0.09 gm/d. While we cannot account for the endogenous production of EPA and DHA intake from ALA, we can assume conversion is very low (0-4%) and likely did not contribute largely to overall availability in the body. Current recommendations, as proposed by the World Health Organization (WHO) [250], for combined intake of EPA and DHA are 0.25-0.5 gm/day in healthy individuals and is thought to be much higher in those with chronic inflammatory conditions. Therefore, although these individuals met the IOM recommendations for n-3, they only consumed 36% of the lower end of threshold for these FAs that are vital in the brain.

Total dietary n-6 intake of our cohort was 13.7 gm/d with 12.7 gm (93%) of total intake consumed as LA and 0.16 gm/d as AA. LA intake met the IOM AI recommendation of 12 gm/d for women between the ages of 19 and 50 (Figure 14). Median total intake of n-6 and n-3 FAs resulted in an n-6:n-3 ratio of 8.8:1. While the n-6:n-3 ratio of our cohort was above the recommendation of 5:1, it fell far below the average ratio of a human consuming a Western diet which has been estimated at approximately 16:1. Generally, the higher ratios seen in this diet pattern are due to high concentrations of n-6 FAs in meats, eggs and processed foods made with soy oils, in combination with low intakes of n-3 containing nuts, fish and vegetable oils.

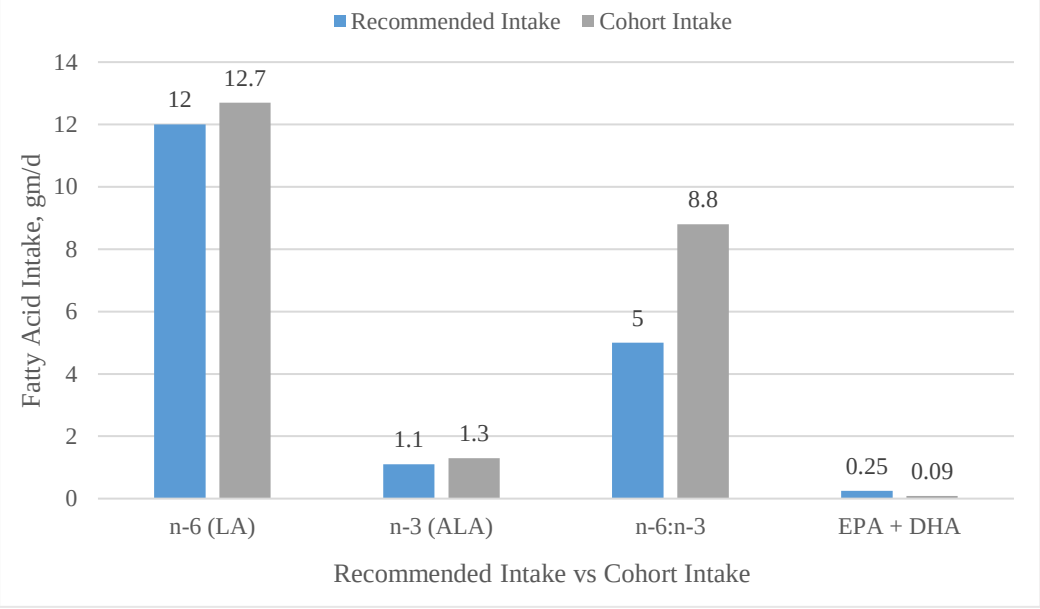

Figure 14: Recommended vs Cohort Dietary PUFA Intakes, gm/d. *Recommendations for n-6 (LA) and n-3 (ALA) based on AIs for adult females between 19 and 50 as proposed by the IOM. Recommendations for n-6:n-3 of 5:1 based on the recommendation by Simopoulas et al. [65]. Recommendations for combined EPA and DHA intake of 0.25 gm/d from the WHO [250].*

Although SCFAs, MCFAs and LCFAs are considerably different in both structure and function, they are often thought of a single SFA group. As previously discussed, there are currently no governmental recommendations for daily SFA intake as they are not seen as essential to the prevention of chronic diseases. However, recent findings, which show specific SFAs are beneficial in multiple disease processes from obesity and diabetes to neurological diseases, may require recommendations to be formed. With a median total fat intake of 72.2 gm/d, our cohort consumed 25.29 gm/d, or 35%, of fat calories as SFA. Here, intake was considerably higher than the 10% of fat calories per day recommended by the American Heart Association.

