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Dietary composition affects the development of cognitive deficits in WT and Tg AD model mice

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Abstract

Clinical and epidemiological evidence suggests that lifestyle factors, including nutrition, may influence the chances of developing of Alzheimer's disease (AD), and also likely affect the aging process. Whereas it is clear that high-fat diets are increasing both body weight and the risk of developing Alzheimer's disease, to date, there have been very few studies comparing diets high with different sources of calories (i.e., high fat versus high protein versus high carbohydrates) to determine whether dietary composition has importance beyond the known effect of high caloric intake to increase body weight, AD pathology and cognitive deficits. In the current study we examined the effects that different diets high in carbohydrate, protein or fat content, but similar in caloric value, have on the development of cognitive impairment and brain pathology in wild-type and Tg AD model mice. The results demonstrate that long term feeding with balanced diets similar in caloric content but with significant changes in the source of calories, all negatively influence cognition compared to the control diet, and that this effect is more pronounced in Tg animals with AD pathology.

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Conflicts of interest

The authors declare no conflict of interest.

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Keywords

Long-term diet; macronutrients; cognition; adiposity; Alzheimer's disease

1. Introduction

Nutrition affects development, but also has an impact on brain structure and function throughout life. Therefore it is of importance to clarify which of the micro- or macronutrients in the diet will affect brain/cognitive functioning, and identify the possible mechanisms underlying such effects (Solon-Biet and others 2014). Unbalanced dietary exposure can have consequences for health years or decades later and this raises questions about the mechanisms through which such exposures are 'remembered' and how they result in altered disease risk. Furthermore, clinical and epidemiological evidence suggests that lifestyle factors, especially nutrition, may influence the chances of developing of Alzheimer's disease (Grant and others 2002; Pedrini and others 2009; Solfrizzi and others 2011b). For instance, in experimental studies it has been shown that high levels of cholesterol in the diet increase the chance of developing Alzheimer's disease (Kirsch and others 2003). On the other hand, epidemiological studies have shown that increased levels of fish oil (docosahexaenoic acid, DHA) in the diet decrease the risk of developing Alzheimer's disease (Morris and others 2003). Furthermore, as our and other studies have shown, low caloric intake has also been associated with a decreased risk of developing AD (Dhurandhar and others 2013; Wang and others 2005). Finally, while the "Mediterranean diet" is seemingly protective in the development of Alzheimer's disease (Solfrizzi and others 2011a), it is not clear which component(s) of the diet is/are protective (Frisardi and others 2010; Pedrini and others 2009).

It has been demonstrated that high fat diets, and especially high cholesterol diets (Morris and Tangney 2014; Sparks and others 2003), increase Alzheimer pathology and that supplementation of these diets (i.e., the "Typical Western Diet") with DHA improves the outcome (Hooijmans and others 2007; Oksman and others 2006) in AD mouse models. Similarly, population studies have indicated that high cholesterol levels elevate the risk of developing AD (e.g., (Harris and Milton 2010; Martins and others 2006; Morris and Tangney 2014), in contrast the "Mediterranean diet" has been shown to be protective against Alzheimer's disease (Feart and others 2013; Solfrizzi and others 2011a). Further, the high intake of refined carbohydrates, particularly sugars, has been linked to increased risk of obesity, diabetes, and Alzheimer's disease (Seneff and others 2011; Solfrizzi and others 2011b; Walker and others 2015), on the other hand some studies (Cuenca-Sanchez and others 2015; Westerterp-Plantenga 2003) have shown that long-term ingestion of high amounts of protein decreases food intake and body weight. Thus, though it is clear that the "fat" composition of the diet is important for the risk of developing AD, the role of the amount of protein or carbohydrate intake is currently much less clear (Solon-Biet and others 2014).

In this study we evaluated the effects of four different diets (similar in caloric value, but different in content; Harlan Teklan; Table 1), i.e., regular chow, and three diets relatively

high in respectively, fat, carbohydrate, and protein content, on the development of cognitive defects and pathology in a mouse model of Alzheimer's disease and in non-transgenic littermates.

2. Materials and Methods

2.1 Experimental design

Two months old male AD (APPSweDI; n=12/group; Tg (Davis and others 2004)) mice from our mice colony and non-transgenic littermates (n=12/group; WT) were put on four different diets (similar in caloric value, but different in macronutrient content; Harlan Teklan) i.e., control diet (Con), high fat (HF; 15P, 43C, 42F; TD.120084), high protein (HP; 35P, 50C, 15F; TD.120083), and high carbohydrate (HC; 15P, 70C, 15F; TD.120082; Table 1). The mice were housed three per cage in all groups. Average food intake per cage and individual body weight were monitored biweekly during the period of treatment with the different diets (except the last three weeks during behavioural testing). Following 6 months of the experimental feeding protocol all mice were analyzed for body mass composition using Dual-energy x-ray absorption. A battery of behavioural and cognitive tests was carried out to analyze cognitive differences, after that the animals were sacrificed for biochemical and histopathological analysis. By including groups of non-transgenic mice we were able to separate any possible amyloid beta – diet induced changes from diet only induced changes. The animals were on the diets from 2 months of age (well before AD pathology is present in these Tg mice) to 8 months of age (when robust AD pathology and cognitive deficits are present in these Tg mice (Davis and others 2004)). All animal protocols were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC). All methods were carried out in accordance with the approved guidelines and protocols.

