Final degree project

KETOGENIC DIETS: NEURODEGENERATIVE AND RARE DISEASES

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ABSTRACT

The ketogenic diet (KD) is a high-fat, low-carbohydrate, and moderate-protein diet that was first described in 1921, with the goal of mimicking the anticonvulsant effects of fasting. KD causes an increased ketone bodies production, allowing the brain to obtain an alternative fuel to glucose. Recently, its clinical use has expanded notoriously, as well as scientific interest, due to its possible positive effects on various diseases. Based on a bibliographic research, this work aims to analyse the current information on KD and its variants, in addition to review some studies that assess its application in two illnesses: glucose transporter type 1 deficiency syndrome (GLUT1DS) and Alzheimer's disease (AD), as examples of rare and neurodegenerative illness, respectively. On one hand, GLUT1DS is an encephalopathy with a wide spectrum of manifestations, with seizures being the most common. In this disorder, KD-derived ketone bodies represent an efficient alternative energy source. On the other hand, AD is the most frequent cause of dementia and its incidence is expected to increase in the coming decades. Currently, there is no cure, so the neuroprotective effects of ketone bodies may decrease the mitochondrial dysfunction, oxidative stress, and neuroinflammation present in AD. Although overall, both the reported cases of GLUT1DS and preclinical and clinical studies in AD demonstrate clinical benefits with KDs, more research is needed to better understand their role in diverse diseases.

***Keywords**: ketogenic diet, ketone bodies, GLUT1 Deficiency Syndrome, Alzheimer*

La dieta cetogénica (DC) es una dieta alta en grasas, baja en carbohidratos y moderada en proteínas que fue descrita por primera vez en 1921, con el objetivo imitar los efectos anticonvulsivos del ayuno. La DC provoca un aumento en la producción cuerpos cetónicos, permitiendo al cerebro obtener un combustible alternativo a la glucosa. Recientemente su uso clínico se ha expandido notoriamente, así como también el interés científico, por sus posibles efectos positivos en diversas enfermedades. A partir de una búsqueda bibliográfica, este trabajo pretende analizar la información actual de la DC y sus variantes, y revisar algunos estudios que evalúen su aplicación en dos enfermedades: el síndrome de deficiencia del transportador de glucosa tipo 1 (SDGLUT1) y la enfermedad de Alzheimer (EA), como ejemplos de enfermedad rara y neurodegenerativa, respectivamente. Por una parte, el SDGLUT1 es una encefalopatía con un amplio espectro de manifestaciones, siendo las crisis convulsivas las más comunes. En esta enfermedad, los cuerpos cetónicos representan una eficaz fuente alternativa de energía. Por otra parte, la EA es la causa más común de demencia y se prevé que su incidencia aumente en las próximas décadas. Actualmente, no existe una cura, por tanto, los efectos neuroprotectores de los cuerpos cetónicos podrían disminuir la disfunción mitocondrial, estrés oxidativo y neuroinflamación presentes en la EA. Aunque en general, tanto los casos reportados de SDGLUT1 como los estudios preclínicos y clínicos en EA, demuestran beneficios clínicos con las DCs, se necesita más investigación para comprender mejor su papel en diversas enfermedades.

***Palabras clave**: dieta cetogénica, cuerpos cetónicos, Síndrome Deficiencia GLUT1, Alzheimer*

TABLE OF CONTENTS

INDEX OF FIGURES

INDEX OF TABLES

ACRONYMS

acetyl-Coa: acetyl coenzyme A
AD: Alzheimer's disease
ADAS-Cog: Alzheimer's Disease Assessment Scale-Cognitive Subscale
APOE: apolipoprotein E
APP: amyloid precursor protein
ATP: adenosine triphosphate
Aß: amyloid-beta
ß-OHB: beta-hydroxybutyrate
BBB: blood-brain barrier
BDH: beta-hydroxybutyrate dehydrogenase
CoA: coenzyme A
CSF: cerebrospinal fluid
g: gram/s
GABA: γ-aminobutyric acid
GLUT1: glucose transporter type 1
GLUT1SD: glucose transporter type 1 Deficiency Syndrome
K_{ATP}: ATP-sensitive potassium
KD: ketogenic diet
LCT: long-chain triglyceride
LGIT: low-glycemic index treatment
MAD: modified Atkins diet
MCI: mild cognitive impairment
MCT: medium-chain triglyceride
MCT1: monocarboxylate transporter 1
MCTKD: medium-chain triglyceride ketogenic diet
mmol/L: millimole/litre
mPTP: membrane permeability transition pore
NAD: nicotinamide adenine dinucleotide
NFT: neurofibrillary tangle
NIA: National Institute on Aging
NMDA: N-methyl-D-aspartate
Nrf2: nuclear factor erythroid 2-related factor 2
OXCT1: 3-oxoacid CoA-transferase 1
PED: paroxysmal exercise-induced dyskinesia
PET: positron emission tomography
ROS: reactive oxygen species
SLC2A1: solute carrier family 2 member 1
TCA: tricarboxylic acid

1.INTRODUCTION

A ketogenic diet (KD) is defined as a diet high in fats and low in carbohydrates, with an adequate amount of proteins (1). The original KD emerged in the early 1920s when several patients suffering from epilepsy were treated with a type of diet regimen that mimicked the effects of fasting, an anticonvulsant strategy already used by some contemporary physicians. Actually, the control of seizures through sustained fasting dates back to the time of Hippocrates, but the first scientific observations were recorded by two French physicians in 1911 (2).

In a fasting state, the energy expended by the tissues firstly comes from the glucose metabolism and stored glycogen. If fasting is prolonged, fatty acids can also serve as an energy source through their breakdown in the liver, and the excess of acetyl coenzyme A (acetyl-Coa) produced is then utilized as a substrate for ketone bodies production: beta-hydroxybutyrate (ß-OHB), acetoacetate and acetone. At the end, a new metabolic state named ketosis occurs, where ketone bodies levels in serum increase in detriment to glucose levels, so there is a fuel shift (3).

In 1921, doctor Wilder, from Mayo Clinic, suggested that the ketosis could be reached by a different dietary strategy. He proposed that a high-fat and low-carbohydrate diet could be maintained for a much longer period of time than fasting, and he was the first one to refer to this type of regimen as “Ketogenic Diet”. Over the following two decades, KD was widely administered to epileptic children. But the appearance of the first antiepileptic drugs, like diphenylhydantoin in 1938, were relegating its use, most probably due to the simplicity of prescribing a pill as opposed to a strict dietary regimen (2,4).

However, in recent years, the clinical use of KD has experienced a resurgence because although several anticonvulsant drugs are available, some patients with epilepsy still fail to achieve significant relief of convulsions. Interestingly, in the mid-1990s, a successful treatment of intractable generalized seizures in a child called Charlie was reported in the news media. The patient’s father, a famous film director, created the C*harlie Foundation* in order to contribute to disseminate this therapy through courses and audiovisual material. Moreover, this foundation supported the first multicenter prospective study testing the efficacy of the KD and since then, the role of KD in refractory epilepsy have been evaluated in numerous studies (2).

The appearance of adverse effects, in addition to the restrictive nature of the diet can lead patients to low compliance. In an attempt to increase variability, palatability and tolerability of the diet, various variants with a lower fat-to-protein and carbohydrate ratio have been designed: the ketogenic medium-chain triglyceride ketogenic diet (MCTKD), the modified Atkins diet (MAD) and the low-glycemic index treatment (LGIT) (5).

Currently, there is a growing scientific interest in the KD since investigations performed to clarify the mechanisms underlying the anticonvulsant effects of KD have allowed to consider its application in other diseases (4).

KD is the treatment of choice for type 1 glucose transporter deficiency syndrome (GLUT1DS). This syndrome is caused by a default in the protein responsible for transporting glucose across the blood-brain barrier (BBB), which results in an energy deficiency of the brain. It is manifested in convulsions in the early stages of life and impaired brain growth, often related to developmental delay and movement disorders. The entrance of KD-derived ketone bodies allows the brain to obtain energy by a different mechanism than glucose (6,7).

Ketone bodies not only serve as energy substrate but are also able to interact with a variety of receptors, channels and metabolic enzymes. The diverse mechanisms of action have been studied and ketone bodies have been seen to play a neuroprotective role through various pathways such as (8):

- Maintenance of energy metabolism
- Modulation of synaptic transmission
- Reduction of oxidative stress
- Modulation of inflammation

Some of these processes are characteristic of certain neurological conditions such as Alzheimer's disease (AD), a neurodegenerative disease that is characterized by progressive loss of memory and sense of orientation, cognitive impairment, language difficulties and changes in personality and behaviour. Nowadays, the only available pharmacological therapies just appear to be useful to alleviate symptoms (9). So, according to some studies carried out lately -that will be reviewed in this final degree project-, KD could also mean a potential alternative for this disease treatment.

2. OBJECTIVES

Since there has been recently an increasing interest in the application of KD in other diseases than refractory epilepsy, this work aims to examine the current knowledge of KDs to later understand its role in GLUT1DS and AD. To this end, a bibliographic research has been done about:

- KD's definition, associated metabolic changes, neuroprotective effects, variants, clinical protocol and adverse effects
- GLUT1DS' definition, manifestations, diagnosis and treatment
- AD's definition, manifestations, etiology, pathogenesis, diagnosis and treatment
- Evidence of the implementation of ketogenic therapies on GLUT1DS and on AD, through different studies

3. METHODS

An exhaustive bibliographic research has been carried out in order to achieve the objectives exposed before.

Firstly, a general research about the KD was done using the databases *PubMed* and *Sci Finder*. Articles since 2010 to the present were limited, and only studies that the *Centre de Recursos per a l'Aprenentatge i la Investigació* facilitated the open access, are included in this work.