Dietary Intake of FAs across Seizure Absence and Seizure Presence Groups

When comparing dietary FA intake in subjects with seizure absence against those with seizure presence, no statistically significant differences were seen in any PUFA or LCFA intake and seizure activity groups. However, there was a marginally significant difference in dietary intakes of SCFA and MCFA between seizure groups (both $p = 0.07$), with higher intakes of SCFA and MCFA in the seizure presence group. Due to the ability of SCFAs to inhibit HDACs and decrease inflammation along with MCFAs ability to inhibit AMPA receptors, we may suspect increased dietary intake of both SFAs to increase seizure thresholds and reduce seizure occurrence [123, 144, 175, 176, 182, 210]. Yet, contrary to the aforementioned literature, data in this analysis may be initially interpreted as individuals consuming higher amounts of SCFAs and MCFAs experience seizures.

Upon further evaluation, however, the marginally significant difference in dietary SCFA and MCFA intakes are likely more reflective of the overall variation in dietary intake between seizure presence groups rather than an effect of the FAs themselves. While no statistically significant differences in median calorie and SFA intake are seen among seizure absence or presence groups ($p = 0.18$ and $p = 0.16$, respectfully), a clinically significant difference was observed. Median calorie intake was 194.5 kilocalories, or a substantial 10% higher in the seizure presence group. Median intake of SFA was a total of 4.7 gm higher in the seizure presence group with SCFA and MCFA contributing 0.36 gm/d and 0.91 gm/d to that total, respectfully. Although the median intake of total fat was only 4.2 gm/d higher in the seizure presence group, median intake of SFA was 4.7 gm/d higher, indicating a disproportionally high amount of the increased fat consumed included SFA (Figure 9). Interestingly, although median calorie intake was 10% higher in the seizure presence group, there was extremely little variation in n-3 and n-6 FAs intakes. In fact, median intakes of EPA, DHA and AA were nearly identical between groups.

Dietary FA Intake and Risk of Seizure Presence or Seizure Absence

In this analysis, univariate and multivariate logistic regression models were unable to show dietary FA intake increased or decreased risk of seizure activity in this cohort. Small variation in PUFA intake along with a disproportionally high SFA intake in the seizure presence group likely contributed to the null results of the logistic regression model. However, results were likely also influenced by the amounts of FAs consumed in the seizure presence group.

Previous research suggests appropriate n-3 dose and adequate duration of administration are integral to observe the therapeutic effect of the FA in people with epilepsy. While consistent n-3 intakes of greater than 1.1 to 3.2 gm/d have been proposed, an optimal dose is currently unknown and clinical trials have produced inconsistent results [216]. Recently, data have indicated that EPA and DHA may be responsible for many of the beneficial effects seen from n-3 FAs in neurological diseases. EPA and DHA serve as precursors to SPM, which are responsible for the endogenous mediation of inflammation. Decreased concentrations of plasma SPM have been found in individuals with neurological disease compared to healthy controls, possibly implying that these individuals utilize more SPM in an attempt to decrease the negative effects of neuroinflammation [173]. DHA has also specifically been shown to reduce neuronal hyperexcitability by suppressing conductance of currents in neuronal cells. As previously discussed, up to 60% of lipids incorporated in neural membranes consist of DHA. However, decreased consumption of n-3 PUFAs results in a partial replacement of DHA by AA in the neuronal cell membrane, likely eliminating the potential anticonvulsant mechanism [75]. Therefore, adequate substrate must be available to saturate the neural cell membrane and contribute to the pool of SPM available to decrease neuroinflammation. Many studies indicate a sustained intake of a least one gm of DHA per day is needed to impact neurological outcomes. Therefore, although median n-3 intake is above the minimum threshold suggested by Auvin et al.,

perhaps the proportion of that intake from DHA or the conversion of ALA to DHA was too low to stimulate a protective effect [216].

