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A Review of a Ketogenic Diet In the Treatment of Autism Spectrum Disorder

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LOMA LINDA UNIVERSITY
School of Behavioral Health
in conjunction with the
Faculty of Graduate Studies

A Review of a Ketogenic Diet In the Treatment of Autism Spectrum Disorder

by

Eugene Reznik

A Project submitted in partial satisfaction of
the requirements for the degree
Doctor of Psychology

June 2024

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Each person whose signature appears below certifies that this doctoral project in his opinion is adequate, in scope and quality, as a doctoral project for the degree Doctor of Psychology.

_____, Chairperson
Richard E. Hartman, Professor of Psychology

_____
Aarti Nair, Assistant Professor of Pyschology

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CONTENT

ABBREVIATIONS

ASD	Autism Spectrum Disorder
ABA	Applied Behavioral Analysis
ADHD	Attention Defecit Hyperactivity Disorder
ADOS	Autism Diagnostic Observation Schedule
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ATEC	Autistic Treatment Evaluation Test Scores
BHB	Beta-hydroxybutyrate
BTBR	Black and Tan Brachyury
CARS	Childhood Autism Rating Scale
CD	Control Diet
DIR	Developmental Individual-Difference Relationship
EL	Epileptic
GFCF	Gluten Free Casein Free
KD	Ketogenic Diet
KO	Knockout
LCT	Long Chain Triglyceride
LGIT	Low Glycemic Index Diet
MADS	Modified Atkins Diet
MCT	Medium Chain Triglyceride
MIA	Maternal Immune Activation
SD	Standard Diet

TEACCH	Treatment and Education of Autistic and Related Communication Handicapped Children
VPA	Valproic Acid
WT	Wildtype

ABSTRACT OF THE DOCTORAL PROJECT

A Review of a Ketogenic Diet In the Treatment of Autism Spectrum Disorder

by

Eugene Reznik

Doctor of Psychology, Department of Clinical Psychology
Loma Linda University, June 2024
Dr. Richard E Hartman, Chairperson

Autism Spectrum Disorder effects millions of people every year, however pharmacological and behavioral treatments remain limited. The need for adjunctive therapies such as diet invervention that target autism spectrum disorder symptoms Is needed now more than ever. A connection between a ketogenic diet, which is high in fat and low in carbohydrates, and autism spectrum disorder can be made as the diet has shown potential in ameleriotating common comorbidities within the autism spectrum disorder population such as metabolic dysfunction, gut-microbiome dysfunction, medication resistant epilepsy, and various psychiatric disorders. Hence, this review focuses on the results and methods of various animal and human studies that implicate the benefits of a ketogenic diet in the treatment of autism spectrum disorder. The data suggest that implementation of a ketogenic diet improves core and associated psychiatric symptoms of autism spectrum disorder such as repetitive behaviors, social behaviors, communication, anxiety, speech, hyperactivity, and cognition.

CHAPTER ONE

INTRODUCTION

Autism spectrum disorder (ASD) is neuro developmental disorder characterized by impaired social interaction, problems with verbal and nonverbal cues, and severely restricted activities and interests (American Psychiatric Association, 2013). Clinicians can diagnose autism reliably starting at 20 months to 24 months of age (Guthrie et al., 2012). The prevalence of autism is about one in 160 individuals and the rate for males is significantly higher in the general population (Elsabbagh et al., 2012). Notably, the disorder affects individuals across social and ethnic backgrounds (American Psychiatric Association, 2013). While the etiology of autism is unknown, evidence suggests that its pathogenesis involves both environmental and genetic components (Buxbaum et al., 2001). Individuals with ASD have higher rates of numerous other psychiatric illnesses or comorbidities in comparison to the general population (Joshi et. al 2010; Joshi et. al 2012; Simonoff et. al 2008). The cost associated with the treatment of autism spectrum disorder is rapidly growing and the need for adequate treatment that is effective, sustainable, and accessible is needed more than ever (Blaxil et al., 2021).

Pharmacologic intervention can address ASD patients who face significant, and often debilitating, social and cognitive deficits (Kent et al., 2012). A review of various pharmacological interventions demonstrates the efficacy and promise of stimulants, psychotropics, antipsychotics, and antidepressants in the treatment of autism spectrum disorder (LeClerc et al., 2015). However, drug interventions are not without potential ramifications; for example, a longitudinal study of adolescent patients with autism showed that a commonly used antidepressant fluoxetine may cause side effects such as

appetite suppression, depression, and hyperactivity (Fatemi et al., 1998). In addition, a commonly used anti-psychotic medication such as risperidone have shown to cause significant weight gain in the autism population (Hellings et al., 2001). Medication often targets psychiatric comorbidities associated with autism spectrum disorder and not its core deficits (Ruskin et al., 2017). Hence the need for other treatment alternatives that target autism spectrum core symptomatology without harmful side effects such as behavioral therapy should be considered (Zachor et al., 2007).

An alternate avenue of treatment for ASD, behavioral therapy, has been shown to be highly successful in alleviating the symptoms of autism such as emotion regulation and adaptive behaviors, and may be even more effective than medication alone (Orinstein et al., 2014). Historically, early intensive home-based behavioral therapies were often the most widely used and the need for early intervention and diagnosis was emphasized (Lovaas 1987). Currently, there are numerous special education behavioral therapies such as the Developmental approach, the Developmental Individual-Difference Relationship (DIR), The Treatment and Education of Autistic and Related Communication Handicapped Children (TEACCH) and Applied Behavioral Analysis (ABA) (Zachor et al., 2007). ABA is often the gold standard for treatment as it targets the core behavioral symptoms of autism spectrum disorders (Zachor et al., 2007). However, like behavioral therapies in the past Its time commitment of 30-40 hours per week and cost is often a heavy burden on families (Zachor et al., 2007). While medication and behavioral approaches may be seen as necessary to combat symptoms associated with ASD, they do come with concrete limitations and adjunctive therapies need further exploration (Fatemi et al., 1998; Hellings et al., 2001; Lovaas 1987; Zachor et al., 2007).

Although adjunctive diet interventions for ASD have been overall lesser explored, a more commonly studied diet intervention for autism spectrum disorder is the gluten free and casein free (GFCF) diet (El-Rashidy et al., 2017; Knivsberg et al., 2002; Whiteley et al., 2010). In one randomized control study, the use of a casein free and gluten free diet demonstrated a reduction in autistic behavior in all ten children after diet intervention (Knivsberg et al., 2002). In another double-blind control study a study found that there was evidence of sustained behavioral improvement that plateaued, but the study concluded that without a placebo they may have not been able to control for confounding variables in the participants' environment (Whiteley et al., 2010). Another study showed that the GFCF diet and a ketogenic diet both improved psychometrically reliable measures of autism: Childhood Autism Rating Scale (CARS) and Autistic Treatment Evaluation Test Scores (ATEC) (El-Rashidy et al., 2017). Thereby, there is some evidence to suggest that nutritional intervention and specifically the ketogenic diet may be a feasible means to combat core behavioral deficits often seen in ASD (El-Rashidy et al., 2017; Knivsberg et al., 2002; Whiteley et al., 2010). Hence, this review will first outline the metabolic, gut microbiome, and treatment resistant epilepsy overlap between a ketogenic diet and autism spectrum disorder; followed by the results and methods of various animal and human studies that implicate the benefits of a ketogenic diet in the treatment of autism spectrum disorder.