Some review articles in which neuroprotective effects of the KD were explained, allowed me to decide about the illnesses I could associate with the KD: GLUT1 Deficiency Syndrome and Alzheimer's disease. More detailed information about these them were consulted in the mentioned databases and some websites from official organizations. At the same time, more specific research about the relationship between KDs and the two diseases was done through the databases using, for instance, the keywords "ketogenic diet", "variants", "neurodegenerative", "Alzheimer" or "GLUT1". Several studies were excluded owing to its low relevance for the objectives of this work.

In an attempt to find out the original sources of certain specific information, some bibliographic references of selected articles were analysed and included. By this method, I also found interesting studies associating KDs with the two diseases. Finally, I looked up ongoing trials in *Clinicaltrials.gov*.

This bibliographic research has also permitted me to elaborate an informative article, as an example of dissemination activity.

4. RESULTS

4.1. Ketogenic diet

Characterized as a high-fat, adequate-protein and low-carbohydrates diet, the KD induces ketone bodies production through fat metabolism. The aim of this diet is to mimic a fasting response, replacing glucose as the predominant caloric source, as well as facilitating enough protein to sustain growth and development in pediatric patients (1,10).

The classic KD, designed by Wilder, consists of a macronutrient ratio, also termed ketogenic ratio of 4:1 (4 grams (g) of fat to 1 g of protein and carbohydrates combined) (11). In a traditional Mediterranean diet (figure 1), the predominant macronutrients are carbohydrates (12). Glucose represents the main energy source for the body and the not-used-glucose is stored as glycogen (3). However, with a classic KD, the proportions of macronutrients vary: 90% from fats, 4% from carbohydrates and 6% from proteins (figure 2) (10).

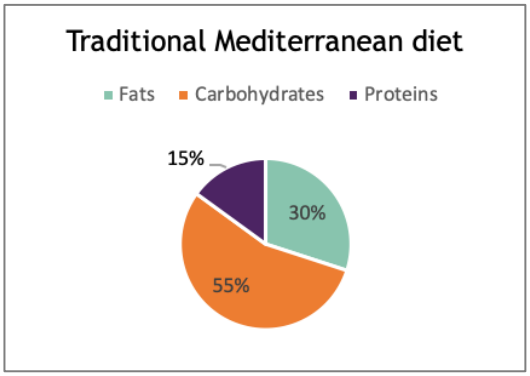

Figure 1. Macronutrient proportions in a traditional Mediterranean diet (12)

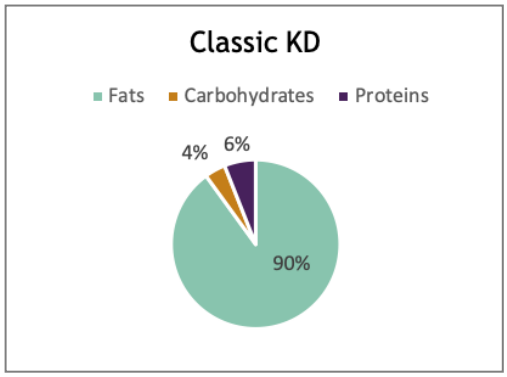

Figure 2. Macronutrient proportions in a classic KD (10)

4.1.1. Metabolic changes associated with the ketogenic diet

By reducing carbohydrate intake, glucose availability is reduced, and glycogen deposits are depleted. This situation triggers the breakdown of triglycerides to free fatty acids and glycerol, and the mobilization of lipids stored from adipose tissue to the liver. Fatty acids submit to ß-oxidation to produce acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle, and then condenses with oxaloacetate to form citrate. Meanwhile, glycerol acts as a substrate for gluconeogenesis (3). It should be noted that gluconeogenesis also requires oxaloacetate as an intermediate (4). Thus, the high rate of ß-oxidation generates a great amount of acetyl-CoA, that exceeds the capacity of the TCA cycle to synthesize citrate. In these circumstances, the surplus of acetyl-Coa serves as a substrate for the ketone bodies synthesis: acetoacetate, ß-OHB and acetone (3).

KD brings the body into a state of ketosis, where ketone bodies preferably feed cellular metabolism in place of glucose (8). Adequate ketosis is reached when ß-OHB levels in blood are approximately 4-5 millimole/litre (mmol/L) (13). Some tissues with high-metabolic demands, such as the heart, skeletal muscle or central nervous system can benefit from this energy source switch. For example, since fatty acids can not directly penetrate the BBB, ketone bodies become the optimal alternative fuel for the metabolism in the brain (figure 3) (4,14).

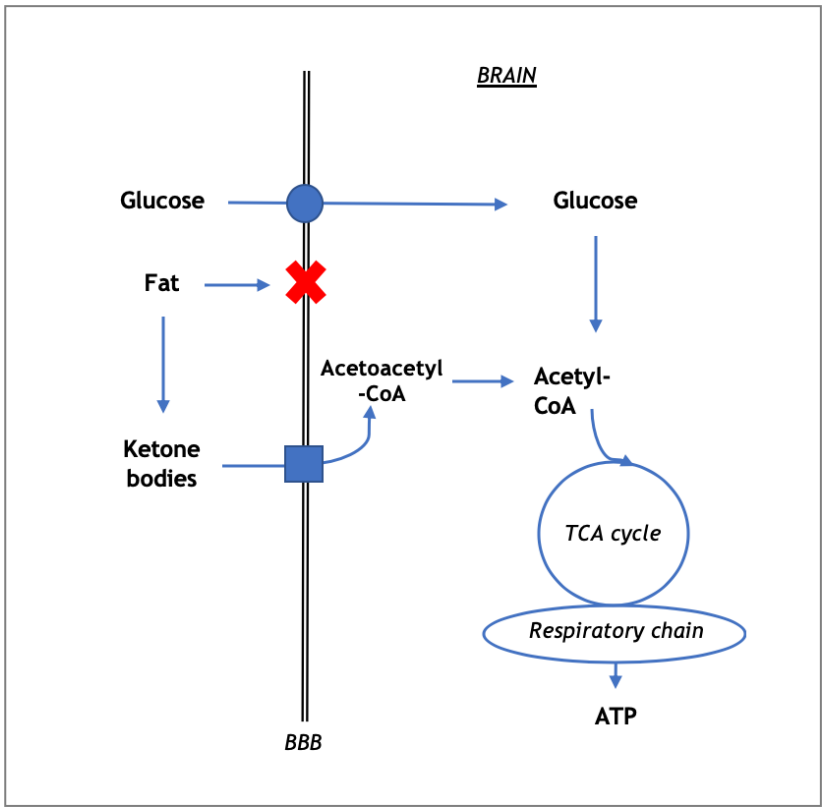

Figure 3. Ketosis and brain energy metabolism. *Glucose enters the brain via the facilitated glucose transporter GLUT1 (●), fatty acids can not enter (x), and ketone bodies penetrate the BBB via the monocarboxylate transporter 1 (MCT1) (■). In the brain, glucose and ketone bodies enter the TCA cycle as acetyl-CoA for energy production.* Adapted from (15).

Ketone bodies production, or ketogenesis, takes place primarily in the mitochondrial matrix of hepatic cells. The acetyl-Coa excess permits the generation of the first ketone body, acetoacetate, from which the other two ketone bodies derive. On one hand, it is largely reduced to ß-OHB, by ß-OHB dehydrogenase (BDH). On the other hand, the third ketone body, acetone, is produced due to a spontaneous decarboxylation of the remaining fraction of acetoacetate in some tissues. Acetone is a volatile product that is mostly exhaled through the lungs, whilst ß-OHB and acetoacetate are released into the blood circulation thanks to the monocarboxylate transporter 1 (MCT1) of the liver.

Extrahepatic tissues can internalize circulating ketone bodies through the MCT1 in order to get acetyl-CoA and, subsequently, to produce energy. Ketone bodies utilization, or ketolysis, occurs in the mitochondria and starts with the participation of BDH, which converts ß-OHB back to acetoacetate. Then, acetoacetyl-CoA is formed by the conjugation of acetoacetate with Coenzyme A (CoA) thanks to 3-oxoacid CoA-transferase 1 (OXCT1). Finally, two molecules of acetyl-CoA are generated, and then oxidized via the TCA cycle and the electron transport chain to obtain energy, in the form of adenosine triphosphate (ATP) (3). The fact that OXCT1 is not expressed in the liver and that differing enzymes are implicated in these two opposite metabolic pathways, prevents a worthless cycle of ketone bodies synthesis and degradation (14).

4.1.2. Neuroprotective effects of the ketogenic diet

Despite quite a century of use, the mechanisms underlying the efficacy of the KD have not yet been totally elucidated. Based on the documented biochemical pathways of KD in numerous studies -mainly carried out in epilepsy-, several mechanistic theories have been proposed (8). This work aims to focus on the most observed neuroprotective effects.

Improvement of energy metabolism in the brain

KD involves an enhancement of the metabolic pathways implicated in energy production occurring in the mitochondria of brain neurons. Ketone bodies are a more efficient energy source compared to glucose, because they are metabolized faster than glucose and are able to access directly to the TCA cycle, whereas glucose has to undergo glycolysis. As a consequence of a greater metabolism of ketone bodies, glycolysis is inhibited (16–18). Moreover, KD helps to restore intermediates of the TCA cycle by facilitating high amounts of acetyl-CoA (4). Long-term KD administration has also found to stimulate the mitochondrial biogenesis and significantly upregulate the expression of genes encoding many enzymes that are responsible for mitochondrial energetic metabolism.

All these mechanisms improve ATP generation, which in turn lead to an increase in energy reserves as ATP, and the excess is stored as phosphocreatine (16).