While marginally significant differences of SCFAs and MCFAs in seizure activity groups were expected to be due to variation in intake, logistic regression modeling appears to confirm this assumption. In the multivariate model, total SFA, SCFA, MCFA and LCFA intake was unable to predict seizure activity, as evidenced by each 95% confidence interval for odds ratios (OR) containing the null (one). The 2019 publication by Citraro et al., notes unpublished data have indicated that a NaB dose of 30 mg/kg only minimally modifies seizure activity. Median SCFA intake of the seizure presence group was 1.01 gm/day or approximately 11.5 mg/kg [240]. Although we cannot account for endogenous production of SCFAs in the intestine, we may suspect that intake is not high enough to drastically impact outcomes.

Direct inhibition of AMPA-receptor activity has been well established as an effective therapeutic mechanism in focal seizures and generalized tonic–clonic seizures. In fact the antiepileptic drug perampanel acts directly on AMPA receptors but at a different site from MCFA [251]. Mean serum concentrations of decanoic acid (C10:0) in patients on a MCTKD have been reported as 157 μmol/L. In the work by Chang et al., data indicate that 100 μmol/L decanoic acid (C10:0) results in a 3-fold increase in the inhibition of the AMPA receptor [144]. The report by St-Pierre et al. found provision of MCFA with a carbohydrate-rich diet could dose-dependently increase corresponding plasma levels after ingestion [111]. Here, providing 20 mL, or approximately 18.5 gm, of MCFA produced an 80-90 μmol/L increase after a single dose. Median intake of MCFAs in the seizure presence group was 3.8 gm/d, which likely corresponds to a relatively low serum concentration. While this intake may be beneficial in inhibiting AMPA receptors, there is currently a paucity of data to indicate a MCFA dose needed in order to influence clinical outcomes.

N-3 PUFAs, DHA, SCFA and MCFA are all associated with anti-seizure properties. Consequently, a plausible explanation for our inability to observe the ability of FA intake to predict seizure activity is the simple fact that dietary intake wasn't high enough to elicit anti-seizure attributes.

Dietary Intake of the FAs across Seizure Severity Groups

In subjects with seizure presence, no statistically significant differences were seen in FA intake among seizure severity groups. However, clinical differences may provide insights on the relationships between dietary FA intake and seizure severity.

The median n-6:n-3 ratio in subjects experiencing a seizure severity score of 4 was 10.1:1, which was higher than those receiving a score of 1 (8.36:1), 2 (8.87:1) or 3 (8.34:1). The n:6-n:3 ratio is of particular importance in the brain due to the competitive absorption of the two essential FAs. As previously described, small changes in dietary intake of FAs can alter brain lipid composition. Even a small increase in n-6 consumption may result in higher levels of brain AA and lower levels of DHA. Further, the higher n-6 intake and competitive absorption may negate the potential for beneficial effects to be seen by n-3 FAs and potentially increase dosage recommendations even more. This may be one explanation as to why seizure severity group 4 did not see beneficial effects of consuming higher amounts of n-3 FA, EPA and DHA (1.61, 0.02, 0.1 gm/d, respectively) as compared to group 1 (1.11, 0.00, 0.04 gm/d, respectively).