CHAPTER TWO

WHAT IS THE KETOGENIC DIET?

The ketogenic diet is a low carbohydrate and high fat diet that allows the body to use ketone bodies (3-hydroxybutyrate and acetoacetate) as the preferred fuel source (Politi et al., 2011). When there is an imbalance of glucose availability and increased fatty acids in the blood, the initial phases of a ketogenic state begin to impact metabolic processes (Politi et al., 2011). The liver will take the excess fatty acids and convert them into ketone bodies or primarily acetoacetate and beta-hydroxybutyrate (BHB) (Politi et al., 2011). For the human body to use fatty acids instead of glucose an individual must generally consume a diet that consists of a 4:1 or 3:1 ratio which means that for every 3 or 4 grams of fat one must consume 1 gram of carbohydrates and proteins combined (Wirrell 2008). There are several variations of the classic ketogenic diet such as a medium chain triglyceride diet, modified Atkins diet, and John Radcliffe diet (Wirrell 2008). Animal models oftentimes show great efficacy with even higher or more strict ratios of 6:1 (Wirrell 2008).

Ketogenic Diet and Metabolism

Autism spectrum disorders do not stand alone but often have underlying metabolic and gastrointestinal pathologies. For example, there are many metabolic dysfunction disorders that overlap with an ASD phenotype such as Phenylketonuria, Profound Biotinidase Deficiency, mitochondrial dysfunction, and disorders of purine metabolism (Zecavati et al., 2009). Mitochondria are key cellular machinery that the body's metabolic system uses as its new fuel source of ketones (Danial et al., 2013).

Hence, when a ketogenic diet is implemented, the body must more efficiently use mitochondria to perform cellular respiration that primarily utilizes fatty acids to produce adequate amounts of ATP to sustain bodily functions (Danial et al., 2013). The therapeutic potential of ketone metabolism revolves around their unique metabolic pathways involving the mitochondria and ability to be used as fuel by the brain (Veech 2004). Improved metabolic understanding of the ketogenic diet has led to an increase in research surrounding its potential therapeutic effects in the pathogenesis of autism (Ahn et al., 2014; Castro et al., 2016; El-Rashidy et al., 2017; Evangeliou et al., 2003; Herbert & Buckley 2013; Lee et al., 2018; Mantis et al., 2009; Mu et al., 2019; Ruskin et al., 2013; Ruskin et al., 2017a; Ruskin et al., 2017b; Spilioti et al., 2013;Verpeut et al., 2016; Zarnowska et al., 2018). The impact of the ketogenic diet on ASD metabolic pathways is still in its infancy but a connection is reasonable because a review showed children with autism were more likely to have mitochondrial dysfunction than typically developing children (Giulivi et al., 2010). Additionally, a recent study examined how the ketogenic diet contributes to the restoration of mitochondrial morphology (Ahn et al., 2020). Hence metabolic dysfunction leads to gastrointestinal problems within ASD because cellular machinery and metabolic pathways dictate the proper functionality of the gastrointestinal system (Molly & Manning-Courtney 2003). Gastrointestinal problems such as diarrhea, constipation, and abdominal pain are often highly comorbid in the ASD population (Molloy & Manning-Courtney 2003). Gastrointestinal within the ASD population further exacerbate ASD symptomatology as the gut-brain axis theory is implicated (Adams et al., 2011).

Ketogenic Diet and the Gut Microbiome

Autism spectrum disorder individuals have different gut microbiomes than typically developing individuals, which may be at play for further exacerbating autism spectrum symptomatology (Adams et al., 2011). For example, the stool samples of 58 autism spectrum disorder children were assessed in comparison to typically developing children (Adams et al., 2011). Within this sample gastrointestinal issues highly correlated with severity of autism and short chain fatty acids were much lower in autistic children (Adams et al., 2011). Additionally, the autistic children had lower levels of species of Bifidobacterium ($p < 0.01$) and higher levels of species of Lactobacillus ($p < 0.001$), but similar levels of other bacteria and yeast using standard culture growth-based techniques (Adams et al., 2011). The study concluded that higher severity in autism may be at play for gastrointestinal issues, which then leads to a further exacerbation of autism spectrum symptoms (Adams et al., 2011). This study is paramount to the idea that autism symptomatology needs to be further explored regarding its effects on gut microbiota (Adams et al., 2011).

In another experiment, a maternal immune activation (MIA) mouse model of ASD was used to showcase the unique microbiota environment that also displays an altered serum metabolic profile (Hsiao et al., 2013). The MIA mouse model is a valid representation of ASD core symptomatology because the mice are deficient in social and communicative behavior and have high levels of repetitive behavior (Malkova et al., 2012). Oral treatment of MIA mouse offspring with the human commensal Bacteroides fragilis corrects gut permeability, alters microbial composition, and ameliorates defects in communicative, stereotypic, anxiety-like, and sensorimotor behaviors (Hsiao et al., 2013). Hence the study shows that the gut microbiome has significant implications in

modulating ASD behaviors and that proper intervention of a specific microbe or B. fragilis (often modulates many metabolites) can have beneficial effects on ASD symptomatology in an MIA mouse model (Hsiao et al., 2013). Therefore, the microbiome composition of ASD individuals needs to be further analyzed as interventions targeting microbes in ASD individuals may help with symptomatology (Hsiao et al., 2013).

Another experiment used the BTBR mouse model of ASD to create two feeding groups, a standard diet group and a ketogenic diet group, to better understand the possible effects of a ketogenic diet on the gut microbiome (Newell et al., 2016). These BTBR mice have reduced communication, low sociability on several tests, and elevated self-directed behavior that are analogous to core ASD symptomatology (Newell et al., 2016). This experiment highlighted that the gut microbiota compositions of BTBR mice were in fact different compared to control mice, which may provide some good confidence for using BTBR models to exam gut-brain relationships in autism (Newell et al., 2016). Additionally, after a ketogenic diet was fed to the BTBR mice their overall gut microbiome decreased causing an antimicrobial effect (Newell et al., 2016). Also, the ketogenic diet showed its ability to improve the ratio of low Fimicutes to Bacteroidetes, a common ASD gut phenotype characteristic (Newell et al., 2016). Thereby preliminary animal model studies, although limited, show that a ketogenic diet may play a significant role in improvement of the gut microbiome in those with ASD (Adams et al., 2011; Hsiao et al., 2013; Newell et al., 2016).

