The Libyan International Medical University

Faculty of Basic Medical Science

The ketogenic diet can prevent the Alzheimer's disease?

Fatma almahdi abeida -2nd year dentistry student

Supervised by: ibtisam alkzairi

Assisted by:nawar

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that primarily strikes the elderly. Studies in both humans and animal models have linked the consumption of cholesterol and saturated fats with amyloid-β (Aβ) deposition and development of AD. Yet, these studies did not examine high fat diets in combination with reduced carbohydrate intake. This report is aimed to reveal the effect of a high saturated fat/low carbohydrate diet on a transgenic mouse model of AD,Starting at three months of age, two groups of female transgenic mice carrying the "London" APP mutation (APP/V717I) were fed either, a standard diet (SD) composed of high carbohydrate/low fat, or a ketogenic diet (KD) composed of very low carbohydrate/high saturated fat chow for 43 days. Animals fed the KD exhibited greatly elevated serum ketone body levels, as measured by β-hydroxybutyrate, compared to SD fed animals. In addition, animals fed the KD lost body weight. In contrast to earlier studies, the brief KD feeding regime significantly reduced total brain Aβ levels by approximately 25%. Despite changes in ketone levels, body weight, and Aβ levels, the KD diet did not alter behavioral measures. Previous studies have suggested that diets rich in cholesterol and saturated fats increase the deposition of Aβ and the risk of developing AD. Here we demonstrate that a diet rich in saturated fats and low in carbohydrates can actually reduce levels of Aβ. Therefore, dietary strategies aimed at reducing Aβ levels should take into account interactions of dietary components and the metabolic outcomes, in particular, levels of carbohydrates, total calories, and presence of ketone bodies should be considered.

Introduction

Alzheimer's disease (AD) is an age-associated neurodegenerative disease that is very common in the US, affecting up to 50% of people between the ages of 75 to 84 years [1]. The number of cases of AD will increase dramatically in the next 50 years due to the aging population of the developed world and will present an increasing medical challenge Clinically, AD is characterized by progressive impairment in memory and language and is frequently accompanied by behavioral symptoms, such as anxiety and depression. Pathologically, AD is characterized by accumulation of senile plaques, dystrophic neurites, and neurofibrillar tangles. The plaques contain large amounts of the β-amyloid (Aβ) peptide derived from cleavage of the amyloid precursor protein (APP). Mutations in APP that result in increased generation of a particular form of Aβ (Aβ42) have been identified in familial cases of AD and this connection has led to the hypothesis that Aβ is central to the etiology of AD . However, APP functions as a vesicular transport protein and the etiology of the disease may not be directly related to Aβ, but rather to abnormal cleavage of APP and failure to efficiently move vesicles in the axons [4]. While the precise role of Aβ in AD remains unresolved, it is clear that Aβ serves as a pathological marker for the disease. The development of AD and the accumulation of Aβ have been linked to dietary factors. Diets rich in saturated fat have been repeatedly implicated in epidemiological studies , though they have been difficult to reproduce . In addition, several experiments in mouse models seem to confirm the link between lipid rich diets and AD. Using transgenic mouse models of AD several groups have reported that high fat diets or diets with added cholesterol increased levels and deposition of the Aβ peptide . However, these studies did not examine the effects of lipid rich diets in combination with low carbohydrate intake. Diets that contain very low carbohydrate and high fat content are well known to induce the hepatic production of ketone bodies (β-hydroxybutyrate, acetoacetate and acetone) and are often referred to as ketogenic diets (KD). Ketogenic diets in some aspects mimic starvation and were developed for use in humans to treat epilepsy based on the long record of observations that fasting reduces seizures . The experimental KD is calorie restricted and has fixed composition and is thus different from low carbohydrate diets used for weight loss, which are usually *ad lib* and variable in composition. Despite these differences, low carbohydrate diets may also be effective in preventing seizures and may work through

similar mechanisms as a KDThe precise mechanism for the anti-convulsant properties of these diets is still unknown. The low carbohydrate content of both diets induce many metabolic changes that may be protective, such as elevated circulating ketone body levels, increased oxidation of fats, changes in protein metabolism, and changes in gene expression .