Modulation of synaptic transmission

Higher energy reserves enable a better synaptic transmission. This boosts the adaptability of neurons to challenge stressful conditions (16). One possible mechanism implicated could be the opening of ATP-sensitive potassium (K_{ATP}) channels, which raises the seizure threshold. K_{ATP} channels open when levels of glycolytic ATP (produced by glucose oxidation) are low, and this is precisely what happens with KD therapy (19). Another mechanism could involve ketone bodies in the mitochondrial permeability mediated by the membrane permeability transition pore (mPTP). Prolonged excitotoxicity can initiate the opening of this pore, through which

pro-apoptotic factors are released that promote cell death. Ketone bodies, by reducing the reactive oxygen species (ROS), may inhibit the mPTP opening (20).

Elevated ATP levels may also alter the concentrations of various neurotransmitters, such as adenosine, glutamate and γ-aminobutyric acid (GABA), contributing to the stability of synaptic transmission. An increased ATP production drives to a rise of adenosine levels that ultimately, derive in a decrease of neuron excitability via adenosine A1 receptors (21). Glutamate is the principal excitatory neurotransmitter of the brain, whilst GABA is the main inhibitor. Ketone bodies may modulate the metabolism of these neurotransmitters, promoting inhibitory GABA neurotransmission. In neurons, glutamate can be transformed into either GABA or aspartate in a reaction that also requires oxaloacetate. Because a KD induces metabolic changes that need available oxaloacetate to be condensed with acetyl-CoA for incorporation into the TCA cycle, aspartate creation is diminished (4). Moreover, aspartate inhibits glutamate decarboxylase, an enzyme that catalyses the conversion of glutamate to GABA. Therefore, a decline in aspartate levels promotes a further GABA synthesis (22). Additionally, ketone bodies can directly compete with chloride for allosteric activation of vesicular glutamate transporters, resulting in lower glutamate release (4). Finally, ß-OHB may boost the concentration of neurotrophins, responsible for the activation of multiple proteins involved in neuronal biogenesis (23).

Mitigation of ROS

ROS production from metabolism pathways is physiological. However, when generation overcomes antioxidant systems, ROS accumulate, causing oxidative stress. KD promotes mechanisms that mitigate ROS. For instance, KD activates the Nuclear factor erythroid 2-related factor 2 (Nrf2), a transcriptional factor that regulates genes involved in antioxidant mechanisms, such as those related to glutathione, an antioxidant molecule (24,25). KD also contribute to protect against ROS through an increase in the ratio between the oxidized and reduced forms of nicotinamide adenine dinucleotide (NAD+/NADH), and an improved expression of uncoupling proteins. Besides, ß-OHB has been shown to inhibit histone deacetylases class I (HDAC1), which is associated with a higher resistance to oxidative damage, by inducing the expression of detoxifying genes (4).

Anti-inflammatory effects

KD also exerts neuroprotective effects through anti-inflammatory mechanisms. The great amount of fatty acids facilitated by KD induces the activation of peroxisome proliferator-activated receptor gamma (PPARγ), which can reduce the expression of the nuclear factor κB (NF-κB), implicated with the release of pro-inflammatory cytokines (8). It has been discovered that ß-OHB activates the hydroxycarboxylic acid receptor 2, expressed in microglia, dendritic cells and macrophages, and also may diminish the release of pro-inflammatory cytokines, through the inhibition of the innate immune sensor NOD-like receptor 3 inflammasome.

There has been controversy about whether ketone bodies are responsible for the anticonvulsant effects of KD, mainly because, according to a few clinical observations, ketone bodies levels in blood inconsistently correlate with seizure control. However, researchers suggest that the differences may be related to heterogeneity of the diet or differences in methodology between studies (11). To generalize, the subjacent mechanisms of KD are likely multiple and synergistic, and include a variety of molecular, genetic, cellular and metabolic factors (26).

4.1.3. Types of ketogenic diets

In the classic KD, fats provide approximately 90% of energetic diet value and are principally composed of long-chain fatty acids, which have 16–20 carbon atoms. Despite the documented efficacy of the classic KD against convulsive disorders, its implementation may pose a challenge. Drastic changes in eating habits are needed to introduce, and are difficult to maintain in the long term. Hence, over time, in order to increase flexibility and palatability, and consequently adherence, other KDs variants have been developed, allowing patients to achieve a similar effect (27). These variants have a lower macronutrient ratio (3:1, 2:1 or 1:1), resulting in less strict KDs. They are chosen based on age, individual tolerability, goal level of ketosis and protein requirements (11).

In 1971, the medium-chain triglyceride ketogenic diet (MCTKD) was designed to deliver 60% of its calories from medium-chain triglycerides (MCTs), which have two or three fatty acid chains comprised of 6-12 carbon atoms, such as caprylic acid, the main component of the MCTKD (5,28). MCTs can be consumed as coconut oil or as an emulsion. As they are metabolized faster than long-chain triglycerides (LCTs), less fat intake is needed to induce ketosis, therefore, a greater consumption of protein and carbohydrate is possible. With this diet, patients consume more varieties of food (1,6). Nevertheless, comparing with the classic KD, an higher rate of gastrointestinal problems may appear, such as diarrhea, vomiting, bloating and abdominal cramps (28). In order to obtain better tolerability, a modified version was suggested, beginning only with a 30% of calories from MCTs, and a larger LCT content. With this modified MCTKD, the MCT percentage is required to be incremented gradually, in detriment of LCT percentage (1). MCT oil has also been applied as a supplement to the classic KD to boost ketosis and improve lipid abnormalities (6).

In 2003, a more flexible variety was described, the Modified Atkins Diet (MAD). “Modified” because its aim was not the weight loss, but the increase of adherence, especially in adults. The MAD is based on a ratio of 1:1, has no restriction of protein, fluids or calories, and contains 10-30 g of carbohydrates/day. All carbohydrates are permitted and can be eaten throughout the day or at one meal. The initial amount of carbohydrates, 10 g/day in children and 15 g/day in adults, can be elevated to 20-30 g/day after a couple of months depending on the response (29). With the MAD, the weighing of food portions or an initial hospital stay are not necessary (5).

In 2005, Pfeifer et al. designed the low-glycemic index treatment (LGIT), that includes approximately 40-60 g of carbohydrates/day, only allowing those foods with a glycemic index below 50. By its features, the LGIT prevents large postprandial rises in blood glucose, permitting more stable circulating glucose levels (30).

The distinct compositions of these diets are shown in Table 1. In addition, some ketogenic dietary supplements, like ketone esters, are currently being target of interest as potential substitutes for KD (11).

Diet	Ketogenic ratio	% carbo-hydrate	% protein	% fat (LCT)	% fat (MCT)
Classic KD	4:1	4	6	90	0
MCTKD	3:1	19	10	11	60
Modified MCTKD	3:1	19	10	41	30
MAD	1:1	10	25	65	0
LGIT	0,6:1	10	30	60	0

Table 1. Composition of the KDs and its variants (5)

4.1.4. Protocol

Dietary plan requires a well-defined protocol of implementation and maintenance.

Previous assessment

Prior to initiation of the KD, a visit with a KD-trained multidisciplinary team is important for providing counselling, as well as for nutritional and laboratory evaluation. Moreover, it is recommended to carry out ancillary testing, such as electroencephalogram, echocardiogram or renal ultrasound (6). This team usually consists of dietitians, nurses and a licensed clinical social worker. They should advise the family about the lifestyle implications of the diet, the efficacy rate and the most common adverse events (31).

Furthermore, during the visit, possible contraindications must be considered. For instance, patients should undergo a metabolic diagnostic in order to exclude ß-oxidation defects, liver disease or metabolic disorders interfering with glucose or ketone bodies homeostasis (6). Also, interactions of KD with other treatments are important to consider. For example, valproic acid, an anticonvulsant drug, may interfere with the therapeutic objective of KD and contribute to carnitine deficiency (which can appear with both KD and valproic acid use) (11). Accordingly, it may

provoke a liver failure (28). In addition, a concomitant use of carbonic anhydrase inhibitors (acetazolamide, topiramate and zonisamide) may worsen metabolic acidosis, which can occur after KD treatment. Other contraindications to consider are inability to sustain adequate nutrition (like anorexia), and non-compliance by parents or caregivers. Finally, it is important to minimize medications, parenteral and intravenous fluids containing carbohydrates and sugar, which could reverse ketosis (6,11).

After discarding any contraindications, dietitians explain to patients and caregivers how to start the treatment and calculate energetic requirements of the patient, basing on KD administration route, as well as age, sex, stress factor, baseline weight and height, level of activity, and the nutrition intake history (31). Regardless of the type, KDs must include mainly foods rich in fats, whilst those protein foods must provide high-quality proteins (32). Examples of them, in addition to various nutrient-dense foods able to optimize KDs, are shown in table 2. For patients or caregivers, preparing tasty and variable meals can suppose a challenge, so counsellors can propose meal plans and recommend websites, videos or publications about KD from support groups such as the *Charlie Foundation* or *Matthew's Friends* or *The Daisy Garland* (33–35). Moreover, the first two have created the "Keto Diet Calculator" and the "Electronic Ketogenic Manager", respectively, to assist professionals and caretakers in the management of this dietary therapy (6).

Recommended foods for KDs	
Commonly-used foods	**Nutrient-dense foods**
Animal fats (pork lard, cow butter…)	Asparagus
Avocado	Arugula
Cheese (mascarpone, brie, gorgonzola, cheddar…)	Blackberries
Eggs	*Brassica* vegetables (broccoli, cauliflower…)
Green olives in brine	Celery
Nuts	Green tea
Oily fish (eel, salmon…)	Radishes
Processed meat	Spinach
Vegetable oils	Sunflower seeds

Table 2. Recommended foods for KDs. Adapted from (13,32).