2.2 Activity and Cognition

To assess how the different diets influence activity and cognitive patterns in the mice, in the last three weeks of the treatment period the mice were tested in the following tests. The open field and zero maze tests, to determine if differences in anxiety or motor performance are present in the any of the groups of mice. For the cognitive testing we used the water maze (spatial learning) and social recognition tasks (non-spatial learning). All of these measurements were performed in the UAB Behavioral Assessment Core.

Open field; the maze consisted of a 42 by 42 cm² arena with clear sides (20 cm high). The animal was placed in the arena and observed for 4 minutes with a camera-driven tracker system (Ethovision XT9.5, Noldus, The Netherlands). The arena was subdivided into the open center area and the side(s). The system records the position of the animal in the arena at 29 frames/second, and the data was analysed in, e.g., time spent in each area, speed of locomotion, rearing, defecation. The test measures anxiety in the animal, i.e., how much time does the animal spent in the center vs the side of the maze, and the speed of locomotion is measured.

Zero Maze; the maze consist of a circular maze (70 cm diameter) that is raised 80 cm above the floor, the circle is divided into four equal parts. Two parts have 15 cm high sides of opaque material (enclosed, or closed space), and two parts have only a 0.5 cm high wall (open space). The animal is put in the arena, and observed for 4 min, with a camera driven tracker system, i.e., Ethovision XT9.5 (Noldus, The Netherlands). The system records the position of the animal in the arena at 29 frames/second, and the data can be analysed in, e.g., time spent in each area, speed of locomotion, rearing, defecation etc. The test measures anxiety in the animal, i.e., how much time does the animal spent in open versus the closed, “safe” areas of the maze.

Social recognition; the social test apparatus consists of a polycarbonate box with partitions separating the box into three chambers. The partitions have openings that allow the animal to move freely from one chamber to another. The animal is monitored by the Noldus Ethovision XT 9.5 tracking system, the time spent in each chamber and the transitions between each chamber are recorded. The three chambered apparatus is in the center of a table (in the center of the room) to minimize gradients in light, temperature, sound and other environmental conditions that could produce a side preference. The black wire cages used to contain the stranger mice are cylindrical, 11 cm in height, bottom diameter 10.5 cm (Galaxy Cup, Spectrum Diversified Designs, Inc., Streetsboro, OH). The test animal is placed in the middle chamber and allowed to explore the apparatus for five minutes. After this five-minute habituation period, an unfamiliar adult C57BL/6J male (stranger 1) is placed inside a small wire cage in one of the side chambers. An identical empty wire cage is placed in the opposite chamber. The test subject is put in the central chamber and allowed to move freely throughout all three chambers of the apparatus over a 10-minute test session. The wire cage allows nose contact between the “bars”, but prevents the stranger mouse from initiating any social contact and limited the possibility of aggressive interactions. Thus, initiation of social contact is attributable to the test subject only. This method allows the test subject to be monitored for lack of initiation of social interaction. After a 24h period, an unfamiliar adult C57BL/6J male (stranger #2) is placed inside a small wire cage in one of the side chambers. The identical wire cage with the former “stranger 1” is placed in the opposite chamber. The test subject is put in the central chamber and allowed to move freely throughout all three chambers of the apparatus over a 5-minute test session.

Water maze; the water maze apparatus and procedure were described in detail before (Liu and others 2002). Briefly, we used a blue plastic pool, 120 cm in diameter, and a see-through round platform, 10 cm in diameter, located 0.5 cm below the water surface. During days 1 through 5 of the testing period, the mice were trained to find the hidden platform, which was kept in a constant position throughout these 5 days. Four trials were run per day; all starting positions were used equally in a pseudo-random order. The mice were given 60s to find the platform and 10s to stay on the platform. If the mouse did not find the platform in the assigned time, it was manually put onto the platform. The inter-trial interval during which the mouse was placed in a towel-bedded drying cage lasted 1 minute. Learning of the task was evaluated by recording the latency time to find the platform, but distance, speed and other parameters were recorded. At the end of the four trials on day 5 of the testing period, the mice were tested in a 60-s probe trial with no escape platform present. Mice that had learned the platform position predominantly searched in the “correct” quadrant of the pool

during the probe trial or entered the correct quadrant faster. Trials were recorded by using a camera-driven tracker system (Ethovision 9.5, Noldus, The Netherlands).