Ketogenic Diet and Treatment Resistant Epilespy

The ketogenic diet has been studied extensively in the treatment of epilepsy, a common comorbidity in the ASD population (Freeman et al. 1998; Ijff et al., 2016; Lambrechts et al., 2016; Neal et al., 2008; Packer et al., 2016; Rogawaski et al. 2016). A randomized control study implementing the ketogenic diet in a pediatric population has been shown to be highly effective in decreasing seizure frequency. For example, 59% of a group of 150 children experiencing seizures ages one to 16 years old, had a higher than 50% reduction in seizures after implementing a ketogenic diet (Freeman et al., 1998). The study showed issues with dropout rate as tolerability of the diet regarding being restrictive was an issue (Freeman et al., 1998). The authors note that if families of patients did not see a reduction in seizure control, then the diet was more likely deemed restrictive (Freeman et al., 1998).

Although diet restrictiveness may be an issue with implementing a ketogenic diet, adjusting the diet to fit the needs of subjects is possible. For example, a randomized control study separated children with treatment resistant epilepsy participants into two groups: classical long chain triglyceride (LCT) ketogenic diet and a medium chain triglyceride (MCT) ketogenic diet (Neal et al., 2008). Generally, side effects of the diet were minimal and ameliorated by adjusting the diet but did include constipation, lack of energy, and hunger (Neal et al., 2008). Overall results showed that 38% of individuals in the diet group had a 50% reduction in seizure frequency and 7% had a 90% reduction, in comparison to controls (Neal et al., 2008). The study highlights that variability amongst studies regarding reduction in seizure frequency, may be due to selection bias because choosing children who may be more receptive to diet intervention increases efficacy

results (Neal et al., 2008). The study did not highlight differences in efficacy and tolerability between those in the medium chain versus long chain ketogenic diet groups, which needs further exploration (Neal et al., 2008).

There remains debate over the efficacy and tolerability of different diets that are versions of high fat low carbohydrate protocols. Specifically, one publication argues that a diet based in long chain fatty acids is not as effective as diets that place an emphasis on medium chain fatty acids (Rogawski 2016). The biochemical underpinnings of this article states that a medium chain fatty acid ketogenic diet blocks AMPA receptors, which is a receptor implicated in the pathogenesis of seizure disorders (Rogawski 2016). Therefore, ketogenic diets high in medium fatty chain acids should be considered more effective in treating drug resistant epilepsy (Rogawski 2016).

A ketogenic diet implemented in additional randomized control studies involving pediatric epilepsy and dogs, also had additional psychological benefits (Ijff et al., 2016; Packer et al., 2016). For instance, in a study a total of fifty participants that ranged from children to adolescents with refractory epilepsy were split into two groups: ketogenic diet or control (Ijff et al., 2016). Those in the ketogenic diet group, after a four month follow up, showed improvements in social emotional functioning, higher work productivity, improved seizure control, and improved cognition (Ijff et al., 2016). Thereby, this study shows promise in not only treating seizures in children with refractory epilepsy but also sheds light on behavioral and cognitive improvements while on a ketogenic diet. Additionally, another six-month double blind control study implemented a ketogenic diet based on medium chain triglycerides in epileptic dogs who were also comorbid with attention defecit hyperactivity disorder (ADHD) (Packer et al., 2016). The results of the

study noted that there was a symptom reduction in seizures, fear related activities, and behavior in the epileptic dogs (Packer et al., 2016). The reduction in symptomatology not only from seizures but fear related activities and ADHD behavior build the case that a ketogenic diet may be important for targeting multiple pathologies (Packer et al., 2016).

A ketogenic diet is often criticized for gastric distress but there is evidence to suggest otherwise (Lambrechts et al., 2016). In another randomized control study of refractory epilepsy patients aged one to 18, the efficacy and tolerability of a ketogenic diet was analyzed (Lambrechts et al., 2016). Results showed that those participants treated with a ketogenic diet showed a 50% decrease in seizure occurrence when compared to baseline (Lambrechts et al., 2016). Gastrointestinal issues did not differ much compared to baseline levels and gastrointestinal issues may be further alleviated if the diet is better fine-tuned (Lambrechts et al., 2016). Thereby, a ketogenic diet may prove to be both effective and tolerable in children with various psychiatric and epilepsy pathology and should be considered in the treatment of ASD (Freeman et al. 1998; Ijff et al., 2016; Lambrechts et al., 2016; Neal et al., 2008; Packer et al.; Rogawaski et al. 2016).

CHAPTER THREE

KETOGENIC DIET AND ANIMAL MODELS OF AUTISM SPECTRUM DISORDER

The relationship between ASD behavioral symptoms and a ketogenic diet intervention has been studied in varying animal models (Ahn et al., 2014; Castro et al., 2016; Mantis et al., 2009; Ruskin et al., 2013; Ruskin et al., 2017a; Ruskin et al. 2017b; Verpeut et al., 2016). One of the first studies to showcase a ketogenic diet's effects on behavioral symptomatology was shown in a male rat model of Rett syndrome: a disorder that closely mimics autism spectrum disorder (Mantis et al., 2009). In this experiment twelve male wildtype (control) and eighteen male Rett mice were selected and placed on a "chow diet" (standard diet) that comprised of a low fat, high-carbohydrate diet in pretrial testing so that a baseline for social behaviors could be established without any diet intervention (Mantis et al., 2009). On the 11^{th,} day both control and male Rett mice were separated into several groups for diet intervention: standard diet (SD), Ketocal diet (ketogenic diet based on soy and comprised of 4:1 ratio of fats to carbohydrates and protein) or restricted ketogenic diet (KD), and restricted standard diet (restricted SD: restricted calories in a standard diet) (Mantis et al., 2009). Male mice in the wildtype group and male Rett mice groups were matched by bodyweight and a 17 hour fast was implemented before diet intervention to maximize a similar metabolic starting point (Mantis et al., 2009). All male mice underwent social testing after one month of diet intervention (Mantis et al., 2009). The open field test measured exploratory behavior through total distance in periphery, total distance in center, number of entries in the

periphery, number of entries in the center, and number of rearing events (Mantis et al., 2009). The light-dark test captured exploration of a novel environment by measuring latency time of male Rett mice going from a dark to light environment, with more time needing to transition meaning decreased exploration of a novel environment (Mantis et al., 2009). Additionally, total time in the light environment during the light-dark test was measured with more time in the light meaning increased exploration of a novel environment (Mantis et al., 2009).

Results showed that male Rett mice on a restricted KD significantly increased the number of entries into the center and periphery as well as rearing events when compared to male Rett mice on SD diet, an indication of increased exploratory behavior ($p < 0.05$) (Mantis et al., 2009). Additionally, male Rett mice on a restricted SD diet had significantly increased the number of entries into the periphery and center as well as total distance into the center when compared to male Rett mice on a SD diet ($p < 0.01$). Results showed that male Rett mice fed a KD and calorie restricted SD diet emerged into the light significantly earlier and had increased total time in the light in comparison to male Rett mice fed a SD unrestricted diet ($p < 0.05$). Thereby this study shows that a KD (4:1 ratio) has the potential to improve social behaviors such as exploratory behavior and exploration of novel environments, deficits often associated with ASD in a male mouse model of Rett syndrome (Mantis et al., 2009). Of note, this study also highlights that a calorie restricted diet that is not necessarily a ketogenic protocol can be just as effective if not more effective in the alleviation of social deficits seen in male Rett Syndrome mice that often mimic those seen in ASD (Mantis et al., 2009).

