Published in final edited form as:

Neuroscience. 2013 December 3; 253: 110–122. doi:10.1016/j.neuroscience.2013.08.044.

Inter-relationships among Diet, Obesity and Hippocampal-dependent Cognitive Function

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Abstract

Intake of a Western diet (WD), which is high in saturated fat and sugar, is associated with deficits in hippocampal-dependent learning and memory processes as well as with markers of hippocampal pathology. In the present study, rats were trained to asymptote on hippocampal-dependent serial feature negative (FN) and hippocampal-independent simple discrimination problems. Performance was then assessed following 7 days on ad libitum chow and after 10, 24, 40, 60, and 90 days of maintenance on WD, on ketogenic (KETO) diet which is high in saturated fat and low in sugar and other carbohydrates, or continued maintenance on chow (CHOW). Confirming and extending previous findings, diet-induced obese (DIO) rats fed WD showed impaired FN performance, increased BBB permeability, and increased fasting blood glucose levels compared to CHOW controls and to diet resistant (DR) rats that did not become obese when maintained on WD. For rats fed the KETO diet, FN performance and BBB integrity was more closely associated with level of circulating ketone bodies than with obesity phenotype (DR or DIO) with higher levels of ketones appearing to provide a protective effect. The evidence also indicated that FN deficits preceded and predicted increased body weight and adiposity. This research (a) further substantiates previous findings of WD-induced deficits in hippocampal-dependent feature negative discriminations, (b) suggests that ketones may be protective against diet-induced cognitive impairment, and (c) provides evidence that diet-induced cognitive impairment precedes weight gain and obesity.

Energy dense diets that are high in saturated fat and sugar are often referred to as western diets (WD) based on their widespread popularity in western and westernized societies. Previous studies using human and nonhuman animal models have shown that consuming WD is associated with not only weight gain and metabolic disease, but also with impaired hippocampal-dependent memory and the emergence of hippocampal pathologies (for details see reviews by Benoit et al., 2010, Kanoski and Davidson, 2011). In addition, some of these studies show that rats maintained on WD exhibit reductions in hippocampal brain-derived neurotrophic factor (Molteni et al., 2002, Kanoski et al., 2007) and hippocampal

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neurogenesis (Park et al., 2010), as well as increased hippocampal inflammation (Puig et al., 2012, Herculano et al., 2013) and blood-brain barrier (BBB) permeability (Kanoski and Davidson, 2011, Freeman and Granholm, 2012). Furthermore, under at least some test conditions, the effects of WD on hippocampal-dependent memory and BBB permeability depend on obesity phenotype (Davidson et al., 2012). Specifically, some rats (i.e., diet-induced obese (DIO)) maintained on a WD exhibit substantially more weight gain compared with rats fed standard low-fat, high carbohydrate chow, whereas other rats (i.e., diet-resistant (DR)) do not (Madsen et al., 2010). Davidson et al., (2012) reported that the obesity-prone DIO rats fed the WD showed impaired performance on a hippocampal-dependent discrimination problem and exhibited significantly higher BBB permeability compared to both DR rats on the same diet and chow-fed controls.

The present research attempts to expand what is known about the relationship between diet composition and the emergence of hippocampal-dependent cognitive dysfunction by addressing the following questions: (1) What types of diet-induced changes in physiological parameters (e.g., body weight, body adiposity, metabolic, hormonal, BBB permeability) are most predictive of diet-induced deficits in hippocampal-dependent memory? (2) What are the effects of a diet very high in fat and low in carbohydrate (i.e., ketogenic (KETO) diets) on hippocampal-dependent cognitive functioning? (3) What is the direction of the relationship between diet-induced changes in body weight, adiposity, and hippocampal-dependent cognitive performance?