2.3 Body composition

In vivo body composition (total body fat and lean tissue) of mice was determined by Dual-energy x-ray absorption (DXA, GE Lunar PIXImus, Lunar, Madison, WI) in the UAB Small Animal Phenotyping Core. Dual energy x-ray absorptiometry (DXA) uses two different X-rays to measure fat, soft-lean tissue and bone in vivo. Animals are anesthetized (Isoflurane) and placed prostrate in the imaging area with the front and back legs extended away from the body.

2.4 Pathology

At the end of the experiment mice were anesthetized with ketamine/xylazine (100/10 mg/kg) and perfused transcardially with cold saline. The brains were removed and cut in half sagittally, and the right hemisphere of the brain was placed in 4% paraformaldehyde overnight. The left hemisphere was dissected into four pieces (rostral cortex, caudal cortex, hippocampus, and midbrain/brainstem) and stored frozen at -80°C for protein analysis (i.e., ELISA, Western blot). Following fixation, the right half of the brain was put for 24h in 30% sucrose for cryoprotection; and 30- μm thick coronal sections were cut on a freezing-sliding microtome. Free-floating sections were pre-treated for 30 minutes in 85°C sodium citrate solution ($\text{pH}=6.5$), washed three times in TBS-T, incubated in TBS-T overnight with an anti-A β antibody (human A β 4-10; 1:2000; The Genetics Company, Schlieren, Switzerland). Additional series of sections were stained with Iba-1 (1:5000; Wako, Richmond, VA) marker for microglia) and GFAP (1:1000; Sigma-Aldrich, St. Louis, MO, marker for astrocytes) to assess inflammation levels. For each antibody staining, sections from all animals were processed in the same staining tray. After staining, sections were mounted on the slides, coverslipped, imaged and measured.

2.5 Measurements

The appropriate areas of the brain were digitized using an Olympus DP70 digital camera. To avoid changes in lighting, which might affect measurements, all images were acquired in one session. Further, to avoid differences in staining density between sections, the measurements were performed on sections that were stained simultaneously, i.e., in the same staining tray. For the amount of amyloid deposits the appropriate area was digitized by the above procedure, and the percentage of area covered by the reaction product to A β (in the W0-2 stained material) was measured in triplicate (i.e., at three rostrocaudal levels for the hippocampus). The percentage of area covered (A β load) is the area occupied by A β in the total area of the hippocampus measured in that section. The density of GFAP and Iba-1 staining was calculated by measuring the optical density in the appropriate area of the hippocampus using the ImageJ (NIH) program. All density measurements were done in triplicate, i.e., measuring the same area at three different levels in the dorsal hippocampus. Density measurements were performed by an investigator who was blinded to the treatment of the animals.

2.6 Statistics

Amyloid beta load and the density of GFAP and Iba-1 density measurement data were analysed by ANOVA (SPSS version 10.0), and appropriate post-hoc tests (Tukey and Scheffe) were carried out to determine the source of a significant main effect or interaction. Most data were analysed using ANOVA with Bonferroni's multiple comparing test. Sample size based on prior pathological and cognitive analyses were calculated to achieve a power of 80% at $\alpha = 0.05$. Some statistical analyses were performed using the SysStat software.