The aim: report is made in order to evaluate the effect of ketogenic diet involvement in treatment of Alzheimer disease

Methods

Sixteen APP [V717I] C57Bl × FVB female mice of 3 months of age were used for this study. Mice were housed under a reversed day-night rhythm: 14 hours light/10 hours darkness starting at 7 p.m. in standard metal cages type RVS T2 (area of 540 cm2). All mice were genotyped by polymerase chain reaction (PCR) at the age of 3 weeks. Mice were blind randomized and age-matched and had free access to pre-filtered and sterile water (UV-lamp). Mice had free access to either ketogenic (KD) (code F3666, Bio-Serv, Frenchtown, US) or standard (SD) chow (Muracon-G, Trouw Nutrition, Gent). The F3666 chow is a runny paste and was given in special designed liquid food suppliers and was refreshed daily. F3666 is a liquid chow and the animals frequently spilled the chow in the cage. Also, since all the animals in a given group were housed together in a single cage, measuring chow intake per animal was not possible and was not recorded. Due to some problems with weight loss in animals in the KD group, these animals were fed a mixed chow 1(SD):3(KD) starting at day 16 until day 20. From days 21–27 the amount of SD chow was reduced to a few crumbs sprinkled over the KD chow. After day 28 the animals were returned to KD chow only. However, one mouse in the KD group refused food intake and died despite attempts of feeding via gavage. One control animal died during blood draw. Blood was collected from anesthetized mice from either the orbital plexus or via a heart puncture. β-hydroxybutyrate levels were measured spectrophotometrically using the Stanbio liquicolor β-hydroxybutyrate kit (Stanbio Inc., Boerne, Texas).

Results

The present study tested experimentally the effects of a KD composed of extremely low carbohydrate and very high saturated fat content in a transgenic mouse model of AD. The mice express a human APP gene containing the "London" APP mutation (APP/V717I) driven by a *thy-1* gene promoter. APP/V717I transgenic mice produce significant levels of soluble Aβ in the brain as early as 3 months of age and exhibit extensive plaque deposition by 12–14 months. The animals demonstrate early behavioral deficits and represent a model of early-onset familial AD

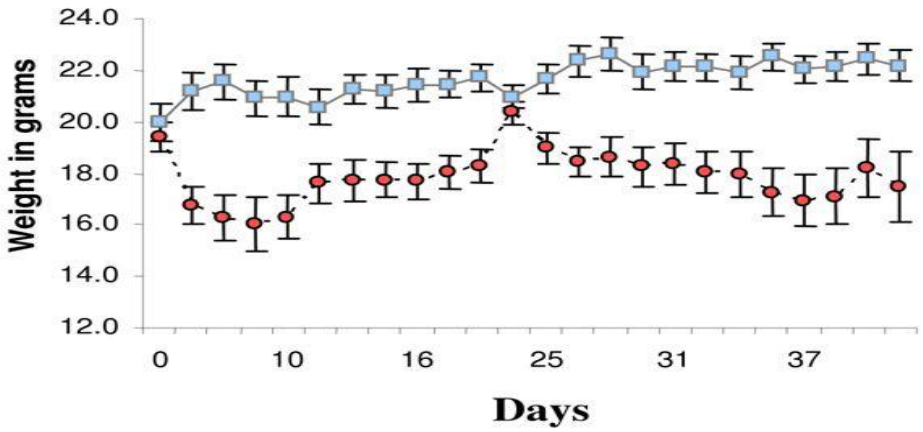
Diet

Sixteen female APP/V717I mice were fed *ad libitum* on a standard diet (SD) comprised of a high carbohydrate/ low fat chow (Muracon-G chow: 35% carbohydrate, 21% protein, 4.5% fat, 39.5% water, fiber). The predominant fatty acid in Muracon-G is linoleic acid (18:2) and it comprises 1.4% of the chow by weight. At three months of age half the group (8 animals) was switched to a ketogenic diet (KD) comprised of very low carbohydrate/high fat chow while the remaining 8 animals remained on the SD. In both cases the animals had free access to chow at all times and intake was not experimentally limited. For the KD we used Bio-Serv Inc. F3666 chow: 0.76% carbohydrates, 8% protein, 79% fat, 12% water, fiber, and ash. F3666 is a ketogenic chow composed of lard, butter fat, dextrose, casein, fiber, corn oil, mineral mix, and a vitamin mix. F3666 is rich in saturated fats. Greater than 29% of the F3666 chow is composed of saturated fats by weight: 2.4% myristic acid (C14:0), 18.9% palmitic acid (C16:0), and 8.4% stearic (C18:0). The animals fed the F3666 chow are referred to as the KD group. The mice that remained on the Muracon-G chow are referred to as the SD group.