These questions are of interest for several reasons: because KETO diets contain much higher levels of saturated fat and much lower levels of carbohydrate compared to the WD (Kinzig et al., 2005), if maintenance on the KETO diet produces disruptive effects that are similar to or of greater magnitude than those of the WD, this would suggest that dietary saturated fat may be a more important determinant of those effects than dietary carbohydrate. On the other hand, if the KETO diet has weaker or no adverse effects on hippocampal-dependent performance, this would suggest that dietary carbohydrate may be the more important factor. In addition, rats that consume ketogenic diets have been reported to show elevated levels of body fat without exhibiting significantly higher overall body weight compared to rats maintained on standard chow (Kinzig et al., 2005). Thus, the effects of a KETO diet on a behavioral task that depends on the hippocampus could help to evaluate the relative roles of body weight and body adiposity, *per se*, in the manifestation of those hippocampal-dependent cognitive effects. Finally, because the amount of carbohydrate in the diet is low, rats maintained on the KETO diet utilize ketone bodies that are converted from fat as the brain's primary energy (Bielohuby et al., 2011). This shift, which is indexed by elevated levels of circulating ketone bodies (i.e., ketosis), has been reported to have therapeutic effects on several brain disorders such as epilepsy (e.g., Lutas and Yellen, 2013) and mild cognitive dementia (Krikorian et al., 2012), and may also have neuroprotective effects in the hippocampus (Samoilova et al., 2010). Thus, it is important to assess if the effects of consuming a KETO diet on cognitive functions that rely on the hippocampus depend on level of ketosis.

In addition to measuring circulating ketone bodies, we will also assess the relationship between diet-induced changes in blood glucose and triglyceride levels and in levels of the hormones, insulin, and GLP-1. These measures are of special interest because the release of and sensitivity to each of these blood-borne factors have been associated with both hippocampal-dependent cognitive functioning and energy regulation (Farr et al., 2008, Malone et al., 2008, Isacson et al., 2011, Schioth et al., 2012)

While previous results have demonstrated that obesity and hippocampal-dependent cognitive dysfunction are related (e.g., Davidson et al., 2005, Davidson et al., 2007, Kanoski and

Davidson, 2011) the question of “What comes first--does obesity predict cognitive deficit or does cognitive deficit predict obesity?” remains open. To approach this question, we used a cross-lagged panel design (Kenny, 1975). The logic of this design is that if a given Factor A at early time point 1 predicts the value of another Factor B at later time point 2 better than Factor B at time point 1 predicts the value of Factor A at time 2, then based on the assumption that causes precede effects (i.e., temporal contiguity), the more likely direction of the relationship is that changes in Factor A precede and cause changes in Factor B. We performed several of these analyses using measures of obesity (e.g., body weight, body adiposity), ketosis, and cognitive performance as Factors A and B.

1.1 Experimental Procedures

1.1.1 Subjects

The subjects were 48 naïve male Sprague-Dawley rats, aged 60–75 days, and weighing between 275–300 g upon arrival from Harlan Inc. (Indianapolis, IN, USA). The animals were housed individually in a climate-controlled environment under a 12:12 hr light:dark cycle with the light phase beginning at 0700 hrs each day. The rats had free-access to water throughout the study, except during experimental sessions as described below. The rats were weighed daily during pre-training and immediately prior to each experimental session. The care and use of all animals in this study was reviewed and approved by the Purdue University Animal Care and Use Committee.

1.1.2 Apparatus

All behavioral training was conducted in 8 conditioning chambers purchased from Med Associates (Georgia VT, USA). The chambers were identical, each with aluminum end walls and clear plexiglass side walls, measuring $21.6 \times 21.6 \times 27.9$ cm and with floors consisting of stainless steel rods (0.48 cm in diameter) spaced 1.9 cm apart. A recessed food cup was located in the center of one end wall, and a 6-W jeweled panel light was located approximately 6 cm above and to the left of the opening for the food cup. Diffuse tone (1500 Hz), white noise, and clicker (3Hz) stimuli (all approximately 78 dB) were produced by auditory stimulus generators (Med Associates, ANL-926) located outside the conditioning chambers near the end wall opposite of the food magazine. A motorized pellet dispenser attached to each chamber was used to deliver 45 mg sucrose pellets to the food cup. A computerized infrared monitoring system with a photo transmitter and receptor was positioned in each chamber so that photobeam interruptions would index entries into the recessed area with the food cup. All experimental events (e.g., stimulus presentations, pellet delivery, recording of beam breaks) were controlled using MED PC IV (Med Associates) computer software. The body adiposity of each rat was measured using an EchoMRI-900 magnetic resonance body composition analyzer (Echo Medical Systems, LLC, Houston, TX, USA).