Initiation

To begin the diet, two approaches can be considered: with or without fasting. Originally, patients had to fast for 12-24 hours, and be hospitalized to prevent hypoglycemia and dehydration. With this approach, calories and fluids are restricted. It is recommended for patients with a greater need for a rapid response, because fasting may lead to a faster seizure reduction. Calories are added gradually according to the tolerance (6,36). The necessity of fasting was discussed later, and it was proved not to be essential, since this method can generate stress on the patient and may have immediate side effects. Without fasting, hospitalization is not required and ketogenic ratio increases gradually, from 1:1 to 4:1. It results in fewer side effects and a better tolerance, whereas efficacy is maintained (37). For these reasons, nowadays, patients tend not to fast (36).

Follow-up

To test efficacy, KD should be tried for at least 3 months from ketosis is reached. For the first months, patient's progress should be evaluated monthly by the KD-trained team, and then every 3–6 months. During all the treatment, it is important that the KD team can be easily to contact in case of doubts or problems (6,36).

Withdrawal

It has been seen in children with epilepsy, that diet should be sustained for at least 2 years. Children with GLUT1DS or pyruvate dehydrogenase deficiency likely require KD treatment for longer, until adolescence. Although KD can be interrupted abruptly in an emergency, it is more frequently tapered over several months, by gradually lowering the ketogenic ratio from 4:1 to 2:1, and then relaxing restrictions on measuring carbohydrate, calories and fluids intake (6).

4.1.5. Side effects

Many of side effects associated with the use of ketogenic diets that have been reported in the literature refer to those that appear with the classic KD and in patients with epilepsy (table 3).

Most reported side effects of KDs	
Gastrointestinal effects	Kidney stones
Metabolic abnormalities	Vitamin and mineral deficiencies
Weight loss	Growth retardation
Dyslipidemia	

Table 3. Most reported side effects of KDs (11,38)

The most commonly adverse effects are gastrointestinal symptoms, including constipation, diarrhea, nausea, vomiting, and abdominal pain. These effects are usually transient and mild, and rarely need pharmaceutical intervention or diet discontinuation, but may require a lower ketogenic ratio. To prevent or relieve them, it is recommended also taking multiple small meals throughout the day, daily exercise and an increased intake of fiber, sodium and fluids (10,11).

The shift in macronutrient ratio can also trigger metabolic abnormalities, such as dehydration, hypoglycemia, metabolic acidosis or electrolyte imbalance (38). It is worth noting that acidosis and dehydration have been recorded to be more typical with protocols beginning with fasting (37). Weight loss is also frequently reported. Inasmuch as many adults suffering from neurologic disorders are overweight or obese, weight loss could mean a positive effect for them (11).

With a long-term KD therapy, there may be a transient elevation of lipids, increasing the risk of cardiomyopathy and atherosclerosis (27). However, lipid levels tend to normalize with continued treatment (11). To prevent dyslipidemia, a higher proportion of unsaturated to saturated fats, the addition of MCT oil, a lower ketogenic ratio, and carnitine supplementation may be helpful (38). KD may involve a larger risk of developing kidney stones, that can be avoided by adequate fluid intake, alkalization of the urine and with potassium citrate administration (7,38).

With the regimen, vitamin and mineral deficiencies may be prevailing because of the limited consumption of fruit, vegetables, enriched grains, and foods rich in calcium. The principal deficiencies observed concern vitamin B, vitamin D and calcium. This is of particular importance in postmenopausal women, since such deficiencies can exacerbate the risk of osteopenia and osteoporosis (11,32). Apart from that, in children, an inadequate calcium intake can further impair bone mineralization, so a correct growth can be affected. Nevertheless, the results concerning the KDs impact on growth retardation are conflicting (32).

4.2. Different applications of the ketogenic diets

Despite most of the studies proving the neuroprotective role of the KD have been carried out on patients suffering from refractory epilepsy, beneficial effects of KD have been suggested to may extend to other disorders such as GLUT1DS, pyruvate dehydrogenase deficiency, Parkinson's disease, AD, amyotrophic lateral sclerosis, cancer or obesity. This wide variety of disorders can be related to the fact that KD may exert benefits beyond seizure control (11,14). This final degree project focuses on the application of KD in the GLUT1DS and the AD.

4.2.1. Glucose Transporter 1 Deficiency Syndrome

Glucose transporter type 1 (GLUT1) Deficiency Syndrome is a rare genetic metabolic disorder that predominantly affects children. Its prevalence estimates to be ranged from one case per 90000 to one case per 24000 people. This gap could be explained because GLUT1DS may go unrecognised or misdiagnosed (39). GLUT1DS is characterized by an impaired transfer of glucose across the BBB and into the brain cells. GLUT1 deficiency leads to a low availability of glucose, the main energy source for the brain and, consequently to a dysfunction in cerebral metabolism and neuronal activity (40).

It is also known as "De Vivo disease" because it was first described in the medical literature in 1991 by doctor De Vivo and his colleagues. They reported two children who presented the same clinical manifestations: early-onset and drug-resistant seizures, developmental delay, acquired microcephaly and movement disorders. Furthermore, the analysis showed low concentrations of glucose in cerebrospinal fluid (CSF) (hypoglycorrhachia) but no hypoglycemia. Also, low CSF lactate concentrations were detected. Based on these findings, a defect in the protein responsible for glucose transport across the BBB was proposed. Later, these speculations were ratified by observing an impaired glucose uptake into erythrocytes (in which GLUT1 is also expressed), and through genetic analysis (41,42), in which mutations in the gene encoding GLUT1 (solute carrier family 2 member 1 -SLC2A1) were identified. GLUT1DS was originally classified in the group of epileptic encephalopathies, in which convulsions are associated with progressive psychomotor dysfunction (39).

4.2.1.1. Manifestations

The manifestations described by De Vivo represent the classic phenotype of GLUT1DS. Nowadays, it has been recognised that this syndrome has a wider spectrum of manifestations, with variable degrees of severity, from mild motor dysfunctions to harsh neurological complications (43).

The most typical symptoms are seizures, which usually emerge within the first months of life. A deceleration of head growth may occur, and affected individuals can develop mild-to-moderate delays in development. Movements disorders such as hypotonia, ataxia, spasticity and dystonia, may cause difficulty walking, whereas cognitive alterations, ranging from mild learning disability to severe intellectual impairment, may drive to difficulty speaking. Some patients with GLUT1DS may suffer from paroxysmal exercise-induced dyskinesia (PED), which commonly begin in late childhood and adolescence (39). It is characterised by episodes of sudden, transient and involuntary movements, that are triggered by prolonged exercise, such as walking or running long distances (39,44).

Moreover, although less common, some patients may develop the atypical or non-classic phenotype, that includes movement disorders and cognitive impairment without epilepsy, or asymptomatic cases (39).

4.2.1.2. Diagnosis

Individuals with a suspected clinic of GLUT1DS are recommended to undergo a fasting lumbar puncture. Diagnosis may be established with a low CSF glucose concentration, termed hypoglycorrhachia (< 2.2 mmol/l), in the absence of hypoglycemia, and in combination with low to normal CSF lactate levels. Hypoglycorrhachia represents the biochemical hallmark of GLUT1DS, but other specialized tests may help to establish an accurate diagnosis. For instance, carrying out a molecular analysis of the SLC2A1 gene. Nevertheless, only 70%-80% of patients carry SLC2A1 mutations. Likewise, patients sharing identical mutations often do not exhibit the same manifestations, suggesting alternative disease mechanisms (45). Another useful test could be a positron emission tomography (PET) scan, where a diminished chemical activity in the brain (hypometabolism) could be detected. As GLUT1DS is also expressed in erythrocytes, glucose uptake tests in these cells may also contribute to clarify the diagnosis, since GLUT1 activity is reduced by approximately 50% in individuals with GLUT1DS (39).

At all events, relying on one only method of diagnosis can lead to false-negative results, especially with mild manifestations. Hence, the most convenient is to submit to various tests (45).

4.2.1.3. Treatment

The diagnosis of GLUT1DS is often made later than the onset of clinical manifestations such as seizures, which are, therefore, typically and mistakenly treated with antiepileptic drugs, such as phenobarbital, sodium valproate, carbamazepine, lamotrigine, topiramate or clonazepam (40). They are generally ineffective in GLUT1DS and in fact, drugs including phenobarbital, narcotics and caffeine can exacerbate the frequency of convulsions, through the inhibition of GLUT1 (39). Since GLUT1DS is associated to a low glucose availability in the brain, seeking an alternative energy source remains central for an optimal brain growth and development in the long term (43). To achieve it, the KD is considered the first choice of for GLUT1DS, because the KD-derived ketone bodies are able to cross the BBB providing thereby sufficient fuel for the brain. When KD is not feasible or sufficient, another appropriate therapeutic approach should be contemplated.

Recently two compounds, alpha lipoic acid and triheptanoin have been proposed as potential supplementary treatments of GLUT1DS. Alpha lipoic acid is an antioxidant molecule believed to help cellular glucose uptake (45). Triheptanoin is an MCT that is metabolized to ketone bodies with 4 or 5 carbon atoms, unlike KD, which uniquely provide ketone bodies with 4 carbons. Thus, triheptanoin permits to obtain more intermediates of the TCA cycle (46).

4.2.2. Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disease with a high impact on global public health. It is the most common form of dementia and may contribute to 50–60% of cases. Dementia is a generic term for several progressive illnesses that mainly affect elderly people and may cause alterations in memory, thinking, behaviour and emotion, in an enough manner to interfere ability to perform everyday activities (47).

World Health Organization estimates that 50 million people worldwide suffer from dementia and this number is projected to reach 82 million in 2030 and 152 in 2050 (48). This dramatic rise may be explained by an increasing life expectancy of the population (49). Because of its neurodegenerative nature, AD entails burdens not only on people suffering from this disease, but also on their caregivers, families and society in general. Besides, it has significant repercussions in terms of medical care costs (50).