Another useful animal model that has an autism-like phenotype is the juvenile BTBR mouse model (Ruskin et al., 2013). These BTBR mice do not present as epileptic so ketogenic diet (KD) intervention behavioral effects may be analyzed independently of its anti-seizure effects (Ruskin et al., 2013). At five weeks of age BTBR mice were randomly assigned to control diet (CD) and keto diet groups and testing occurred at eight and ten weeks after dietary treatment (Ruskin et al., 2013). To measure social behavior three phases of social testing were implemented in a 3-chamber apparatus (Ruskin et. al, 2013). The first phase consisted of a social measurement called "side bias" and looked at if BTBR mice preferred a particular side at baseline but did not measure anything related to social behavior (Ruskin et. al, 2013). The second phase introduced a new BTBR mice and measured "sociability" as increased time spent with new BTBR mouse in the target chamber (Ruskin et. al, 2013). The third phase introduced another new BTBR mouse and measured preference for social novelty by time spent in target chamber containing new mouse or increased total frontal contact with new BTBR mouse (Ruskin et. al, 2013). Self-repetitive behavior was quantified by measuring self-grooming within the three-chamber sociability test and alone in an empty cage (Ruskin et al., 2013). Communication was assessed by social transmission of a food where there is one demonstrator BTBR mouse that is fasted for 18 hours and presented with a prearranged flavor of food or "trained" food. The demonstrator mouse is later returned to its home cage with another BTBR "observer" mouse, who has not been exposed to any food, for 30 minutes (Ruskin et al., 2013). The BTBR "observer" mouse is then fasted for 18 hours and must choose between the "trained" flavor food and "untrained" flavor food.

Increased social communication is measured as BTBR "observer" mice who choose the "trained" food (Ruskin et al., 2013).

Results showed that in phase 2 of the experiment, KD-fed BTBR mice were significantly more social when given a choice between an empty chamber and another mouse-containing chamber in comparison to CD fed BTBR ($p < 0.001$) (Ruskin et al., 2013). In phase 3, a KD did not influence preference for social novelty, however, BTBR mice fed the KD did spend significantly more time in frontal contact with mouse-containing cages ($p < 0.001$) (Ruskin et al., 2013). Also, a KD decreased self-directed repetitive behaviors (grooming) in social contexts of the experiment (phase 2 and phase 3) but did not have any effect on grooming in phase 1 of the experiment when other mice were not present (Ruskin et al., 2013). Lastly, there is evidence to support that a KD improved the construct of social communication as KD fed BTBR mice ate significantly more of the "trained" food ($p < 0.001$) (Ruskin et al., 2013). This study was able to showcase the amelioration of ASD symptoms through a KD such as repetitive behaviors in social context, sociability, and social communication in a BTBR mouse model of autism but lacked evidenced for reducing repetitive behaviors in isolation and increasing social novelty behaviors (Ruskin et al., 2013).

Another useful animal model for ASD was the pre-natal valproic acid (VPA) rodent model. In one experiment Sprague-Dawley Dams (type of rat) were administered valproic acid (experimental dose of 500mg/kg) or saline (control) on gestational day 12.5 (Ahn et al., 2014). The rats treated with valproic acid had offspring that showed significant reduction in number of play initiation/attacks, a deficit that is analogous to social deficits seen in ASD symptomatology (Ahn et al., 2014). The offspring of the rats treated with

valproic acid, and the offspring of the rats treated with saline were both placed on a standard diet (details regarding the macromolecule ratios for the standard diet were not given) and a ketogenic diet (6:1 fat to carbohydrates and protein) at postnatal day 21 (Ahn et al., 2014). There were four groups of rats that were used for a statistical analysis of a social test: Saline with standard diet, Saline with ketogenic diet, VPA with standard diet, and VPA with ketogenic diet (Ahn et al., 2014). Animal social testing began at 35 and 38 days of life so diet intervention occurred for about two weeks (Ahn et al., 2014). One such test consisted of placing two rats that were previously cage mates into plexiglass boxes for a period of ten minutes (Ahn et al., 2014). Increased play behavior consisted of increased attacks to the nape (back of the neck) and the response of the other playmate that is categorized into: complete rotations, partial rotations, horizontal rotations, and evasions (Ahn et al., 2014). It is important to quantify responses as engaging in play is an important development milestone often deficient in those with autism spectrum disorder (Ahn et al., 2014).

Results showed that the KD was able to recover some of the social interactions that were altered in the prenatal VPA model of ASD as there was a significant main effect of the diet ($p < 0.05$) (Ahn et al., 2014). VPA rats had an increase rate of complete rotations or defensively responding, an effect that the VPA rats treated with KD did not help resolve (Ahn et al., 2014). Additionally, the KD did increase the probability that a play partner had a partial rotation, regardless of treatment ($p < 0.05$) (Ahn et al., 2014). Lastly although not significant there was a strong main effect identified toward the KD group normalizing the percentage of times VPA animals evade (Ahn et al., 2014). This study showcases that a strict ketogenic diet (6:1) is effective in reversing some of the negative

social symptomatology of VPA rats that are often analogous to the social deficits observed in ASD, however a KD did not significantly influence all aspects of reciprocal play (Ahn et al., 2014).

Another model that aimed to replicate the neurodevelopmental deficits seen in autism was the Engrailed 2 null mice model (KO): deletion of the En2 gene produces behavioral deficits consistent with ASD symptomatology (Verpeut et al., 2016). Specifically, KO mice display diminished social interaction, decreased play, reduced social sniffing, and depressive behaviors that parallel much of the core ASD symptomatology. Only male KO and wildtype mice (WT) in this experiment were fed a lard-based ketogenic diet (4:1 ratio of fats to carbohydrates and protein) or a control diet (1:2:7 fats to protein to carbohydrates) from post-natal developmental period days of 21-60 (Verpeut et al., 2016). Social testing began on day 62 and focused on the three-chamber social test that incorporates all three phases of testing as previously mentioned (Ruskin et al., 2013). It is important to note that that when testing began mice were placed back on standard chow to mitigate some of the acute effects of the KD (Verpeut et al., 2016).

Results showed that there was an increase in male KO's social behaviors over all three phrases and reduced self-grooming (repetitive behaviors) when fed a KD as compared to male KO's fed a CD ($p < 0.05$) (Verpeut et al., 2016). More specifically, male KO mice fed a KD had a significant increase frontal contact time ($p < 0.05$). Similarly, to the BTBR mice in a previous experiment, phase 3 of the social testing results showed that male KO mice fed an SD spent significantly more time and had more contact with a novel mouse but those fed a KD did not (Verpeut et al., 2016). This study shows that pro-social behaviors and reduction in repetitive behaviors with a standard

ketogenic diet (4:1) intervention is possible in a male KO mouse model but that a KD did not influence preference for social novelty (Verpeut et al., 2016).