1.1.3 Procedures

Pretraining—The rats were fed standard rodent laboratory chow (LabDiets formula 5001, Purina, Framingham MA, USA) ad libitum for 14 days after their arrival in the lab. Food rationing was then used to gradually reduce the body weight of each rat to 85% of the average weight obtained on the last two days of ad libitum feeding. Rats were weighed and handled daily during this period of food rationing. Magazine training began when this 85% ($\pm 1\%$) of ad libitum weight criterion was achieved by all rats.

Magazine training—The rats were assigned to 6 squads of eight rats each with each rat assigned to one of the eight conditioning chambers for the duration of the study. The rats in each squad received one 15-min session of magazine training to help habituate them to the

apparatus and to learn the location of the food cup. All rats received ten presentations of two sucrose pellets, which were delivered according to a variable-time 60-sec schedule (i.e., one presentation of two pellets per min on average). The rats were returned to their home cages and fed their daily ration immediately after the session was over.

Discrimination training—Following the general design and procedures used in previous studies (Kanoski et al., 2010, Davidson et al., 2012), all rats were trained to asymptote on two concurrent discrimination problems. One problem was a serial feature negative (FN) discrimination (referred to as a FN discrimination hereafter). Because FN performance is impaired following selective neurotoxic lesions of the hippocampus, this problem is considered to be “hippocampal-dependent”. The second problem was a simple Pavlovian (CS+/CS−) discrimination. Hippocampal lesions have little or no effect on simple discrimination performance (Jarrard and Davidson, 1991, Holland et al., 1999), so this problem is considered to be hippocampal-independent. The rats were maintained at 85% of their ad libitum body weight throughout training. For FN discrimination training, the presentation of a 5-sec tone terminated with the delivery of two sucrose pellets (on Tone+ trials), whereas no pellets were delivered when a 5-sec illumination of the panel light (L) followed by a 5-sec empty interval (i.e., with no programmed stimulus) preceded presentation of the 5-sec tone (on Light→Tone-trials). For simple discrimination training, a 5-sec presentation of the white noise terminated with the delivery of two sucrose pellets (on a CS+ trial), whereas no pellets were delivered after a 5 sec presentation of the clicker (on CS− trials). Each training session consisted of one Tone+ trial and two Light→Tone-trials along with one CS+ trial and two CS− trials. Trial orders for each session were selected randomly and the intertrial interval was randomized within the range of 300 to 900 sec (average = 600 sec). Training was completed after 44 sessions. All rats were returned to their home cages and fed their daily ration immediately after each session of discrimination training. All animals received one 60 min session of discrimination training per day. Training sessions were conducted between 0900–1530h.

Diets—Immediately after the end of discrimination training all rats were fed standard lab chow ad libitum for 7 days, at which time all rats had regained body weight up 100% of that recorded prior to food rationing. The rats were then assigned to one of four ad libitum fed diet groups (n = 12 each) that were matched on both FN and simple discrimination performance over the last two sessions of training and on body weight and body adiposity at the end of the 7-day period of ad libitum feeding with standard chow. One group (WD) was fed a diet high in saturated fat and dextrose (Harlan Teklad, Indianapolis, IN, USA TD. 04489) that contained the following ingredients (g/kg): 270 g casein, 220.5 g glucose, 200 g cornstarch, 170 g lard, 50 g cellulose, and 15 g safflower oil. This diet had a caloric density of 4.5 kcal/g and contained the following percentages of energy from the three macronutrient classes: 38% kcal from carbohydrate, 21% kcal from protein, and 40% kcal from fat. Group KETO was maintained on a ketogenic diet (Research Diets, New Brunswick, NJ, D06040601A) that had a total caloric density of 6.1 kcal/g with 80% kcal from fat, 5% kcal from carbohydrate, and 15% kcal from protein. This diet contained 151 g casein, 40 g cornstarch, 50 g cellulose, 336 g lard, 15 g safflower oil, and 25 g soybean oil. Standard laboratory rodent chow diet (Lab Diets 5001) was used for the control diet (CHOW). This control diet had a caloric density of 3.0 kcal/g, and contained the following percentages of energy from the three macronutrient classes: 60% kcal from carbohydrates, 28% kcal from protein, and 12% kcal from fat.