Another interesting clinical difference was the intake of n-6 PUFAs and LCFAs across seizure severity groups. Median intakes of n-6 and LCFAs were higher in seizure severity group 4 (SCFA: 16.15 gm/d; LCFA: 22.21 gm/d) than in group 1 (SCFA: 9.8 gm/d; LCFA: 18.98 gm/d) (Figure 15). High intakes of these FAs are highly prevalent in a standard Western diet and both have been shown to increase neuroinflammation by triggering the release of cytokines in the brain. As increased cytokine levels lead to a decrease in seizure threshold and an increase in

spontaneous seizure frequency [159], it is entirely possible that increased n-6 and LCFAs perpetuated seizure activity and resulted in increased seizure severity in the group.

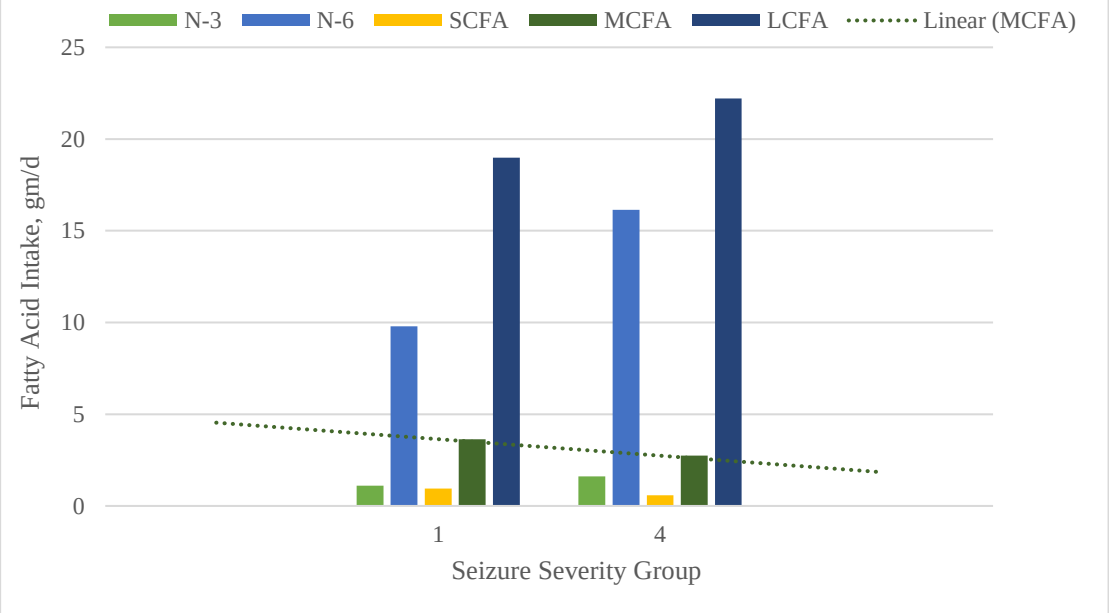

Figure 15: Median Daily FA Intake (gm/d) of Seizure Severity Groups 1 and 4. *Group 1: Single or few brief seizures with minimal impairment of awareness or preserved awareness; Group 4: >1 Tonic Clonic or >1 Grand Mal seizure. Dotted line represents the trend toward significance of the inverse linear relationship between MCFA intake and seizure severity score (β = -0.11, p = 0.16).*

In contrast, median intakes of SCFAs and MCFAs were lowest in subjects experiencing the most severe seizures (seizure severity group 4). Median intakes of SCFAs in seizure severity groups 1, 2, 3 and 4 were 0.95, 1.03, 1.08 and 0.58 gm/d, respectively, while median MCFAs in groups 1-4 were 3.64, 4.11, 4.08, and 2.74 gm/d, respectively. From these results, we may hypothesize that SCFA or MCFA above a certain threshold may inhibit the progression of multiple, severe seizures. Higher intakes may be especially significant in this cohort, where ASMs have been reduced or stopped for continuous EEG assessment of seizure activity during admission.