3 Results

3.1 Dietary influence on body weight and food intake

At the end of the study there were no significant differences between the eight groups of mice in overall health, i.e., none of the mice showed any signs of disease; only one mouse in the Tg HC group died prematurely. After perfusion, organs (heart, liver, and kidney) were weighed and checked for pathology. None of the mice displayed any gross pathology of heart, liver or kidneys. No differences were present in the heart weight/lean body weight ratios or in the kidney weight/lean body weight ratios in the WT or Tg animal diet groups (Supplemental Table 1). Liver weight/lean body weight ratios were significantly lower ($p < 0.05$) in the WT HP diet group to compare with other diet groups. In the TG HF diet group liver weight/lean body weight ratios were significantly higher ($p < 0.001$) compared to other Tg animal diet groups. There were significant differences in body weight between the HF (heaviest; $41.5 \pm 1.3\text{g}$; $p < 0.0001$) and the other three diets, in the WT groups (end weights: Con, $33.6 \pm 1.1\text{g}$; HC $35.7 \pm 1.0\text{g}$; and HP, $32.0 \pm 0.7\text{g}$; not significantly different; Figure 1, Table 2). Similarly, in the Tg groups the HF diet animals were the heaviest ($38.7 \pm 1.0\text{g}$; $p < 0.0001$) compared to the other three groups (end weights: Con, $31.5 \pm 0.5\text{g}$; HP, $31.7 \pm 0.5\text{g}$; and HC $32.1 \pm 0.9\text{g}$; not significantly different; Figure 1, Table 2). The HF animals started to significantly differ in body weight at week 12 in the WT group, and at week 14 in the Tg group (Figure 1). The average daily food intake during the last four weeks on the diet was for the WT animals, Con, $3.2 \pm 0.2\text{g}$; HF, $2.6 \pm 0.2\text{g}$; HC, $3.1 \pm 0.2\text{g}$; HP, $3.0 \pm 0.2\text{g}$, respectively (Table 2). The average daily caloric intake in these weeks was: Con, $11.9 \pm 0.5\text{Kcal}$; HF, $11.5 \pm 1.0\text{Kcal}$; HC, $11.3 \pm 0.7\text{Kcal}$; and HP, $10.9 \pm 0.7\text{Kcal}$, respectively; not significantly different. The average daily food intake in the Tg animals groups in the last four weeks on the diets were, Con, $3.4 \pm 0.3\text{g}$; HF, $2.6 \pm 0.2\text{g}$; HC, $3.1 \pm 0.2\text{g}$; and HP, $3.1 \pm 0.2\text{g}$; respectively (Table 2). The average daily caloric intake in these weeks was: Con, $12.5 \pm 1.2\text{Kcal}$; HF, $11.3 \pm 0.7\text{Kcal}$; HC, $11.3 \pm 0.6\text{Kcal}$; and HP, $11.3 \pm 0.5\text{Kcal}$, respectively; not significantly different.

3.2 Dietary influence on body composition

Following all behavioral/cognitive tests the animals were analyzed for body composition, the *in vivo* body composition (total body fat and lean tissue) of mice was determined by Dual-energy x-ray absorption (DXA, GE Lunar PIXImus, Lunar, Madison, WI). The results demonstrated that there were significant differences in body composition between the treatment groups and between the two genotypes (WT and Tg; Figure 2). In the WT animals Control and HP groups were not different (21.3% and 17.5% body fat, respectively; Figure

2A), but both the HC and the HF groups had significantly ($p<0.05$) higher body fat (25.5% and 38.6% body fat, respectively; Figure 2A). In the Tg animals the HF group had significantly ($p<0.05$) more body fat (32.5%) than the other three groups (that were not significantly different from each other; Figure 2B). It should be noted that the Tg animal groups all had significantly less body fat than the WT animals (Figure 2), even if the body weights for each dietary group were not significantly different between these two genotypes.

3.3 Dietary influence on behaviour and cognition

In the behavioural assessments, there were no differences between the diet groups in the open field test, i.e., in the times spend in the center of the maze compared to the side (Figure 3, top). It should be noted that the average speed of the Tg mice is significantly slower compared to the WT mice ($3.28\pm0.21\text{cm/s}$ and $6.88\pm0.45\text{cm/s}$, respectively; $p<0.001$). The WT animals move a significant larger distance compared to the Tg animals (1642 vs 762 cm, respectively; $p<0.001$), but part of the difference is caused by the amount of time the animals do not move (i.e., are sitting still), which is significantly higher ($p<0.001$) in the Tg animals (supplemental Table 2). Similarly, in the zero maze test, no differences were present in the time spend in the open vs closed parts of the maze between the diet groups and between WT and Tg mice (Figure 3, bottom), but the WT mice move faster than the Tg animals ($3.82\pm0.12\text{cm/s}$ and $2.69\pm0.09\text{cm/s}$, respectively, $p<0.001$). The WT animals move a significant larger distance compared to the Tg animals (915 vs 647 cm; $p<0.001$), but part of the difference is caused by the amount of time the animals do not move (i.e., are sitting still), which is significantly higher ($p<0.001$) in the Tg animals (supplemental Table 2). In contrast, in the social recognition test there were significant ($p<0.001$) differences between the diet groups, with the HF diet group performing worse than the other three diet groups (Figure 4), in the WT animal groups ($p<0.001$) and significantly worse than the Con animals in the Tg animal groups (Figure 4; $p<0.05$). In the water maze, in the WT mice there were small differences in the learning curves between the diet groups, but on day 5 of the water maze the HF mouse group performed significantly ($p<0.05$) worse than the other three groups (Figure 5), the other three groups were not different from each other. Similarly, in the Tg animals the HF diet group performed the worst of all groups ($p<0.05$), however, in contrast to the WT mice, the Control group performed significantly ($p<0.001$) better than all other groups (Figure 5), i.e., the HC and HP groups were also impaired compared to control diet ($p<0.05$). Further, all groups of WT mice performed significantly better than their Tg littermates (Figure 5). The time in quadrant probe trial data did not show significant differences between WT and Tg groups or between diets. However, in the Tg animals, Con animals had significantly more platform crossings in the probe trial than all other groups ($p<0.05$). It should be noted that in contrast to the open field and zero maze tests, there were no differences in swimming speed between any of the groups, neither between diet groups or genotypes (WT swimming speeds: Con, $15.1\pm0.9\text{cm/s}$; HF, $15.0\pm1.2\text{cm/s}$; HC, $14.8\pm1.0\text{cm/s}$; and HP, $14.9\pm1.1\text{cm/s}$; respectively, and Tg swimming speeds: Con, $14.5\pm1.2\text{cm/s}$; HF, $14.7\pm1.0\text{cm/s}$; HC, $14.6\pm1.0\text{cm/s}$; and HP, $14.6\pm1.3\text{cm/s}$; respectively; Supplemental Table 2).