KD diet and weight loss

During the first 7 days many of the animals in the KD group were reluctant to eat the new chow and lost weight. To improve consumption and mitigate weight loss, SD chow was mixed with KD chow at a ratio of 1:3 starting at day 16 until day 20. For the seven days following day 20 the amount of SD chow was reduced to a few crumbs sprinkled over the KD chow. After day 28 the animals were returned to KD chow only. The mixed

chow restored body weights of the KD group to approximately the level of the SD group, at about 20 grams (Figure 1). When the animals were fed KD chow exclusively, body weights again dropped, yet tended to stabilize at approximately 18 grams (Figure 1). At the conclusion of the experiment mean weights were significantly different



KD diet elevates serum β-hydroxybutyrate levels

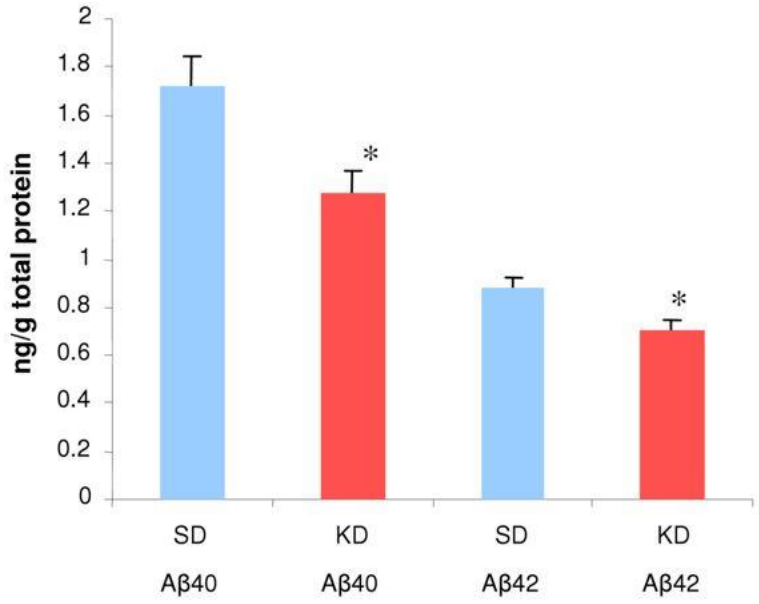
To measure the effectiveness of the chow to induce a ketogenic state, blood samples were taken weekly and examined for levels of β-hydroxybutyrate (BHB). Eight days after switching the chow animals in the KD group had greatly elevated BHB levels compared to the SD group, possibly due to some animals not eating. As expected, feeding the mixed chow on days 16–28 reduced serum ketone bodies. Yet, at all-time points examined after day 0 ketone levels were significantly greater in the KD fed group compared to the SD group.

Cognitive testing

After 38 days on the diet animals were tested for behavioral deficits using object recognition tests as previously described [18], see methods. Despite the differences in chow, BHB levels, and weight loss, no difference in behavioral measures were detected between the groups

Aβ levels

At four months of age APP/V717I mice do not possess Aβ positive plaques and all the Aβ is present in the soluble fraction [19]. Therefore, 43 days after dietary change levels of soluble Aβ in the brain were measured in both groups of animals. Brain homogenate was isolated ,One hemisphere from each animal was analyzed for both Aβ 40 and 42 levels using the Amyloid Aβ40 or Aβ42 ELISA High Sensitivity Kit (The Genetics Company, Zurich, Switzerland). Levels of both soluble Aβ 40 and 42 were found to be significantly lower in the KD fed group (Figure 3). In cases of familial AD excess Aβ42 is produced relative to Aβ40 thereby increasing the Aβ 42/40 ratio. We examined the ratio of Aβ42 to Aβ40 and found no difference between groups (SD 0.51 ± 0.024 vs. KD 0.56 ± 0.026, p= 0.2872), suggesting that the diet did not alter cleavage sites on APP, but instead promoted a general lowering of Aβ species.



Total protein levels were examined to determine if a general decline in brain protein in the KD group could explain the decrease in Aβ levels. However, protein levels, measured as mg/ml of brain homogenate, did not differ between the two groups (SD 0.56 ± 0.035 mg/ml vs. KD 0.51 ± 0.017 mg/ml, p= 0.213). Since most (though not all) of the animals in the KD group lost weight, the animals with the greatest weight loss may have been expected to have the lowest Aβ levels. However, the levels of Aβ40 or

Aβ42 did not correlate with weight change across all groups. Both of these measures suggest that the Aβ lowering effect is not the result of a general lowering of protein levels due to weight loss.

A better measure of the effectiveness of the ketogenic diet was serum BHB levels, since all the animals in the KD group exhibited elevated BHB levels. When Aβ40 and 42 levels from all animals were correlated with the average serum BHB over the course of the experiment, a significant correlation was observed. However, this correlation was most likely driven by the large differences in BHB and Aβ levels between the two groups, since there was no significant correlation between Aβ and BHB levels in the KD group alone

Discussion

This study demonstrated the unexpected result that a brief treatment with a low carbohydrate/high saturated fat diet reduced total Aβ levels in a mouse model of Alzheimer's disease. Previous studies had suggested that diets rich in saturated fats or cholesterol increased both the production and deposition of Aβ in mouse models of AD, leading to the suggestion that diets rich in lipids were a factor in AD [10, 12–14]. However, these diets were not low carbohydrate diets. In the high cholesterol diets, cholesterol was added to the diet without reduction in other components [10, 14]. In the studies of high fat diets, carbohydrate content was still relatively high. For example, Ho *et al*. used a diet of 60% fat, 20% carbohydrate, 20% protein. This diet was sufficiently high in carbohydrate to cause large increases in body weight in the animals [11].