4.2.2.1. Manifestations

Initially, individuals may experience the termed "mild cognitive impairment (MCI) due to AD", in which they suffer a cognitive decline greater than expected for their age, but it does not significantly interfere with daily activities (50), so MCI precedes dementia. These first symptoms may be overlooked and are characterized by forgetfulness and confusion, for example, having problems with remembering newly learned information, losing track of the time or becoming lost in familiar places. Symptoms of AD gradually worsen and become clearer, including severe disorientation, a deterioration in cognitive abilities (such as decision-making and difficulty in performing previously routine tasks), and behaviour, personality and mood changes (47,48,51). More and more, patients have problems recognizing family and friends, difficulty swallowing, speaking and walking, and a larger need for help with personal care. At the end of their lives, individuals are usually in bed and require complete care (50,51).

4.2.2.2. Etiology

More than a century after Alois Alzheimer first described AD, its etiology is not entirely understood yet (14). Numerous factors are involved in the development of AD, both genetic and environmental, that seem to interact with each other (52).

Advanced age is the most important risk factor. The percentage of people with AD rises dramatically with age (figure 4) (14,49). As people get old, there is a deterioration in protective mechanisms for the brain, such as levels of growth factors, optimal energy metabolism and efficient repairing processes, that may lead to a greater AD risk. With ageing, there is also a higher prevalence of cardiovascular diseases and diabetes that can promote AD through vascular or inflammatory mechanisms (52).

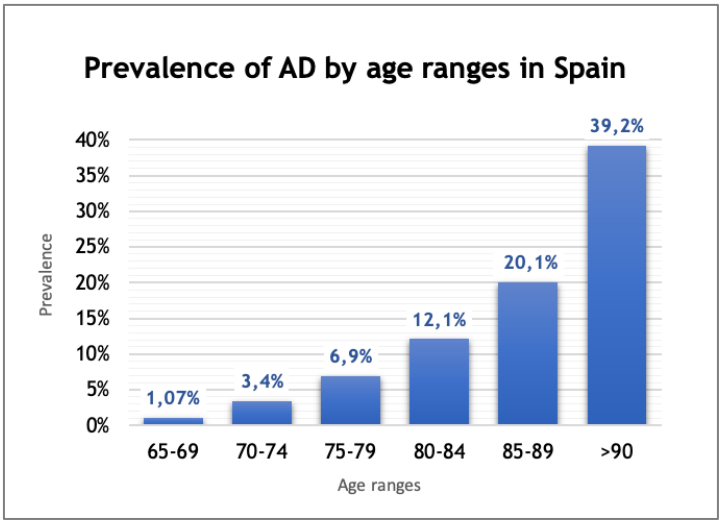

Figure 4. Prevalence of AD by age ranges in Spain (49)

Even though most patients suffering from AD begin to suffer from symptoms after age 65, a very low proportion of total AD cases (probably fewer than 1%) have manifestations earlier. It is called "early-onset" AD, has a faster progression compared to the predominant "late-onset" AD, and is caused by the transmission of autosomal dominant mutations in genes that encode proteins involved in the generation of amyloid plaques, features in AD.

The most established genetic risk factor of the "late-onset" AD is the apolipoprotein E (APOE) ε4 allele, which encodes the APOE4 lipid-carrier. In contrast, the more common ε3 and the rare ε2 alleles are relatively protective against AD (52). APOE has a key role in the maintenance of lipid homeostasis in the brain. ε4 allele carriers have reduced levels of APOE compared with ε4 non-carriers (53). In any case, it should be emphasized that ε4 allele is not essential to develop AD (50).

The female gender may also be a risk factor, as approximately two-thirds of AD patients are women (53). Additional risk factors include depression, low educational attainment, social isolation, cognitive inactivity, toxicants (like aluminium), repeated head injury, or a diet with a high-glycemic index (associated with increased insulin resistance) (11,14,48).

4.2.2.3. Pathogenesis

There are two histopathological hallmarks in the brain that are associated with AD:

- Amyloid plaques, deposits build up in the spaces between neurons. They consist of amyloid-beta (Aß) peptides generated from the amyloid precursor protein (APP), by the enzymes ß-secretase and γ-secretase (51,52).
- Neurofibrillary tangles (NFTs), found inside neurons, result of abnormal hyperphosphorylation and aggregation of tau protein (54).

Although people without AD also develop some plaques and tangles as they age, those with AD accumulate greater amounts (51), due to an imbalance between deposition and clearance.

Aß peptides provoke a loss of synapses that results in a dysregulation of some neurotransmitters, for example, they may promote a decreased release of acetylcholine and neurotrophins (54). Moreover, cells exposed to Aß have found to suffer from disruption in calcium homeostasis, which may lead to an increase in calcium influx via N-methyl-D-aspartate (NMDA) receptors. Thus, because of high intracellular calcium levels, there is an atypical prolonged release of glutamate, leading to excitotoxicity and subsequently cell death (55). As neurons die, the affected regions atrophy or shrink, causing ultimately the AD manifestations. AD progresses from these first symptoms to widespread and more severe neurological complications because the first neurons affected are those in regions involved in memory. The other complications arise as a consequence of the destruction of neurons in other brain regions (50).

In the pathogenesis of AD, other highlight mechanisms seem to be implicated, including mitochondrial dysfunction, hypometabolism of glucose, oxidative stress and cytokine-mediated inflammation, among others.

Aß peptides inhibit relevant mitochondrial enzymes in the brain (54). Mitochondrial dysfunction in turn brings about diminished ATP production from the oxidation of glucose. A reduced uptake and metabolism of glucose may contribute to the progression of AD (14). It correlates with a lower concentration of GLUT1 observed in the brain of individuals with AD (56). Mitochondrial dysfunction also promote oxidative damage, since the main site of ROS generation is, in fact, mitochondria (57). Oxidative stress can trigger an increased Aß deposition, by inducing ß-secretase activity (58). Cytokine-mediated inflammation appears owing to a chronic response of the immune system against brain damage. Also, the BBB suffers from a dysfunction in its effort to protect the brain from oxidative stress and inflammation (54). Additionally, APOE ε4 can accelerate the neurodegenerative course of AD, by inducing an incremented production of Aß peptide and an impairment of its clearance (52).

Many of the pathological features of AD described have been detected even prior to the first clinical symptoms. This stage has been denominated “preclinical AD”, and remains still under investigation (50).

4.2.2.4. Diagnosis

An AD diagnosis with 100% certainty requires a microscopic autopsy of the brain, where 'tangles' and 'plaques' can be detected in damaged areas. But nowadays, AD may be diagnosed in living patients with more than 95% of accuracy, by exclusion of other potential causes for dementia. Prior steps consist of taking the clinical history from patients and their families and evaluating cognitive function by neuropsychological

tests. Then, other causes of dementia are ruled out, such as low thyroid function, vitamin deficiencies, infections, cancer or depression, through brain PET scans and tests of CSF (52).

Since it is known that neuropathology of AD emerges before symptomatology, identifying "preclinical AD" biomarkers has become a challenge, because they would allow an early diagnosis of AD to be established (50).

4.2.2.5. Treatment

On average, people with AD aged 65 and older live four to eight years after diagnosis, but some live up to 20 years with AD. To date, there is no available treatment to prevent AD or modify its progressive course (51). Only a few approved drugs by *Food and Drug Administration* (FDA) may ameliorate the symptoms by regulating the activity of the neurotransmitters. These approved drugs are acetylcholinesterase inhibitors (donepezil, galantamine and rivastigmine), which increase the concentration of acetylcholine at synapses and are indicated for the symptomatic treatment of mild-to-moderately severe AD, and an uncompetitive NMDA receptor antagonist, memantine, that blocks the excitatory effects of glutamate and is indicated for moderate to severe AD. (59).

Still, these medications seem to help patients in a limited duration. Furthermore, it takes a long time to observe whether investigational treatments are effective (50). So, it is of utmost importance to offer support therapies to patients and their families and carers in order to obtain an optimal management of AD and overall quality of life (48). People with AD may also suffer from other disorders, such as depression, apathy, wandering, sleep disturbances, agitation and aggression along AD pathogenesis, which should be treated (50).

4.3. Impact of the ketogenic diets on neurological diseases

4.3.1. Studies associating the ketogenic diets with GLUT1 Deficiency Syndrome

To date, many GLUT1DS patients have been effectively treated with a KD. Most of the documented effects of KDs on this disorder that can be found in the literature come from case reports and series.

Klepper et al. (15) assessed the application of a ketogenic formula in four infants between 6-28 weeks of age suspected of GLUT1DS, who presented seizures and hypoglycorrhachia. The treatment, beginning with an initial fast, was based on a 3:1 ketogenic ratio. Additional sugar-free supplements of vitamin D, iron, fluoride and calcium were administered when necessary. Adequate ketosis was achieved within 24

hours and all four patients did not suffered from seizures during the diet. In general, the therapy was well tolerated, and parental compliance was good. As GLUT1DS diagnosis was only confirmed in two patients, the diet was interrupted in the others. In one infant, MCTs were substituted for LCTs to reverse development failure. Adverse effects were limited to kidney stones in one patient, which were reverted following oral rehydration and alkalization of the urine.

Various researchers evaluated the effects of a MAD therapy on GLUT1DS patients. In all studies, the diet was initiated without fasting, total calories and fluids were not restricted, and carbohydrates were limited to approximately 10g/day.