Another study also used the VPA model of ASD and separated the offspring of the mice into four groups: control group with standard diet, control group with ketogenic diet, VPA group with standard diet, and VPA group with ketogenic diet (Castro et al., 2016). Additionally, only male offspring were used as subjects as the female offspring of the parental mice treated with VPA were euthanized (Castro et al., 2016). The mice were fed the diet intervention at 21 days of life and completed the diet for seven weeks (Castro et al., 2016). The mice in the experiment were fed a moderate ketogenic diet of 3:1 or fats to carbohydrates and proteins, while the control group had a diet that consisted of 1:2:5 ratio of fats to proteins to carbohydrates (Castro et al., 2016). The rats were then assessed for stereotypic behaviors using the marble burying test, anxiety based on self-grooming within the three-chamber test, and social behaviors using the three-chamber test (Castro et al., 2016). It is important to note that there are differences between this experiment's three chamber test and those previously mentioned, as this experiment also used objects (Ruskin et. al 2013; Verpeut et al. 2016).

Results showed that marble burying which measured stereotyped behaviors was significantly reduced in VPA mice fed a KD in comparison to VPA mice fed a standard diet ($p < 0.05$) (Castro et al., 2016). Regarding self-grooming behavior measuring the construct of anxiety, VPA mice fed a KD had a reduction in time spent self-grooming ($p < 0.05$) (Castro et al., 2016). Additionally, results showed that for the three-chamber social test VPA mice that were fed a KD were able to completely prevent the effects of VPA. Specifically, their time spent exploring novel mice 1 and object, as well as time

spent with novel mice 1 and object, were like controls ($p < 0.05$) (Castro et al., 2016). In another social test measuring social novelty preference, VPA mice treated with a KD spent a more significant time exploring another novel mouse than a known mouse ($p < 0.05$) (Castro et al., 2016). Overall, this study showed the positive potential of a KD on some of the core behavioral aspects of autism particularly repetitive behaviors and social behaviors, in addition to comorbidities such as anxiety within a VPA model of ASD (Castro et al., 2016).

In another study, sex differences and strictness of KD played an integral role when looking at a ketogenic diet and its effects on ASD behavioral symptomatology (Ruskin et. al, 2017a). This study used a ketogenic diet on epileptic (EL) mice: a mouse model of autism with an unknown etiology that often complements other ASD models because these mice develop epilepsy, a common comorbidity with ASD (Ruskin et. al, 2017a). At five weeks of age EL mice were fed three different diets: regular diet (0.08:1 fat to carbohydrates), a strict keto diet (6.6:1 fat to carbohydrates), and a less strict keto diet (3.0:1 fat to carbohydrates) (Ruskin et. al, 2017a). It's important to note that male mice were only given the strict 6.6:1 KD and females were given both the strict 6.6:1 KD and moderate 3.0:1 KD (Ruskin et. al, 2017a). Also, all testing was done at eight to nine weeks of age, so diet intervention lasted between three to four weeks (Ruskin et. al, 2017a). To test sociability mice were given the three-chamber test in three phases as previously described in another mice study (Ruskin et al., 2013).

Results showed that feeding with KD formula highly increased sociability in female mice ($p < 0.001$) (Ruskin et. al, 2017a;). Also feeding with a 6.6:1 KD increased sociability in male mice ($p < 0.05$) (Ruskin et. al, 2017a). Results showed that the 6.6:1

KD female mice showed significant preference for novel mice ($p < 0.05$), although the effect size is small so this result may need to be interpreted with caution (Ruskin et. al, 2017a). Also, female mice in the 3:1 KD group did not show increased preference for novel mice ($p > 0.05$) (Ruskin et. al, 2017a). Additionally, male mice in phase 3 of the experiment may trend towards novel mice but there was no statistical significance ($p = 0.053$) (Ruskin et. al, 2017a).

Another test for sociability in the three-chamber test was social contact and results showed that for either KD sociability significantly increased for females for both phases 2 and 3 ($p < 0.001$) (Ruskin et. al, 2017a). However, there was no significant result for male mice when fed with a 6.6:1 KD in either phase 2 or phase 3, which was generally low and in the same range as CD fed female mice ($p > 0.05$) (Ruskin et. al, 2017a;). Therefore, male mice preferred to spend time in the chamber with a stranger mouse but did not interact directly with novel or familiar mice ($p > 0.05$) (Ruskin et. al, 2017a;). Another domain that was measured by this study was the core autistic symptom of repetitive behaviors (Ruskin et. al, 2017a). Self-grooming was the repetitive behavior used to measure this construct and results were mixed based upon environment and sex (Ruskin et. al, 2017a). For example, in a solitary environment male EL mice who were fed a KD significantly reduced self-grooming activity ($p < 0.05$), but female mice fed KD did not exhibit reduced self-grooming activity ($p > 0.05$) (Ruskin et. al, 2017a). In the 3-chamber social test the repetitive behavior of grooming was also measured (Ruskin et. al, 2017a). Specifically, females in phase 1 who were alone and were fed KD showed significant reduction in grooming ($p < 0.05$) (Ruskin et. al, 2017a). Females in phase 2 did not show a marked reduction in grooming activity when fed with a 6.6:1 KD but did

show marked reduction when fed a 3:1 KD (Ruskin et. al, 2017a). Males did not show marked reduction in grooming activity for either phase 1 or phase 2 of the experiment ($p > 0.05$) (Ruskin et., al 2017a). Results showed that CD-fed female EL mice preferred the trained flavor, and this preference was not altered when fed with either KD (results not shown on graph), hence no significant change ($p > 0.05$) (Ruskin et al., 2017a). Similarly, there was no significant results for social communication and a 6.6:1 KD for male EL mice ($p > 0.05$). This study shows the nuances of both dose dependent, context dependent, and sex dependent variables as it pertains to a ketogenic diet and helping ameliorate core ASD symptomatology in an EL mouse model (Ruskin et., al 2017a).

In another study, a maternal immune activation (MIA) model of ASD was used to show the potential benefits of a ketogenic diet in ASD symptomatology (Ruskin et., al 2017b). Specifically, all offspring of MIA mice were fed until week five of development when they were separated into two groups: control diet or a ketogenic diet that consisted of 6.6:1 ratio (fats to carbohydrates and protein) (Ruskin et., al 2017b). The experiment had three groups: mice fed a control diet, MIA mice fed a control diet, and MIA mice fed a ketogenic diet (Ruskin et., al 2017b). Social testing on mice occurred after three to four weeks of diet intervention (Ruskin et al., 2017b). Social behaviors were tested using the three-chamber test, single chamber test, social transmission of food preference, and quantifying social contact time within the three-chamber test as previously seen in BTBR and EL mice models (Ruskin et al., 2013; Ruskin et., al 2017a). Repetitive behavior was measured by quantifying self-grooming both in isolation and in a social setting (Ruskin et., al 2017b).