The interaction of different macronutrients, in particular fats and carbohydrates, is known to influence the metabolic state of the animal. For example, Marsset-Baglieri et *al*. examined if fat in the diet alone was sufficient to shift energy balance toward fat storage. Yet, rats fed *ad libitum* high fat (50%) diets devoid of carbohydrates did not increase energy intake and did not gain in body adiposity, while animals fed high fat diets (30%) in the presence of carbohydrates (56%) increased energy intake and gained in body fat. Such studies support the view that when fat and carbohydrates are consumed simultaneously, the carbohydrates stimulate insulin secretion and thereby

promote storage of fat ,Therefore, it is important to consider the macronutrient profile of the diet when examining the effects of dietary fat on biological processes.

In the present study, transgenic animals were fed *ad libitum* a very high fat (79%) diet that was practically devoid of carbohydrates (0.76%). The KD resulted in ketone body production, weight loss, and decreased Aβ levels. Hence, the data presented here suggests that it may not be fats in the diet that increases Aβ levels, but perhaps levels of total calories, carbohydrates, or the metabolic state of the animal.

Epidemiological studies in humans have implicated saturated fats in the diet as a risk factor for Alzheimer's disease. For example, Kalmijn *et al*. correlated eating habits with incidence of dementia after a two year follow-up in a large study of 5,386 subjects in Rotterdam, NL. The results from this analysis led the author to suggest that diets rich in saturated fats and cholesterol increased the risk of several types of dementia [5]. However, after a 6 year follow-up of this same population, no correlation between dementia and fat intake could be identified, leading the authors to conclude "High intake of total, saturated, and trans fat and cholesterol and low intake of MUFA, PUFA, n-6 PUFA, and n-3 PUFA were not associated with increased risk of dementia or its subtypes." [9]. More recent studies have also examined the link between dietary fat and cognitive decline. In a study of 2,560 participants ages 65 and older in the Chicago Health and Aging project, fat intake was measured by food questionnaire and correlated with cognitive testing examined after a 3 and 6 year follow- up. This large study found only weak trends between saturated fat and cholesterol intake and cognitive decline . Both the rodent and human studies highlight the complications of trying to link complex environmental factors, such as eating habits or macronutrient intake, with dementia and Alzheimer's disease. In particular, one complicating factor in the human studies is the normal consumption of large amounts of carbohydrates in modern diets. The present study demonstrates that, contrary to expectations, transgenic mice fed ab libituma very low carbohydrate/high saturated fat diet present lower levels of Aβ after only 43 days of dietary change. The KD group exhibited low levels of both Aβ40 and the more amyloidic Aβ42, suggesting that the KD diet did not change or increase the efficiency of cleavage sites within APP. Instead the data suggests the KD regime either reduced processing of APP or increased degradation of Aβ species. Most of the animals administered the ketogenic diet lost body weight as well as exhibited reduced Aβ levels.

However, the reduced Aβ levels may not have been due to a general lowering of protein content. Total brain protein levels did not differ between the groups and Aβ levels did not correlate with weight loss. Interestingly, despite change in diet, weight loss, and Aβ levels, no change in cognitive performance was observed. This observation agrees with the general finding that KD diets are not harmful to mice. Also, the finding that reduction in Aβ did not improve cognitive performance may be due to the modest lowering of levels under these conditions and longer treatment may be required.

Increasing evidence suggests a role for insulin/IGF-1 in regulating APP and modulating Aβ levels. Receptors for both insulin and IGF-1 are highly expressed in brain, especially in hippocampus and cortex, where they may influence learning and memory. Insulin signaling in the brain increases extracellular levels of Aβ by promoting secretion and inhibiting degradation by insulin-degrading enzyme. This view has also gained recent support in humans. Fishel et al. demonstrated that induced hyperinsulinemia in healthy elderly subjects elevated both serum and spinal fluid Aβ levels, suggesting insulin plays a role in elevating Aβ, especially under conditions such as type II diabetes

Conclusion

Dietary intervention represents a relatively safe and readily available method to combat AD. Yet, the key dietary links remain unclear. Much of the earlier work has focused on the role of high fat or high cholesterol diets and their contribution to AD, foods rich in carbohydrates are relatively recent additions to the human diet and are likely to be more evolutionarily discordant than high fat diets.

Future Work

The recent evolutionary switch to high carbohydrate diets may play an important role in development of AD, Thus complementary researches regarding this issue must be accomplished to get more knowledge about how to prevent or even delay this disease.

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