For instance, a group of Japanese researchers -Ito et al. (60)- described positive outcomes with this therapy, and concluded that the effectiveness of the MAD was similar to the classic KD, as well as MAD seems to be tolerable for a long-term application. They reported the case of a 7-year-old child who suffered from epilepsy at an earlier age and have been treated with anticonvulsants, progressively showed development delay, and episodes of ataxia and loss of consciousness mainly before meals. After several neurological tests and identification of a mutation in the GLUT1 gene, GLUT1DS diagnosis was confirmed and KD therapy was proposed to parents. However, they refused to introduce such a restrictive diet, therefore MAD was chosen. Supplementation of vitamin B1, B6, B12 and calcium was required. After 3 days with MAD, the analysis revealed an increment of ß-OHB levels in blood at over 5 mmol/L. His ataxia and paroxysmal loss of consciousness before meals decreased. After 3 months of treatment, carbohydrates limitation was lowered to 15g/day. MAD was in general well tolerated, without significant side effects. Later, in 2011, the same researchers (61) assessed the response to MAD in six males with GLUT1DS aged 7 to 16 years, during a period ranging from 1 to 42 months. The GLUT1DS diagnosis had been confirmed by mutational analyses or glucose uptake studies. The ketogenic ratio in this study stood at nearly 2.5 to 2.1:1. During all period, urinary ketosis was adequate. The MAD led to an important decrease in seizures and paroxysmal events. Motivation, cognitive function and motor abnormalities improved in most individuals. Only some patients, in the early days after starting the diet, displayed temporarily nausea, vomiting, fatigue, headache, constipation, hyperlipidaemia or hyperuricemia.

Successfully outcomes were also obtained with MAD in a 6-year-old girl in Austria, according to Haberlandt et al. (62). This girl had been diagnosed with GLUT1DS, after detecting hypoglycorrhachia and a mutation in the SLC2A1 gene. At baseline, laboratory testing was performed to exclude metabolic defects. She also underwent to electrocardiography and echocardiography, which were both normal, like the laboratory tests results. ß-hydroxybutyric acid in the initial days of MAD presented values above 2 mmol/L. With MAD introduction, convulsions disappeared, and still remained seizure-free over the follow-up period, that lasted 17 months. Also, her intellectual quotient boosted during the treatment. Although speech problems and motor dysfunction did not enhance, ataxia and muscle hypotonia did.

Interestingly, Kitamura et al. (63) proved that the introduction of a MAD in a 4-year-old girl with GLUT1DS for 18 months permitted to identify a reduction in the levels of oxidative stress markers in CSF and an increment in the phosphocreatine/ATP ratio, suggesting that hypoglycorrhachia may lead to oxidative damage and lipid peroxidation. After the dietary therapy, oxidative damage in the brain was reduced, and energy reserve capacity was improved.

The MAD has also been applied to adolescents or adults suffering from GLUT1DS. Leen et al. (64) evaluated the effectiveness and feasibility of MAD for the treatment of GLUT1DS-related movement disorders, in four patients between 15 to 30 years old. At MAD initiation, they had no seizures or of low frequency. Vitamin supplementation was given, and carnitine was measured in case of suspected deficiency. Carbohydrate intake was raised in steps of 5g if possible, according to clinical judgment. All patients achieved mild-to-moderate ketosis within 1 day to 1 week (ß-OHB values in blood of 0.3– 2.0 mmol/L). With the MAD, paroxysmal movement disorders were effectively treated, as well as cognitive function, according to the caretakers and the examining neurologist. Compliance with the diet was good, and no severe side effects were observed. Lipid profile slightly increased after 3 months on the MAD, but it maintained stable after 6 months and 12 months. Authors concluded that due to compliance is especially difficult for this ranged age, the MAD should be considered as a good and feasible alternative to the classic KD.

Atypical GLUT1DS manifestations have been also demonstrated to respond positively to KD. Friedman et al. (65) informed that a classic KD ameliorated motor function of a 10-year-old boy, who predominantly suffered from movement disorders consisting of ataxia, dystonia and choreoathetosis but, unlike most GLUT1DS patients, he was normocephalic and no clear evidence for seizure activity was noticed. Within one month after KD initiation, ß-OHB levels in serum were stood between 2.86 and 3.12 mmol/L, and an enhancement in motor performance was reached.

Studies comprising larger numbers of individuals have been performed. It is the case of the study by Pong et al. (66) that compiled data from 87 patients with GLUT1DS from August 1989 to December 2010. Seventy-eight (90%) of total patients were confirmed to have epilepsy. A classic KD was used to attain a ß-OHB concentration in blood of 4–5 mmol/L whenever possible. Of the 61 patients with active convulsions at KD initiation, 67% achieved and continued seizure-free with KD, and 83% got seizure freedom with KD alone after withdrawing their pre-existing anti-epileptic drugs. The convulsions resolved within 1 week of initiation of the diet, or within 1 month, although it is worth mentioning that compliance difficulties were reported by 13 of 78 families, and that lower ketogenic ratios were applied to four patients with epilepsy.

With a different method of collecting data, Kass et al. (67) published the experience of 92 patients with GLUT1DS. Information was obtained from the surveys distributed and then collected at the July 2015 GLUT1 Deficiency Foundation biannual parent

conference. The attendant families were primarily from United States, United Kingdom, Italy, Germany, Australia or Japan. In total, ninety-two families completed the survey. GLUT1DS subjects had an age ranging from 1 to 24 years. Diverse types of KD were used: 59 patients were treated classic KD, 29 with MAD, 4 with MCT diet and 2 with LGIT. The diet duration ranged from 1 month to 20 years. Of those patients with seizures, 80% had more than 90% of seizure reduction. The percentage obtained of seizure-free children receiving a KD or an MCT was similar to the percentage from seizure-free cases treated with a MAD or a LGIT.

Published studies associating the KDs with GLUT1DS			
Ref.	**Number of subjects**	**Type of KD**	**Main outcomes**
(15)	4 infants	Ketogenic formula	Disappearance of seizures
(60)	1	MAD	Decrease of ataxia and paroxysmal loss of consciousness
(61)	6	MAD	Reduced seizures and paroxysmal events Improved motivation, cognitive function and motor abnormalities in most individuals
(62)	1	MAD	Disappearance of convulsions Enhancement in intellectual quotient, ataxia and muscle hypotonia, yet not in speech problems and motor dysfunction
(63)	1	MAD	Reduced oxidative damage in the brain Improved energy reserve capacity
(64)	4	MAD	Effectively treated paroxysmal movement disorders and cognitive defects
(65)	1	Classic KD	Enhancement in motor function
(66)	87	Various	Mostly, resolution of the seizures
(67)	92	Various	More than 90% of seizure reduction in 80% of patients with seizures

Table 4. Published studies associating the KDs with GLUT1DS

As triheptanoin is likely to be effective in some patients with GLUT1DS who are refractory to KD, nowadays two active studies explore the compatibility of triheptanoin with KD on subjects diagnosed with GLUT1DS. In one of them (68), patients who tolerate supplies over 50% of calories from fat, have to replace 45% of their daily caloric intake with triheptanoin for 24 hours, dosaged in 4 times. In the other study (69), researchers seek a goal intake of 35% total calories provided by triheptanoin (maximum 100 millilitres of oil/day).

4.3.2. Studies associating the ketogenic diets with Alzheimer's disease

Relatively few studies on the involvement of KD in AD have been done. With this disease, the main studies carried out are both preclinical and clinical trials.

Preclinical trials

Kashiwaya et al. (70) showed that the addition of D-ß-OHB protected cultured hippocampal neurons against the toxic effects of $Aß_{1-42}$, a fragment of amyloid protein. Concretely, in this study, cultured cells were exposed to 5 micromole/L of $Aß_{1-42}$ for 14h, and there was a decrease in neuronal number. However, the addition of 4 mmol/L of D-ß-OHB doubled their surviving.

Other studies made on mice models of AD have tested the effects of the KD or ketone bodies. In a study by Van der Auwera et al. (71), two groups of 8 female transgenic mice each were fed either a standard diet or a KD for 43 days. The mice were carrying the "London" APP mutation, that drives to produce significant levels of soluble Aß in the brain and exhibit further plaque deposition, representing a model of early-onset AD. At all times, animals had totally access to the diet. During the first seven days, many of the 8 mice following KD were reluctant to eat the diet and lost weight, so the standard diet was mixed to KD since day 16 until day 27. During this period, KD-fed mice gained weight. After day 28, these mice were returned to KD only. At all time, ß-OHB levels in blood maintained higher in the KD group in comparison to the standard diet group, yet mixed diet on days 16-28, led to a reduction of ketone bodies. Cognitive performance was also examined, resulting in no differences between the groups. At day 43, levels of soluble Aß were measured. The KD group were found to have significantly lower levels. Another study carried out in symptomatic mice models of AD by Yin et al. (57) revealed an improvement in cognitive function. These mice were overexpressing human APP. After acute exposure to exogenous oligo-$Aß_{42}$, mice had increased levels of $Aß_{42}$, stronger oxidative stress, and mitochondrial dysfunction. However, a ketone bodies delivery through subcutaneous injections blocked oligo-$Aß_{42}$ entry, therefore achieving a reduction in the number of plaques. Mitochondrial dysfunction, oxidative damage and cognitive decline were reversed.

Studzinski et al. (72) described positive effects in brain energy metabolism of aged dogs after short-term administration of MCTs. The animals were fed a 2 g/kilogram/day dose of MCTs for 2 months, and presented ameliorated mitochondrial function, due to a decrease in oxidative stress. APP levels also diminished with ketosis induced by MCTs.

Nevertheless, KD seems unlikely to improve cognition performance according Brownlow et al. (73), who indicated that within 4 months, a KD rich in MCTs did not reverse cognition failure but did enhanced motor function in two transgenic mouse lines, APP/PS1 or Tg4510, models of amyloid and tau deposition, respectively. Moreover, amyloid and tau markers showed no differences between animals fed the

control diet or the KD. In KD-fed mice, ketosis was effectively reached (>1mmol/L ketone body levels) and stood throughout the experiment, whereas plasma glucose levels remained significantly low and body weight, unchanged. Another study, by Beckett et al. (74), drew the same conclusions. After 1 month of daily KD administration, mice carrying APP had a better motor performance and no changes in cerebral or muscle Aß deposition. This diet also elevated ketone bodies levels to 1mmol/L. Furthermore, this study investigated KD effects on oxidative stress, resulting in no effect on it.