In phase 2 of the experiment measuring sociability, results showed that KD fed male mice were significantly social as the KD reversed the lack of sociability found in control diet fed male MIA mice ($p < 0.05$). However, this effect did not occur with female MIA mice ($p > 0.05$) (Ruskin et., al 2017b). In phase 3 of the experiment, treatment groups did not significantly differ from controls as female and male mice all preferred social novelty somewhat equally ($p > 0.05$) (Ruskin et al., 2017b). Results pertaining to social contact in phase two and phase three showed that KD treatment significantly increased social contact in both female and male mice MIA mice when compared to control mice ($p < 0.05$;) (Ruskin et., al 2017b). A test of social communication showed that males and females fed a KD did not perform significantly better than controls ($p > 0.05$) (Ruskin et., al 2017b). Regarding repetitive behavior, KD fed male MIA mice had a complete reversal of increased repetitive behaviors, in phase one and phase two of the three-chamber test, such that MIA mice were no different than control ($p < 0.05$) (Ruskin et., al 2017b). Additionally, male MIA mice showed a partial reversal in the one chamber test by KD feeding for repetitive behaviors (Ruskin et., al 2017b). MIA female mice did not have elevated self-grooming in either the three-chamber test nor single chamber tests ($p > 0.05$). However, female MIA mice fed a KD showed a reduction in self-grooming in phase two of the three chamber test ($p < 0.05$) (Ruskin et., al 2017b).This study shows the nuances of both context dependent and sex dependent variables as it pertains to a ketogenic diet and helping ameliorate core ASD symptomatology in an MIA mouse model (Ruskin et., al 2017b).

Discussion

Overall, there are limited animal studies that examine a ketogenic diet and its effects on ASD behavioral symptomatology and future studies would benefit from a shared protocol to maximize study design and results (Ahn et al., 2014; Castro et al., 2016; Mantis et al., 2009; Ruskin et al., 2013; Ruskin et al., 2017a; Ruskin et al., 2017b; Verpeut et al., 2016). Although various animal models of ASD can add breadth to the overall benefits of a ketogenic diet, one may argue that testing hypothesis on one model at a time is more valid. It may be useful to match animal subjects by weight and implement a fast before commencement of diet intervention to standardize baseline metabolic systems. Studies should be careful to not reintroduce a standard diet before social testing as this may skew results and mice may not be in a full state of ketosis. The age of the mice when given a ketogenic diet needs to be kept more standardized as all studies gave mice a ketogenic diet at various days of the juvenile period (3-8 weeks) and some testing even began in the adult phase of the mice (after 8 weeks). Studies should state their standard diet macromolecule ratios, which could impact standardization of baseline metabolic levels. Future animal studies may benefit from more diet intervention groups based upon human studies that compare the difference in efficacy of a restricted calorie diet, a restricted carbohydrate diet, a gluten free casein free diet with a ketogenic diet, and medium chain versus long chain 3:1, 4:1, and 6:1 ketogenic diets. Of note based on human studies implementing a strict ketogenic diet (6:1) may be impossible as many struggle to maintain a classic ketogenic diet (3:1 or 4:1). The length of diet intervention on animal models varied from four to eight weeks and should also be kept standard as one can argue that staying in ketosis longer may produce greater effect. All future studies

would benefit from incorporating both female and male mice for analysis of ASD behavioral symptoms, as gender differences were observed.

Importantly, creating behavior testing that appropriately measures as many behaviors related to ASD symptomatology in a standardized manner is necessary in future animal study design. Thus far repetitive behaviors were not measured by few of the experiments, a core ASD symptom. Additionally, repetitive behaviors were measured based upon self-grooming in five experiments and marble burying in another. Also, self-grooming was used as a measure of anxiety and in contexts that involved isolation and other mice. Hence, future experiments may benefit from a standardized protocol that measures repetitive behaviors in both a social and isolation context, which incorporate quantification of both marble burying and self-grooming behaviors.

All experiments measured pro social behaviors through a variety of methods such as the three-chamber test, one chamber test, open field test, light-dark latency test, and play behavior in cage. These tests were able to capture social behavior constructs such as exploratory behavior, sociability, social communication, play behavior, and social novelty. Future experiments should consider incorporating all methods of social testing and keeping a consistent testing battery for a more comprehensive and standardized social behavioral analysis. Addtionally, based upon the results of animal testing further exploration should be warranted for all social testing as discrepencies in results were observed based upon animal model, sex, social or isolation context, and diet protocol. Conclusively, all animal studies showed the potential positive benefits of a ketogenic diet for the behavioral symptoms of autism and should call for more human research (Ahn et

al., 2014; Castro et al., 2016; Mantis et al., 2009; Ruskin et al., 2013; Ruskin et al., 2017a; Ruskin et., al 2017b; Verpeut et al., 2016).

CHAPTER FOUR

KETOGENIC DIET AND HUMAN STUDIES OF AUTISM SPECTRUM DISORDER

The ketogenic diet in the treatment of autism spectrum disorder does not have a plethora of robust randomized control human studies but in the existing research the benefits of a ketogenic diet are observed (El-Rashidy et al., 2017; Evangeliou et al., 2003; Herbert & Buckley 2013; Lee et al., 2018; Mu et al., 2019; Spilioti et al., 2013; Zarnowska et al., 2018;). A pilot study had thirty autism spectrum children ages 4-10 who were all treated with haloperidol six months before the start of a ketogenic diet intervention (Evangeliou et al., 2003). It is important to note that Childhood Autism Rating scale (CARS) scores did not change with pharmacological treatment and no behavioral treatments were given during or before the ketogenic diet intervention (Evangeliou et al., 2003). Pharmacological treatment was still used for some participants during the diet intervention (Evangeliou et al., 2003). Initial CARS scores for the twenty-eight of the children were in the severe range, while the other two participants had mild to moderate scores (Evangeliou et al., 2003). Children underwent extensive metabolic testing and a glucose loading test, to better inform researchers of underlying metabolic issues (Evangeliou et al., 2003). Rather than use a strict ketogenic diet researchers opted for the John Radcliffe diet: 30% of energy as medium-chain triglyceride oil, 30% as fresh cream, 11% as saturated fat, 19% as carbohydrates, and 10% as protein (Evangeliou et al., 2003). This diet was given for a period of six months, with continuous administration 4 weeks at a time and that included a break from dieting every four weeks for a period of

two weeks (Evangeliou et al., 2003). Patient ketone levels were tracked daily using ketone strips and extensive lab tests were performed every four-week interval of the diet intervention (Evangeliou et al., 2003).