3.4 Dietary influence on brain pathology

After all behavioural and body analysis the animals were sacrificed, and the brains were collected for histological analysis. In the Tg animals the A β load in the hippocampal formation (both in area CA1 [stratum oriens] and dentate gyrus) was significantly ($p<0.05$) higher in the HC group compared to the other three groups (Figure 6).

Analysis of the inflammation levels (both GFAP levels and Iba-1 levels) in the dorsal hippocampus did not show any significant differences between the four groups of animals in both genotypes (Figures 6 and 7).

4 Discussion

We assessed the impact of four different diets (similar in caloric value, but different in content; Harlan Teklan; Table 1), i.e., regular chow, and three diets relatively high in respectively, fat, carbohydrate, and protein content, on the development of cognitive defects and pathology in a mouse model of Alzheimer's disease and in non-transgenic littermates.

As expected and in accordance with previously published studies, the HF diet caused a significant increase in body weight in both the WT and Tg animals compared to the other diet groups, i.e., Con, HC and HP. Furthermore, both in the WT and Tg mice groups the HF diet induced significant impairments in cognition compared to all other groups, while in the Tg animals the HC and HP mice were also impaired compared to Con mice. In the Tg animals A β load was significantly ($p<0.05$) increased in the HC group, but, surprisingly, the levels of inflammation were not different between any of the treatment groups, both for astrocyte (GFAP) and microglia (Iba-1) staining density. Similarly, in the WT animals no differences in inflammation levels (both for GFAP and Iba-1 staining densities) were present in the hippocampus. Together these results demonstrate that diets with significant changes in the source of calories, but with similar caloric density, all negatively influence cognition compared to the control diet, and that this effect is more pronounced in Tg animals with AD pathology. We would like to note that all four diets had relatively high levels of carbohydrate, since the regular, control diet we use for our animals contains 46.5 g% of carbohydrate. While several studies suggest that a ketogenic diet might be protective for AD, or other neurodegenerative diseases partially due to improved brain glucose metabolism and reduced gliotransmitter release of glutamate (for review (Hertz and others 2015), in our study we did not investigate the effects of low levels of macronutrients, e.g., low protein or low carbohydrate on cognitive outcome.

4.1 Dietary influence on body weight and food intake

While it is well-known that the consumption of a HF diet increases body weight (e.g., (Freeman and others 2014; Hariri and Thibault 2010; Pedrini and others 2009; Roberts and others 2002)), and our HF diet also increased body weight, we would like to point out, that our HF diet is only slightly higher in caloric content compared with the other three diets (4.4 Kcal/g vs 3.7Kcal/g) and it has only 42% fat content which is lower than most HF diets (which often contain more than 60% fat content). On average these animals ate the smallest amount of their food, but their daily caloric intake was not different from the other diet

groups. Even so, they do have the highest % of body fat (Hariri and Thibault 2010; Townsend and others 2008). Together these data indicate that likely a significant change in their metabolism has occurred (Townsend and others 2008).