Together, these clinical observations suggest a potential relationship between FA intake and seizure severity. Even with dietary intakes far below what may be considered a therapeutic dose, the influence of FA intake appeared possible in subjects with seizure presence.

Dietary FA Intake Predicting Seizure Severity

When assessing the ability of FAs to predict seizure severity, we observed a trend toward significance in the ability of dietary MCFA intake to predict seizure severity in the multivariate linear regression model ($\beta = -0.11$, $p = 0.16$). In previous discussion, we note MCFA dose-dependently increased in the serum of subjects on a carbohydrate-rich diet and AMPA receptors were inhibited 3-fold in mice with a serum level greater than 100 μmol/L [111, 144]. Here, results may reflect the impact of increased dietary MCFA intake on AMPA receptor inhibition and the ability to decrease seizure severity.

In this analysis, no significant results were identified in the remaining PUFAs or SFAs in univariate or multivariate modeling. However, the direction of these FAs in the multivariate regression provide interesting insights into relationships with seizure severity. An inverse linear relationship was observed in total n-3, ALA, DHA, AA, total SFA, SCFA and LCFA. The FAs EPA, total n-6, LA and the n-6:n-3 ratio resulted in a positive linear relationship. Once again, the majority of the FAs follow the trends seen in research on FA intake and seizure activity. We anticipate a cohort with increased variability of FA intake would further elucidate the strength and direction of these relationships.

PUFAs, SFAs and KDTs as Epilepsy Treatment

In the fasting state, plasma FAs increase almost entirely from hydrolysis of triglycerides, while FAs are metabolized for energy in the absence of carbohydrates in a KDT. Historically, the production and metabolism of KBs has been considered fundamental to the anti-seizure effects of

a KDT or starvation. However, more recent research questions this position, prompting investigations into alternative theories.

Both KDTs and individual FAs have been associated with potential mechanisms of action to inhibit seizure activity or increase seizure thresholds (Table 13). Namely, the ability to alter neurotransmitters, activate PPARγ, mediate neuroinflammation and inhibit HDACs [252]. While all four KDTs have been shown to be effective, the adverse effects, low retention rates and unknown impact on long-term health outcomes often prevent their use as first-line therapy. Identification of anti-seizure mechanisms of action in KDTs could allow for diet liberalization, increased quality of life and a true treatment for epileptogenesis. Therefore, it will be important to distinguish if the favorable effects of a KDT are truly a function of KB production, increased accumulation and metabolism of FAs, or perhaps both.

Table 13: Common Theorized Mechanisms of Action between KDTs, N-3s, SCFAs and MCFAs

Theorized Mechanism of Action	Dietary Component Catalyst			
	KDT	N-3	SCFA	MCFA
Alter Neurotransmitters	X	X	X	X
Activate PPARγ	X	X	X	X
Inhibit HDAC	X		X	
Mediate Neuroinflammation	X	X	X	

There is a well-established fact that there are both responders and non-responders to KDT. Responders are often classified as subjects with a greater than 50% improvement in seizure activity from baseline within a specified time period. With the exception of specific metabolic disorders, prediction of the response to therapy is currently unknown [253, 254]. One potential explanation for the unpredictable response could be that patient's circulating FA levels aren't high enough to benefit from therapy.

Although the brain represents only 2% of body weight, it consumes 20-23% of whole-body energy in healthy individuals [252]. However, we may suspect that brain energy requirements of an individual with epilepsy may be much higher due to increased activity. Previous research by Wang et al. reported lower levels of FAs after seizures, indicating their increased consumption during seizure occurrence [44]. As increased consumption likely results in decreased levels of FAs in the brain, the ability of a KDT to increase serum levels of PUFAs and SFAs may be associated with its anti-seizure effects. We may speculate lower levels of FAs, specifically in the brain, may decrease many of the aforementioned theorized mechanisms of action [44]. Inadequate circulating levels within a specified timeframe could be due to the patient's baseline dietary pattern, increased brain consumption due to seizure activity or an inability to follow a KDT for a prolonged period of time. As seen in the research of n-3 PUFA supplementation in pre-clinical and clinical epilepsy research, length of supplementation is often considered paramount to ensure efficacy [216, 226, 228, 233, 235]. Although data often suggest a minimum length of supplementation of at least three months for n-3 and MCT, many studies evaluating the efficacy of KDTs begin assessing for a decrease in seizure activity at the one to three month mark [11, 13, 14, 26, 255, 256]. Assessment at these early intervals may not allow enough time to increase the concentration of FAs in the brain. Consequently, some patients may be labeled as a "non-responder" before the possibility of experiencing therapeutic benefit.