In an attempt to assess whether supplementing a 4:1-ratio KD with triheptanoin would boost the effectiveness of the diet, Aso et al. (75), tested the treatment in APP/PS1 transgenic mice for 3 months. The outcomes indicated that this intervention led to a reduction in memory impairment and in the expression of the pro-inflammatory cytokine interferon gamma, as well as an upregulation of genes encoding ROS detoxification. However, Aß production and deposition remained unaltered. Authors concluded that triheptanoin-rich KDs might be helpful for AD.

Preclinical studies associating the KDs with AD		
Ref.	**Model**	**Main outcomes**
(70)	Cultured neurons	The addition of 4 mM D-ß-OHB doubled the surviving of cultured neurons exposed to Aß
(71)	Transgenic mice	No difference in cognitive performance between the KD-fed mice and the standard diet-fed mice Lower Aß levels in the KD-fed mice
(57)	Transgenic mice	Better mitochondrial and cognitive function, whilst reduced oxidative damaged and number of Aß plaques
(72)	Aged dogs	Ameliorated mitochondrial function due to a reduction of oxidative stress, in addition to diminished APP levels
(73) (74)	Transgenic mice	Improvement in motor abilities and energy metabolism No effects on cognition failure and Aß deposition
(75)	Mice	Decrease in memory impairment and pro-inflammatory cytokines. Upregulation of genes encoding ROS detoxification. No changes Aß production and deposition
(76)	Mice	Boosted learning and memory ability and diminished Aß and tau deposition

Table 5. Preclinical studies associating the KDs with AD

Studies evaluating the effects of ketone esters on AD have also been developed. Kashiwaya et al. (76) studied the therapeutic benefits of supplementing a synthetic ketone ester on AD, concretely, (R)-3-ß-OHB-(R)-1,3-butanediol monoester. Two groups of mice models of AD were fed either a diet containing this ketone ester (21.5 % of energy from ketone ester and 43.5%, from carbohydrates) or an isocaloric

carbohydrate diet (with 64,9% of energy from carbohydrates). During the treatment, mice fed the ketone ester diet lost weight and had greater ß-OHB levels in blood. The results of behavioural tests carried out at 4 and 7 months after diet initiation, displayed boosted learning and memory ability on the mice fed the ketone ester, in addition to diminished Aß and tau deposition, compared with the mice fed the other diet.

Clinical trials

Evidence of KDs or ketosis-inducing treatments in patients with AD is very preliminary, holding the treatments with MCTs, the most evidence.

The first randomized controlled trial in humans was published in 2004 by Reger et al. (77). On different days, 20 older adults with MCI or AD consumed a drink containing either emulsified MCTs or placebo. After 90 minutes of MCTs ingestion, ß-OHB levels in serum elevated, and correlated with an improvement in the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) scores but only in subjects without the APOE ε4 allele.

In line with these findings, other 2 studies were performed using MCT oil. On one hand, Henderson et al. (78) performed a large trial including 152 individuals with mild-to-moderate AD. They were offered daily either 20 g caprylic triglyceride (referred to as AC-12020) treatment or placebo for 3 months. Two hours after AC-1202 administration, ß-OHB levels in serum showed a higher elevation compared to placebo, which was associated positively with better ADAS-Cog scores on day 45 and day 90, in relation to the baseline. However again, no such effect was observed in subjects with APOE ε4. Adverse effects were described more frequently in patients receiving AC-1202, although they were principally mild-to-moderate in severity and limited to gastrointestinal problems. On the other hand, in the study published by Rebello et al. (79), six subjects with MCI were approached to participate in a randomized double-blind controlled trial for 24 weeks. However, two participants dropped out of the study. Thus, half of the remaining subjects received 56 g/day of MCT oil (one carrying APOE ε4 and the other not), whereas the other half, the placebo. Subjects easily incorporated the treatment into their diet and did not experience weight loss. MCT oil intake led to an increase in ketone bodies levels in serum and enhanced memory. At the end of the study, placebo-treated subjects had no changes in memory or overall ADAS-Cog scores, whilst the two individuals receiving the MCT oil, had better scores in word recall, word recognition and remembering test instruction. It should be noted that the APOE ε4 non-carrier had an improvement in the overall ADAS-Cog scores, whereas the APOE ε4 carrier had a decline due to a failure in orientation.

Lately, Croteau et al. (80) investigated the effects of two MCT supplements on 15 patients with mild-to-moderate AD. Participants sequentially consumed 30 g/day of the supplements, both for one month: a mixture of caprylic and capric acids, followed by a wash-out and then tricarprylin. Brain acetoacetate and glucose uptake were quantified by PET before and after each MCT intervention. Finally, eleven participants

completed the protocol, and results indicated no variation in cerebral glucose levels, but doubled brain ketone uptake on both supplements, which in turn, correlated with plasma ketone bodies concentrations. Thus, it was demonstrated that both MCT supplements boosted total brain energy metabolism by increasing ketone bodies supply without affecting cerebral glucose uptake.

Very few investigations concerning the KDs have been performed in people with MCI or AD. In a randomized controlled trial by Krikorian et al. (81), 23 older adults with MCI followed either a high-carbohydrate (50% of energy from carbohydrates) or a very low-carbohydrate diet (5-10%) for 6 weeks. The low-carbohydrate diet provoked an increment in ketone bodies levels, that positively correlated with better verbal memory scores from baseline to the sixth week. Despite this diet had a lower caloric value (1000 kcal) compared to the high-carbohydrate diet (1600 kcal), the carbohydrate-restricted subjects lost more weight. In this study, the authors suggested that other mechanisms such as reduced inflammation and enhanced energy metabolism also may have helped to improve cognitive abilities.

More recently, Brandt et al. (82) investigated the feasibility of using a MAD to induce ketosis in patients with MCI or early-onset AD, and the effect of this dietary therapy on memory and other clinical outcomes. In the study, 27 participants were randomly assigned for 12 weeks to either the National Institute on Aging (NIA) recommended diet for seniors (a low-fat, high-carbohydrate diet), or a MAD. In the end, 9 patients following the MAD and 5 with the other diet completed the trial. At week 6, MAD-adherent subjects showed better memory scores, whilst on the contrary the non-adherent subjects, a decline. Regardless adherence influence, within this period, MAD participants increased their energy levels. At 12 weeks, none of the 14 completing participants had a significant enhancement in memory scores. Simultaneously, in a single-arm pilot trial by Taylor et al. (83), 15 patients with mild-moderate AD maintained an MCT-supplemented ≥ 1:1 ratio KD for 3 months. The diet consisted of 70% of energy as fats (including the MCT oil), 20% energy as proteins and less than 10% of energy as carbohydrates, and was characterized by a high intake of non-starchy vegetables, butter, eggs, olive oil, avocados, nuts and seeds. MCT oil contained a mixture of two fatty acids (caprylic and capric acids) and represented approximately 10% of energy from fats during the first week. This proportion raised each consecutive week until 40%. 9 out of 10 compliant patients that completed the treatment and achieved ketosis, obtained higher ADAS-Cog scores. However, after a 1-month suspension of the diet, the mean ADAS-Cog score returned to the baseline.

To date, evidence for treatment of AD with supplementation of a ketone ester has only been reported in one-case study published in 2015 by Newport et al. (84). After a prolonged oral administration of a ketone monoester, a 63-year-old AD patient carrying the APOE ε4 allele improved significantly his mood and demeanour, as well as his ability to perform daily activities. The ketone monoester was well tolerated throughout the 20 months of treatment.

Clinical studies associating the KDs with AD			
Ref.	**Type of study**	**Protocol**	**Main outcomes**
(77)	Double-blind placebo-controlled trial	20 patients with MCI or AD consumed a drink with MCTs	Improvement in memory and cognition function but only in subjects without the APOE ε4 allele
(78)	Randomized, double-blind, placebo-controlled multicenter trial	152 subjects with mild-to-moderate AD took AC-1202 over 90 days	Enhancement in ADAS-Cog scores. Reduced response to AC-1202 in subjects with APOE ε4
(79)	Randomized, double-blind placebo-controlled parallel trial	6 patients with MCI consumed 56 g/day of MCTs over 24 weeks	Improved ADAS-Cog scores in the APOE ε4 non-carrier
(80)	Randomized, placebo-controlled parallel trial	15 subjects with mild-to-moderate AD sequentially took 2 MCT supplements	Boosted total brain energy metabolism by increasing ketone bodies supply, with no effects on cerebral glucose uptake
(81)	Randomized, placebo-controlled trial	23 patients with MCI were given a high carbohydrate or very low carbohydrate diet over 6 weeks	Enhanced verbal memory scores, probably through reduced inflammation and higher energy metabolism
(82)	Randomized, placebo-controlled parallel trial	27 patients with mild AD or MCI consumed either a MAD or the NIA recommended diet for seniors	Increased energy levels but no significant changes in memory scores
(83)	Single-arm pilot trial	15 patients with mild-to-moderate AD maintained an MCT-supplemented ≥ 1:1 ratio KD for 3 months	Greater ADAS-Cog scores
(84)	Single-patient case study	One APOE ε4 carrier with early-onset AD was supplemented with a ketone ester over 20 months	Better mood, demeanour and ability to perform daily activities

Table 6. Clinical studies associating the KDs with AD

Ongoing trials

Several registered trials assessing the efficacy of KD on MCI or AD patients, sponsored by distinct universities, are underway. The findings will contribute to obtain a wider knowledge of ketogenic therapies in AD.