Results showed that twenty-three patients tolerated the diet (76.6%) and of those patients five discontinued between weeks 4-10 due to lack of behavioral improvement (Evangeliou et al., 2003). Of note, patients who either discontinued or did not tolerate the diet were of the more severe CARS scores (Evangeliou et al., 2003). Most importantly, results showed that the overall average improvement of CARS scores was a decrease of 4.77 units ($p < 0.001$). More specifically, two boys experienced a reduction of 12 units in their CARS scores and could attend school for non-mentally handicapped children (Evangeliou et al., 2003). Another eight patients (six boys and two girls) experienced an average improvement of 8-12 units on their CARS score and eight more patients (four boys and four girls) displayed 2–8-unit minor improvements in their CARS scores (Evangeliou et al., 2003). The two patients who displayed the most dramatic improvement in post diet intervention CARS scores were those with the lowest pre-intervention CARS scores (Evangeliou et al., 2003). Additionally, eighteen patients with a more severe pre-intervention CARS rating tended to have minor or moderate improvement post diet intervention (Evangeliou et al., 2003). This study was revolutionary as it set the stage for the possibility that a ketogenic diet could be used as an adjunctive therapy in the treatment of autism spectrum disorder (Evangeliou et al., 2003).

One study in Greece instituted a ketogenic diet for six of their sixteen female patients aged 4-14. Specifically, ASD participants who had an elevated level of serum

Hydroxybutyrate associated with glucose fast loading (Spilioti et al., 2013). Originally the study wanted to implement the diet for all sixteen ASD participants but found that many could not adhere to such a strict diet (Spilioti et al., 2013). One of the six patients showed a remarkable improvement in CARS scale score and was able to stop taking the medications hydroxyzine and risperidone (Spilioti et al., 2013). The patient was able to return to elementary school without clinical issues (Spilioti et al., 2013). Clinical improvement in the remaining five patients were subtle and not as significant (Spilioti et al., 2013). This study was significant because it identified individuals with abnormal levels of hydroxybutyrate, which is often correlated with mitochondrial disorder to understand who would most benefit from a KD (Spilioti et al., 2013). Also, this study showed that adherence to a strict KD protocol is difficult with those in the ASD population but nonetheless one patient showed enough drastic improvement to warrant further exploration (Spilioti et al., 2013).

A case study found that a girl in preschool diagnosed with regressive autism and a CARS score of 49 greatly benefited from implementation of a casein free and gluten free ketogenic diet Herbert & Buckley 2013). Specifically, when the child started to develop into adolescence, she developed seizures and medical personal deemed it appropriate to try a ketogenic diet instead of adding more medication (Herbert & Buckley 2013). The ketogenic diet implemented had a 1.5:1 ratio (fats to carbohydrates and protein) centered around medium chain fatty acids and provided polyunsaturated fats for essential fatty acids (Herbert & Buckley 2013). Due to a high input of medium chain fatty acids, a strict ratio of 4:1 or 3:1 (fats to carbohydrates and protein) typically seen in a ketogenic diet, was not necessary to achieve ketosis (Herbert & Buckley 2013). In addition to

improvement in seizures, the patient had marked improvement in language function, cognition, social skills, anxiety, and even had complete resolution of stereotypies (Herbert & Buckley 2013). This case study provided a solid example that a medium chain ketogenic diet in conjunction with a casein free and gluten free diet may significantly improve core ASD deficits (Herbert 2013).

Another case study (Zarnowska et al., 2018) looked at a six-year-old boy who was diagnosed with ASD, early mental retardation, and ADHD (Zarnowska et al., 2018). Patient scored as severely autistic (score of 43) on the childhood autism rating scale (CARS), a validated measure of autism. Patient started a ketogenic diet that consisted of a classic 2:1 ratio of fats to carbohydrates and protein (Zarnowska et al., 2018), that was slowly introduced over a period of four weeks. After achieving ketosis, the patient was switched to a modified Atkins diet (MADS), which consisted of an allowed 15-18 grams of carbs per day and more protein (Zarnowska et al., 2018). Patient's ketone levels stayed consistent, and the diet was well tolerated for an additional five months (Zarnowska et al., 2018). The patient was then placed on a low glycemic index treatment (LGIT) diet that had him consume 40-60g of carbohydrates per day (Zarnowska et al., 2018). The patient had noticeable behavioral improvement as early as one month when on a classic ketogenic diet such as less hyperactivity and aggressive behaviors (Zarnowska et al., 2018). The patient's next psychological evaluation revealed a significant reduction in CARS score (score of 27), which occurred 17 months after starting the initial ketogenic diet and transitioning into a LGIT diet (Zarnowska et al., 2018). This case study sheds light on the implementation of a less strict diet protocol after implementation of a ketogenic diet that sustains amelioration of ASD symptomatology.

As previously mentioned in the introduction section, a study involving thirty-three ASD males and twelve ASD females ages 3-8 years old were separated into three groups: Modified Atkins diet, Gluten Free Casein Free Diet, and a control diet (El-Rashidy et al., 2017). Specifically, the study implemented the use of ketostix (piece of paper with a reagent that turns a certain color depending upon urine ketone levels) for daily ketone levels to maintain documentation of ketosis state in patients (El-Rashidy et al., 2017). Parents were held responsible for documenting in a journal their children's ketone levels and diets were adjusted on a weekly basis (El-Rashidy et al., 2017). The study made sure extensive lab work was done for those in the keto group as numerous metabolic dysfunction diseases would be contraindicated for participants (El-Rashidy et al., 2017). The study did a great job using validated autism measures, CARS and ATEC, to communicate the statistically significant results pre and post nutritional intervention.

Regarding results, the ketogenic diet group had statistical significance ($p < 0.001$) for CARS scores and ATEC scores ($p < 0.01$) (El-Rashidy et al., 2017). Regarding the subdomains for the ATEC scores speech ($p < 0.01$), social ($p < 0.05$), and cognition ($p < 0.001$) were all statistically significant (El-Rashidy et al., 2017). The subdomain of behavior for the ATEC scores was not significant and the authors hypothesize that the increase in energy that comes with a ketogenic diet may have contributed to the lack of ameliorating behavioral problems within ASD (El-Rashidy et al., 2017). Additionally, when compared with the GFCF diet, the ketogenic diet did not show to be statistically more significant in ameliorating ASD symptoms measured by the validated measures (El-Rashidy et al., 2017). However, when strictly comparing the percent change between each diet in the outcome measures, there was further evidence that a ketogenic diet may

in fact be superior to a GFCF diet when it came to CAR's scores, ATEC scores, and all subdomains within the ATEC scores except for behavior (El-Rashidy et al., 2017). This study is paramount in not only showing general efficacy of a ketogenic diet for core symptoms of ASD but possibly having a superior effect when compared to a GFCF diet.