4.2 Dietary influence on body composition

A new, and interesting, finding was, that disregarding of the diet condition, the Tg animals have a significantly lower body fat mass as determined by DXA, compared to the WT animals. One explanation could be that Tg AD model mice have an increased metabolism compared with their non-Tg littermates (Knight and others 2013; Knight and others 2012; Morgan and Gordon 2008; Sancheti and others 2014). This change in metabolism could be present since obesity leads to upregulated levels of APP in fat cells (Lee and others 2008) and therefore the high levels of APP and/or A β in the Tg mice could positively impact metabolism in fat cells (Freeman and others 2012). On the other hand, there is no clear evidence that the amyloid precursor protein (APP or one of its cleavage products) influences overall metabolism or how peripheral energy metabolism affects APP processing. Further, it should be mentioned that in the brain, the amyloid precursor protein (APP) controls cholesterol turnover needed for neuronal activity (Pierrot and others 2013), and, it has been shown that cholesterol and sphingomyelin metabolism are influenced by A β and presenilin (Grosogen and others 2010). Finally, recent data suggest that amyloid precursor protein transgenic mice consume excess calories relative to nontransgenic mice, yet they weigh less (Morgan and Gordon 2008). The increased body weight of the HF groups is in line with other studies on the effects of high-fat diets in Tg AD model mice (Levin-Allerhand and others 2002; Nishikawa and others 2007; Pedrini and others 2009; Refolo and others 2000). It should be noted that the HF mice had only a moderate increase in fat (42%) in the diet, and therefore only had a modest increase in body weight compared to mice fed high-fat diets (Petro and others 2004). Similarly, the significantly increased percent of body fat in the HF groups is in line with previous studies (Nishikawa and others 2007; Yang and others 2014). Likewise, the significantly higher liver weights in the Tg HF group are as expected (de Meijer and others 2010). Further, in the HC group an increase in body fat was observed in the WT mice, this has been suggested as a compensation for low protein levels in the diet (Le Couteur and others 2016). While some studies (Cuenca-Sanchez and others 2015; Westerterp-Plantenga 2003) have shown that long-term ingestion of high amounts of protein decreases food intake, body weight, and body adiposity, we did not observe this effect in our study, the HP group did not show any significant reduced body weight compared to the other groups. This might be because the changes in our diets were relatively modest compared to the diets used in these studies, furthermore, our WT and Tg animals were young (they were only 8 months old when sacrificed) and if we would have continued the study to a more advanced age, there likely would have been more significant differences between body weights of the four dietary groups. Finally, we did not observe any change in kidney weights in the HP group indicating that the higher level of protein in the diet was not causing kidney damage (Cuenca-Sanchez and others 2015; Kostogrys and others 2015).

4.3 Dietary influence on behaviour and cognition

It is of interest to note that the Tg animal groups have a significantly ($p < 0.05$) lower speed of locomotion in the open field and zero maze behavioral tests (compared to the WT mice),

however, this difference is primarily caused by different behaviors in these tests (Ferguson and others 2013; Filali and Lalonde 2015). The speed when moving is slightly different, but the Tg animals also spend more time “sitting still” than the WT animals (Supplemental Table 2 (Lalonde and others 2012)). This is not observed in the swimming speed in the water maze test (Filali and others 2011; Klapdor and van der Staay 1996; Lalonde and others 2005), where motivation for moving is different compared to the dry mazes. Thus, surprisingly, the significant increase in body weight in the HF groups of mice does not affect speed of movement (Leamy and others 2009; Zhang and Gershenfeld 2003), although there is a trend for this group to move the slowest of all, compared to the other diets.

While the finding that diets high in fat content have a negative influence on cognition (both in social recognition and water maze) in both the WT and Tg animals was not unexpected (Julien and others 2010; Kesby and others 2015; Levin-Allerhand and others 2002; Maesako and others 2012; Petrov and others 2015), one should keep in mind that previous studies have indicated that the high-fat induced memory impairments are not necessarily directly related to the A β load in Tg AD model mice (El Akoum and others 2011; Knight and others 2014). Our data, similarly, do not show a HF diet increase in A β load in the hippocampus, while we do see cognitive deficits. On the other hand, the HC diet significantly increased the A β load. Very few studies have examined the role of HP or HC diet on cognition, somewhat unexpectedly, our data demonstrate that in Tg mice both HC and HP diets also negatively affect cognition (Chepulis and others 2009; Jurdak and Kanarek 2009; Seneff and others 2011). It has been demonstrated that short term exposure (5 to 20 days) to a diet rich in sugar has been shown to impair place but not object recognition in rats (Beilharz and others 2014; Beilharz and others 2015), and the authors suggest a role for oxidative stress and neuroinflammation in the cognitive impairment, especially affecting the hippocampal formation. Similarly, an eight week exposure to 32% sucrose solution has been shown to impair novel object recognition in young rats (Chepulis and others 2009; Jurdak and Kanarek 2009), most likely due to diet induced alteration in glucose metabolism. While in the Tg mouse groups the HF, HC, and HP mice show cognitive deficits, in the WT mice only the HF group is impaired in cognition, but, likely, with longer treatment periods the HC and HP WT animals would have shown a similar effect of these diets on cognition. The current treatment period was likely too short to affect the brain and cognition in the WT mice. In light of our findings that high protein diet fed Tg mice performed significantly worse in cognitive tests compared with Con diet fed mice, brings into question recommendations for increasing the percentage of energy from protein for weight loss (Huang and others 2013; Pesta and Samuel 2014). Our study indicates that while there might be beneficial effects of high protein diet in early life, the use of a long-term high protein diet in adulthood could impair cognition.