For example, a study sponsored by University of Kansas (85), still recruiting, investigates the adherence of 80 participants with AD, for 3 months to either a 1:1 KD (approximately 70% fat, less than 10% carbohydrate, and 20% protein as energy) or a "Therapeutic Lifestyles Changes diet" (20-35% fat, 50-60% carbohydrate, and 15% protein as energy). Moreover, with the KD treatment, 4 or more servings of non-starchy vegetables and ½ cup of berries must be provided daily. The researchers want to measure the changes in cognition performance, brain metabolism, and mitochondrial function.

Another example is a study sponsored by Wake Forest University. The aim of the researchers is examining the effects of a 4-month "Modified Mediterranean KD (MMKD)" in adults with MCI (86). The study is designed to randomly assign 120 people with MCI to receive either the MMKD or a low fat/high carbohydrate diet. The MMKD has a restriction of carbohydrates of less than 20 g/day, and contains plentiful fish, lean meats and nutrient-dense foods. Also, it is supplied with extra virgin olive oil and a multivitamin product. Researchers want to measure concentrations in CSF, cognition performance and cerebral blood flow.

5. Discussion

Since the first description of KD in the beginnings of 1920s, the clinical outcomes derived from a ketogenic therapy have been widely reported and evaluated, especially in the last few decades.

Although the most extended KD use is for drug-resistant epilepsy, the study of the different mechanisms by which it exerts its antiseizure effects (which are not yet fully understood) has allowed the approach of its use as a potential therapy for other diseases. In this work, some neuroprotective effects of KD have been described in order to relate them to the two diseases treated, GLUT1DS and AD, both of which are characteristic of the neurological system. However, other non-neurological illnesses could benefit from KD. For instance, with its anti-inflammatory properties, KD could reduce the risk of cardiovascular diseases or complement their treatment, since they are characterized precisely by a chronic low-grade inflammation.

The effectiveness of the KD has been proved globally on many occasions, in both children and adults, as it is evidenced in the studies reviewed. This effectiveness appears to be associated with reaching an appropriate ketosis state, which is usually measured by ß-OHB levels, and is usually achieved with whatever type of KD is used.

Given that KD emerged to treat epilepsy, it makes sense that KD would be the first-choice treatment for the GLUT1DS, since the latter is often characterized by seizures. In both epilepsy and GLUT1DS, KD represents an alternative fuel for the brain. The most studied effects of KD on GLUT1DS are anticonvulsants. However, there are cases of GLUT1DS where seizures do not occur, like the one reported in the study by Friedman et al. (65), but still, KD enhances motor function. These cases reinforce the idea that KD can exert beneficial effects beyond seizure control.

In general, KD variants have been efficiently applied in both GLUT1DS and AD. Whilst in GLUT1DS mainly the classic KD and MAD have been implemented, MCT supplementation predominate in MCI or AD studies. I could intuit that since GLUT1DS affects mostly young patients, it is easier for them to follow a strict diet as the classic KD is, than patients with AD, typically older people. For patients with AD, supplementation with MCTs would be more advisable and feasible, since they are more susceptible to malnutrition, chewing and swallowing problems, and low compliance. In fact, for adolescent or adult patients with GLUT1, where compliance poses challenging, it seems more feasible to use MAD instead of classic KD.

The diverse studies concerning on GLUT1DS, exemplify the wide range of manifestations and the diagnostic methods described, as well as the protocol followed in the application of KD. The wide spectrum of presentations of GLUT1DS should be known by physicians in order to facilitate both early diagnosis and treatment, keys to improve the long-term neurological outcome. According to the cases explained by Ito et al. (60,61) and Haberlandt et al. (62), the diagnosis of GLUT1DS is confirmed when the mutation of the SLC2A1 gene is detected. In terms of KD protocol, patients usually need dietary supplementation, and it is likely that starting KD without fasting is equally effective at the end. Also, the side effects appeared in patients with GLUT1DS are similar to those observed in the treatment of epilepsy. Examples of it are the kidney stones informed by Klepper et al. (15), the gastrointestinal problems, hyperlipidemia and hyperuricemia from the study of Ito et al. (61), and the lipid increase and later stabilization reported by Leen et al. (64). As children with GLUT1DS must continue the KD therapy until adolescence, the long-term effects of the diet, such as growth impairment and atherosclerosis, would be of more concern. Additionally, the neuroprotective ability of KD on GLUT1DS is demonstrated in the study by Kitamura et al. (63) by lowering oxidative stress and increasing the patient's energy reserves.

Seeing that most of the studies linking KD to GLUT1DS are reports or case series, it seems logical to me that they are of this type -unlike the studies about MCI or AD-, since GLUT1DS mainly affects children. Many of the published studies involve very few patients, making the results difficult to interpret. However, the studies that collect a larger number of cases (66,67), allow to reinforce the idea that ketogenic therapies are effective in GLUT1DS.

It may appear that the two disorders treated in this project have nothing more in common than the beneficial effects of KD. However, they share the impairment of the

GLUT1 transporter, leading to a deficient metabolism of glucose in the brain and subsequently a failure in the nourishment of brain cells. Therefore, the KD would serve as an effective alternative fuel in both diseases. Besides, some researchers have investigated the compatibility of supplementing a KD with triheptanoin. The two about GLUT1DS are still active, but the described study concerning AD have shown positive outcomes. As it is a molecule that provides more intermediaries to replenish the TCA cycle, I think it can be beneficial in both disorders.

The 4 neuroprotective effects explained of the KD are consistent with some of the hallmarks of AD (mitochondrial dysfunction, oxidative damage and inflammation) as it is demonstrated in several of the studies presented. Since these features seem to develop before clinical manifestations, one strategy could be supplementation with MCTs in the preclinical phase of AD for people with a family background of AD.

The study carried out by Kashiwaya et al. (70) in cultured neurons and the studies in mice models of AD focus almost exclusively on the role of ketogenic therapies in Aß pathology and changes in cognition function. Some aspects are worth commenting on. On the one hand, the lack of effect on cognitive function observed by Van der Auwera et al. (71) could be due to the reduction in the level of ketosis from day 16 to day 27 after mixing KD with the standard diet to mitigate the level of weight loss in mice fed KD. On the other hand, the positive results of KD reported by Studzinski et al. (72) on cognitive functioning, mitochondrial function, oxidative damage and plaque formation in aged canines, are reinforced by those informed by Yin et al. (57) in mice, taking into account that the experiments were performed not only on different models of AD, but also using distinct routes of administration -oral route or subcutaneous injection-. However, cognitive improvement is not demonstrated in the studies by other studies (73,74). It is possible that no changes in Aß were detected because of the selected neuronal populations or the use of different mice models.

Trials in humans that investigate the role of ketogenic therapies on MCI or AD are mostly placebo-controlled clinical trials. Therefore, the protocol to be followed is that of the study rather than the KD protocol explained in this project. Besides, these clinical studies focus on determining the effects on cognitive performance, measuring it mainly through ADAS-cog scores. Carriers of the APOE ε4 allele have an increased risk to develop AD. This correlates with the outcomes obtained in the Reger, Henderson and Rebello et al. (77–79) studies, in which people with MCI or AD who do not carry this allele had a better cognitive performance, unlike carriers. Therefore, the APOE4 genotype influences the response to ketogenic therapies.

Few studies test the use of a KD in patients with MCI or AD. The three studies described demonstrate a cognitive enhancement, and also show how not all participants complete the study, mostly owing to lack of compliance. In addition, the study by Taylor et al. (83) exemplifies the type of foods consumed in a KD: non-starchy vegetables, butter, eggs, olive oil, avocados, nuts and seeds.

Even though the exact physiopathology of AD has not been totally elucidated and there are no current treatments that stop AD course, the encouraging outcomes of KD derived from preclinical and clinical trials exemplify the constant interest in searching new effective therapies for such an incident disorder, that causes heavy burdens for affected people and their families or caregivers. In fact, the studies by Kashiwaya et al. (76) and Newport et al. (84) establish the ketone esters as a promising ketogenic therapy. Furthermore, the patient from the second study is an APOE ε4 carrier, which prompts to suggest that a ketone ester could be useful in patients carrying such allele.

To sum up, it is likely that there is more evidence from the implication of KD in GLUT1DS than in MCI or AD. The main studies in humans done about GLUT1DS are case reports, whereas clinical trials controlled by placebo are predominant in patients with MCI or AD. Moreover, it should be noted that GLUT1DS is the principal target of KD, apart from refractory epilepsy. Nevertheless, all preclinical or clinical trials carried out assessing the impact of KD in MCI or AD, and those that are ongoing, collectively, help to establish more evidence for the potential role of KDs in this disorder.

6. Conclusions

The objectives of the project have been consolidated. The KD effectively causes the typical ketosis observed after prolonged fasting. Its implementation involves following a strict clinical protocol to monitor the effectiveness of the diet, as well as its tolerance and compliance, because dealing with the possible appearance of side effects can be challenging. Over the past years, the beneficial effects of KD proved and their diffusion through organisms like the *Charlie Foundation* have contributed to expand the clinical use of KD.

As a consequence of the increasing knowledge of the implicated mechanisms of action of the KD-derived ketone bodies, that appear to go beyond seizure control, several disorders seem to be potential targets of a therapy with KD. Multiple cases of GLUT1DS have reported positive effects of KD on the wide spectrum of manifestations -not only on seizure resolution-. In addition, the ketone bodies exert a collection of neuroprotective effects that could benefit some defects existing in AD. Both animal model studies and human trials with AD present ketogenic therapies as promising alternatives for such an incident disease in our population and without a current cure.

As it has been discussed, more and more, the role of KDs in different diseases is being investigated. Additionally, there is an increasing interest in some ketosis-inducing supplements such as triheptanoin and ketone esters as potential alternatives to the restrictive KDs. Further research will help to a better understanding of the mechanisms through which ketogenic therapies exert on various diseases.

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