In another study, an observer blinded clinical trial testing the effects of a modified clinical trial on ASD had fifteen children (13 males and 2 females), between the ages of 2-17 years of age (Lee et al., 2018). Each participant had bloodwork done to assess baseline status and to rule out any contraindications for starting a ketogenic diet (Lee et al., 2018). Also, caregivers were given instructions with a ketogenic diet by a dietician, required to journal food intake, and had access to additional nutritional guidance online and by phone during the intervention (Lee et al., 2018). The diet applied was a modified ketogenic diet that included being gluten free and incorporating a medium chain triglycerides oil (MCT) (Lee et al., 2018). Total carbohydrate intake was not to exceed 20-25 grams per day and MCT oil was used to account for 20% of energy needs (Lee et al., 2018). Caregivers were instructed to check urine ketones twice daily for the first month and once daily thereafter (Lee et al., 2018). The autistic diagnostic observation schedule second edition (ADOS-2) and the CARS were used to get measures at baseline, months, and six months (Lee et al., 2018). Within this cohort, seven of the participants were classified as having a high level of autism while the other eight were classified as having a moderate level of ASD symptoms (Lee et al., 2018). A total of fifteen subjects completed 3 months on the diet and ten subjects completed six months on the diet (Lee et al., 2018).

Results for the ADOS-2, at three months showed that there was improvement in comparison score, a 19.9% mean improvement in social affect score ($p < 0.01$), 20.7% improvement in total overall score ($p < 0.05$) (Lee et al., 2018), but there was no improvement in the domain of restricted and repetitive behaviors ($p > 0.05$) (Lee et al., 2018). Additionally, significant improvement (more than 7 units decrease on ADOS-2) was observed in six participants, moderate improvement (more than 3 units) was observed in two participants, and minor/no improvement was seen in seven participants (Lee et al., 2018). For the six month follow up, ten participants all maintained improvement in ADOS-2 comparison scores, total score, and social affect subdomain scores ($p < 0.05$), but there was no improvement on restricted and repetitive behaviors ($p > 0.05$) (Lee et al., 2018). For CARS-2 scores, participants score significantly decreased following 3 months, more specifically on measures of imitation, body use, and fear of nervousness ($p < 0.05$) (Lee et al., 2018). Thereby, this study shows promise in improving core ASD symptomatology through a ketogenic diet that was modified to exclude gluten and increased use of medium chain triglyceride oil.

A study looked at 23 children diagnosed with ASD to implement a modified ketogenic diet that included a regimen that was gluten-free and incorporated MCT oil (total net carbohydrates per day was limited to 20-25g and MCT oil comprised of 20% of energy requirement) (Mu et al., 2019). The study provided a dietician for caregiver trainings and made sure to run bloodwork, so no participant involved had contraindications for starting a ketogenic diet (Mu et al., 2019). ADOS-2 and CARS-2 were the psychometric validated measures used for both baseline behavioral assessment and after 3-month completion of diet intervention (Mu et al., 2019).

Results showed that after three months of diet intervention five individuals showed a greater than seven units decrease in ADOS-2 score (high responders) and six individuals showed a less than three unit decrease in ADOS-2 score (low responders). Additionally, this study looked at metabolites and their correlations with ADOS-2, social affect score, and comparison score (Mu et al., 2019). The study found that ornithine concentration was negatively correlated with ADOS-2 overall score and social affect score (Mu et al., 2019). Acetoacetate significantly negatively correlated with comparison score and tended to be negatively correlated with ADOS-2 overall score and social affect score (Mu et al., 2019). It's important to note that higher responders had greatest concentrations of hydroxybutyrate after KD intervention (Mu et al., 2019). This study was able to show that a modified ketogenic diet had significant impact on overall ADOS-2 scores and social affect, as well as established potential avenues of exploration regarding specific metabolites found when implementing a KD intervention (Mu et al., 2019).

Discussion

Current human studies show evience for improvement of core ASD symptoms and further exploration is needed. Generally, based upon current studies of the ketogenic diet and human trials, there is a major need for double blind case-controlled studies. Studies should consider a larger group size for better statistical power as a drop out due to compliance issues is a common theme. Additionally, future study designs should compare a medium chain triglyceride ketogenic diet with a standard ketogenic diet, as well as implementing a gluten free casein free diet in conjunction with a ketogenic diet protocol. Also, severity of autism and underlying mitochondrial or metabolic disorders

should be significant factors in study results as both may influence ketogenic diet efficacy.

The ketogenic diet does not come without its potential for adverse side effects or limitations. For example, a study that implemented a ketogenic diet for the treatment of refractory epileptic encephalopathies showed adverse side effects such as drowsiness, constipation, weight loss, vomiting, gastroesophageal reflux, fever, and hyperlipidemia (Coppola et al., 2009). Additionally, dyslipidemia as well as other metabolic and gastrointestinal issues may need to be monitored amongst ASD children on the ketogenic diet (Kwiterovich et al., 2003). Anemia must also be monitored in ASD children due to systemic ketosis (Kang et al., 2004). Additionally, the ketogenic diet is not easily adhered to especially in a population of young ASD children according to a review (Bostock et al., 2017). Also, agreement on how to standardize or regulate what is consistent in a ketogenic diet is still being debated (Kosoff et al., 2008).

CHAPTER FIVE

CONCLUSION AND FUTURE DIRECTIONS

Both animal and human studies show the positive potential of a ketogenic diet in helping improve the core and associated psychiatric symptoms of autism spectrum disorder such as repetitive behaviors, social behaviors, communication, anxiety, speech, hyperactivity, and cognition. However at present animal studies are not extensive and standardized while human studies often lack proper statistical power due to sample size and compliance. Hence future direction should look at the idea of ketone bodies as an alternative energy fuel, which has been around for a long time (Miller and Dymsza 1967). Ketone bodies themselves may be the reason the ketogenic diet has therapeutic effects because of their role as a respiratory fuel and their cellular communication (Thompson and Wu 1991; Newman and Verdin 2014). Although the question of fitting a ketogenic diet in a pill has been asked, a ketogenic diet is often not necessary to prove that exogenous ketones have a therapeutic effect on pathology (Rho and Sankar 2008). There is proof that the use of exogenous ketones can provide many of the same metabolic benefits of a ketogenic diet (Kesl et al., 2016). Specifically, two experiments using rats showed how the use of exogenous hydroxybutyrate caused sustained and rapid elevation of the ketone BHB along with decreasing anxiety-related behaviors (Ari et al., 2016; Kovacs et al., 2018). Furthermore, a human study suggested that R-3-hydroxybutyl (exogenous ketone) was well tolerated by human subjects and plasma ketone levels were elevated enough to induce states of hyperketonemia (Clarke et al., 2012). Another human study showed that exogenous ketones that induced hyperketonemia could help with

symptoms of Alzheimer's and is safe and convenient (Newport et al., 2015). Additionally, oral administration of ketone salt in doses from 80 to 900 mg/kg/day was enough to get peak blood levels of total d-b-hydroxybutyrate-acetoacetate of 0.19–0.36 mM that were therapeutic for children with acyl CoA dehydrogenase deficiency (Van Hove et al., 2003). Lastly, different types of exogenous ketones (ketone esters and ketone salts) are still being studied in humans, but there is proof that ketone esters have shown to sustain blood ketone levels better than ketone salts (Stubbs et. al, 2017). Thereby it is possible to use exogenous ketones that induce mild ketosis and produce therapeutic effects. Hence, the use of exogenous ketones for ASD pathology should be further investigated and used in addition to the KD because it may offer easier adherence to a strict dietary protocol.

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