4.4 Dietary influence on brain pathology

There is a substantial amount of clinical and research data indicating that high fat diets and, especially, high cholesterol diets increase the chances of developing AD and A β pathology in the brain (Gu and Scarmeas 2011; Morris and Tangney 2014; Pedrini and others 2009; Sparks and others 2003). Surprisingly, in our study we did not observe an increase in A β load in the hippocampus in HF diet mouse group. Further, the high intake of refined

carbohydrates, particularly sugars, has been linked to increased risk of obesity, diabetes, cardiovascular disease, and Alzheimer's disease (Seneff and others 2011; Solfrizzi and others 2011b; Walker and others 2015), this could explain why the HC group had a significantly increased A β load in the hippocampus. In our diets there were no changes in the amount of sucrose between the diets, the increased level of carbohydrates was achieved by increasing the amount of corn starch in the diet (Solfrizzi and others 2011b).

Somewhat surprisingly, the assessment of brain inflammation in the hippocampal formation did not show differences between the different diet groups. This is in contrast to the many studies which have indicated that peripheral inflammation caused by obesity increases central inflammation (Heneka and others 2015; Puig and others 2012; Takeda and others 2014). We expect that the relatively low level of obesity in the HF animals in our study, due to the not very high fat content in the HF diet (and therefore not very energy-rich diet) is the cause of this discrepancy. Another explanation is the region of brain where we were measuring inflammation, the hippocampus, it is possible that other brain regions such as the hypothalamus show a more increased level of inflammation. Finally it should be noted that only in the ageing CNS of mice, rats, and primates, microglia show enhanced sensitivity to inflammatory stimuli (Perry and Teeling 2013), and our mice are still early middle age.

5. Conclusion

In conclusion, the data indicate that any long-term significant deviation from a balanced diet has detrimental effects on body composition, and, especially on cognition. Our data would suggest that a balanced, healthy diet such as the Mediterranean diet, would appear to be a beneficial approach with the least possible adverse effects to slowing the progression of AD.

In future studies, by using the hippocampal tissues from this experiment, we will assess changes in synaptic protein expression and insulin signaling pathways. In a, new separate study we will measure energy expenditure and glucose tolerance in WT and Tg AD animals on these diets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

Long term feeding of diets, with significantly different macronutrient content, negatively affects cognition in WT and Tg AD model mice

Long term feeding of diets, with different macronutrient content, differently affects adiposity in WT and Tg AD model mice

Tg AD mice are more susceptible to diet alterations than WT mice

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Figure 1.

Line graphs showing the increase in body weight in the WT and Tg mice on the four different diets during the 6 months on the diet. The HF diet groups have a significantly higher body weight, after 12 (WT) and 14 (Tg) weeks of diet, respectively; $p < 0.05$.

Figure 2.

Bar graphs showing the quantification of the percent of body weight and body fat from the DXA measurements. **A**, indicates WT and **B** indicates Tg mice. The HF animals are heavier both in the WT and Tg animal groups ($p < 0.001$ and $p < 0.001$, respectively). The HF animals also have a significantly higher percent of body fat compared to all other groups in the WT animals ($p < 0.001$), and in the Tg animals ($p < 0.001$). Further, the HC animals have a significantly higher percent of body fat in the WT animals compared to the Con and HP groups ($p < 0.05$). Finally, all WT animals groups have a significantly higher fat mass compared to the TG animals groups ($p < 0.0001$). Left axis in A and B— weight in grams, right axis - % of fat.

Figure 3.

Bar graphs showing the quantification of the time spend in the center of the open field vs the side of the open field (**top of Figure**), and the time spend in the closed vs open parts of the zero maze (**bottom of Figure**). All animal groups have similar levels of anxiety.

Figure 4.

Bar graphs showing the quantification of the percent of time spend with the new object in the Novel Object Recognition task. The HF animals are significantly impaired compared to all other groups in the WT animals ($p < 0.001$), and compared to the Con group in the Tg animals ($p < 0.05$).

Figure 5.

Top, line graphs showing the improvement in learning and memory in the water maze task over the 5 days of testing, in the WT animals the HF diet animals are significantly impaired to all other groups ($p < 0.05$). Similarly, in the Tg animals the HF mice are significantly impaired compared to all other groups ($p < 0.05$), and the HC and HP groups are significantly ($p < 0.05$) impaired compared to the Con group of mice. **Bottom**, two bar graphs showing the time spend in each quadrant in the probe trial, no significant differences are present in the probe trial data.

Figure 6.