Another cause for variable response to KDT could be that specific anti-seizure mechanisms of action are more proficient among particular seizure types and epilepsy etiologies. If so, the anti-seizure mechanism provoked by the FA content of a KDT may or may not be beneficial in the patient. Specific FAs are more prevalent in some KDTs than others. For example, a cKD is often characterized by high intakes of LCFAs due to its reliance on butter and cream as major fat sources. However, both of these foods also contain a considerable amount of SCFAs. In fact, butter supplies 3-4% of its fat content as butyric acid which may prevent seizure activity by

inhibiting HDACs or decreasing intestinal inflammation. Alternatively, the MCTKD provides 30-60% of total energy intake from MCFAs, which has specifically been proven to inhibit AMPA receptors. We must also consider the possibility that a subset of seizures are directly inhibited by metabolism of KBs. In this case, the presence of KBs from any KDT may be beneficial to the patient and necessary for treatment.

Data presented in this study suggests dietary PUFA and SFA intake may have the potential to influence seizure activity independent of KB production and substantiates the need for further research in this area. Assessing the impact of adequate DHA, SCFA and/or MCFA provision over a prolonged period of time will be necessary to achieve a better understanding of the anti-seizure properties of FAs. Furthermore, analysis of the metabolic signature of seizures via comprehensive metabolic profiling will be an important step in elucidating how dietary intake can impact seizure outcomes. Identifying the metabolic signature of seizures could allow clinicians to treat epilepsy through precision nutrition by providing FAs with theorized mechanisms of action to reverse the metabolic derangement resulting in seizure activity. We posit the provision of targeted nutrition interventions will increase dietary therapy retention with less restrictive requirements, allowing adequate time for FA accumulation in order to produce anti-seizure effects. We also anticipate improved “responder” rates with dietary treatments prescribed based on theorized mechanisms of action and the metabolic signature of the seizure.

Study Strengths and Limitations

The primary strength of this study was the use of the Harvard FFQ to evaluate the impact of FA intake on seizure activity in the absence of ketosis. Furthermore, this study is the first to establish baseline PUFA and SFA intakes in a cohort of subjects with epilepsy. Another major strength of this study was the use of video EEG monitoring to assess seizure presence and activity rather than subjective reporting.

Limitations of this study have been recognized. We had a limited sample size of subjects experiencing seizures (n = 31) with an unequal distribution across seizure severity score groups. The single-site study was completed at a Midwestern hospital with a largely adult, female and Caucasian cohort. The lack of geographic and racial diversity in the study population likely decreased diet variability among participants. Although epilepsy was suspected in all individuals admitted to the EMU, the diagnosis may have been ruled out during admission after continuous EEG monitoring. Therefore, it must be taken into account that some individuals in the seizure absence group may have been unable to experience a seizure.

Conclusion

Epilepsy continues to affect millions of individuals around the world every day. Although medical treatments may improve seizure symptoms, epileptogenesis is not inhibited and many patients do not achieve adequate seizure control. Specific dietary FAs have potential anti-seizure properties that warrant further exploration as epilepsy treatment. A minimally invasive, targeted dietary treatment could decrease the devastating side effects of epilepsy, reduce the staggering cost of treatment and improve quality of life for patients and their families.

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