A, Top, two sets of high power photomicrographs of coronal sections of dorsal hippocampus (area CA1) of the brains of the four diet groups of the mice showing the staining for astrocytes (GFAP), in WT mice (top row) and in Tg AD mice (second row). Bottom, two sets of lower power photomicrographs of the hippocampus stained for amyloid beta (W02 antibody) in the Tg mice, third row – dentate gyrus and bottom row – area CA1. **B**, quantification of the staining densities for GFAP and amyloid beta, there are no significant differences in GFAP expression levels between the groups or genotypes (stats). Note the significantly ($p < 0.05$) increased levels of amyloid beta staining in the DG of HC Tg AD model diet group. so – stratum oriens, sp – stratum pyramidale, sr – stratum radiatum, DG - dentate gyrus. Space bar for top rows - 200 μ m, bottom rows – 50 μ m.

Figure 7.

A, Four sets of photomicrographs of coronal sections of the dorsal hippocampus of the brains of the four diet groups of the mice stained for microglia (Iba-1 antibody). Top row, low magnification images demonstrating the staining for microglia (Iba-1) in WT mice and, second row, in Tg AD mice. The two bottom sets of images show higher magnification pictures from the area outlined in the box in first row. There are no significant differences in microglia upregulation between the different diet groups in WT and Tg AD mice (stats). Space bar for top rows - 1000 μ m, bottom rows - 50 μ m. HIP- hippocampus, so-stratum oriens, sp- stratum pyramidale, sr- stratum radiatum. **B**, Two bar graphs demonstrating the quantification of the Iba-1 density.

Table 1

Contents of the four diets, the Con diet is based on the NIH-31 diet.

	Con		HF		HC		HP	
	g%	kcal%	g%	kcal%	g%	kcal%	g%	kcal%
protein	18.4	20.0	16.8	15.1	13.7	14.9	32.5	35.3
carbohydrate	46.5	50.4	47.7	42.9	64.6	70.0	45.5	49.5
fat	4.5	10.9	20.7	41.9	6.2	15.1	6.2	15.2
Kcal/g	3.7		4.4		3.7		3.7	
Ingredients								
casein			190		155		370	
L-cystine			3		2.4		3	
corn starch			269		458		246	
maltodextrin			130		130		130	
sucrose			100		100		100	
cellulose			50		50		50	
soybean oil			58		17		16.4	
anh. milkfat			65		19		18.4	
lard			47.8		14		13.5	
olive oil			34.2		10		9.7	
mineral mix (98057)			16		13.4		13.4	
ca phosphate			15.3		13		6.2	
ca carbohydrate			6.7		5.4		10.4	
vitamin mix (AIN -93-VX)			12		10		10	
choline bitartrate			3		2.5		2.5	
TBHQ			0.04		0.01		0.01	
food color			0.1		0.1		0.1	

Table 2

Body weights, food intake in grams $\pm$ sem, calorie intake in Kcal $\pm$ sem, and fat mass in % $\pm$ sem

	Final Weight	Daily Food intake	Daily Food intake	Daily Calorie intake	% Body fat
	grams	first 4 weeks	last 4 weeks	last 4 weeks	
Con WT	33.6 $\pm$ 1.1	2.6 $\pm$ 0.1g	3.2 $\pm$ 0.2g	11.9 $\pm$ 0.5 Kcal	21.3 $\pm$ 1.0
Tg	31.5 $\pm$ 0.5	2.9 $\pm$ 0.1g	3.4 $\pm$ 0.3g	12.5 $\pm$ 1.2 Kcal	13.3 $\pm$ 1.2
HF WT	41.5 $\pm$ 1.3 *	1.9 $\pm$ 0.3g *	2.6 $\pm$ 0.2g *	11.5 $\pm$ 1.0 Kcal	38.6 $\pm$ 1.4 *
Tg	38.8 $\pm$ 1.0 *	2.4 $\pm$ 0.2g	2.6 $\pm$ 0.2g *	11.3 $\pm$ 0.7 Kcal	32.5 $\pm$ 4.2 *
HC WT	35.7 $\pm$ 1.0	2.4 $\pm$ 0.2g	3.1 $\pm$ 0.2g	11.3 $\pm$ 0.7 Kcal	25.9 $\pm$ 2.3 *
Tg	32.1 $\pm$ 0.9	2.5 $\pm$ 0.1g	3.1 $\pm$ 0.2g	11.3 $\pm$ 0.6 Kcal	17.3 $\pm$ 2.7
HP WT	32.0 $\pm$ 0.7	2.5 $\pm$ 0.1g	3.0 $\pm$ 0.2g	10.9 $\pm$ 0.7 Kcal	17.5 $\pm$ 1.7
Tg	31.7 $\pm$ 0.5	2.6 $\pm$ 0.1g	3.1 $\pm$ 0.2g	11.3 $\pm$ 0.5 Kcal	14.9 $\pm$ 2.2

* significantly different.