Discrimination probe testing—To assess the effects of the different diets on discrimination performance, all rats were returned to their previously assigned conditioning chambers for probe tests on both the originally trained FN and simple discrimination

problems. The probe tests were given after the rats had been on their diets for 10, 24, 40, 60, and 90 days. Each probe test consisted of two sessions conducted on consecutive days using the same procedures as were used during original training sessions, except that all test sessions were conducted under ad libitum feeding conditions.

Probe test analysis of blood ketone body levels and triglyceride levels—Blood levels of beta-hydroxybutyrate (BHB), an index of blood ketone body levels (Carmant, 2008), and plasma triglyceride levels were assessed at the 0-day baseline and after the conclusion of probe tests following 10, 24, 40, 60, and 90 days on the diets. For blood BHB measurements, a small droplet of whole blood was obtained via tail nick and applied directly to a dry reagent card contained within the StatSite ketometer (StanBio, Boerne, TX). For triglyceride levels, a small amount of blood was also collected into iced EDTA2+ tubes and centrifuged at 200×g for 15m at 4°C. Plasma was aspirated and stored at –80°C for later processing via the Wako Diagnostics L-Type Triglycerides Microtiter Procedure (Wako Diagnostics, Richmond, VA). Briefly, 4μL duplicate samples and serially-diluted calibrators, and 90 μL Reagent A were pipetted into a 96-well plate, incubated for 5m at 37°C, and blanked a SpectraMax M2e plate reader (Molecular Devices, Sunnyvale, CA) at 600 nm with a 700 nm reference wavelength. 30 μL of Reagent B was added to all wells, and the plate was then incubated and read for quantification. The reference values were subtracted from the sample absorbance, and following a correction factor for volume (0.76), the blank value was subtracted from the final absorbance. Standards were plotted according to concentration for all plates, and unknown values were interpolated from the standard curve ($r^2 = 0.98–1.0$).

Analysis of body adiposity—On the day following the conclusion of each probe test, body fat for each rat was assessed as a percent of total body mass. The rats were placed in a constraint tube, which was then inserted into the EchoMRI-900 for a period of approximately 90 sec. During that time, total and % body fat, lean mass, and fluid were calculated and recorded.

Post-behavioral testing measures of fasting blood glucose, insulin, GLP-1, and triglyceride levels—Beginning the day following the completion of the 90-day probe test, the rats were fasted overnight, and baseline blood samples (150–200 ul) were collected from the tail vein into microcentrifuge tubes. Blood glucose was measured immediately in duplicate using a hand-held glucometer (Bayer Contour). The remaining samples were then centrifuged, and the serum was separated and frozen as described above. Insulin, total GLP-1, and fasting triglyceride levels were then assayed in duplicate at a later date using commercial ELISA kits (Crystal Chem, Inc., Millipore, and Wako Diagnostics, respectively).

Analysis of blood-brain barrier (BBB) permeability—Following procedures described previously (Kanoski et al., 2010), at the conclusion of probe testing the rats were anesthetized with an intraperitoneal injection of a mixture of ketamine (75 mg/kg) and xylazine (10 mg/kg). Under full anesthesia, the right femoral artery was exposed from linear incision, and a polyethylene catheter fixed by ligation was introduced into the artery. Sodium fluorescein (10% NaFl, 0.6 ml/kg in saline) was applied from a syringe into the catheter at a rate of approximately 0.2 ml/min. After the NaFl had been applied, the artery was ligated with a suture to prevent backflow, and the dye was allowed to circulate for 45 min. The rats were then perfused intracardially with sterile saline at a rate of 10 ml/min for 5 min to wash out the dye remaining in the brain capillary. To prevent re-circulation of the blood, the left ventricle of the heart was cut before the initiation of the perfusion. Following the perfusion, the rats were decapitated and the brains were quickly removed. After

removing the choroid plexus by hand, the hippocampus, striatum, prefrontal cortex (PFC), and cerebellum were bilaterally extracted over ice and stored at -80°C . For NaFl fluorescence analysis, the tissue samples were weighed, homogenized in 200 μl phosphate buffered saline, and centrifuged for 10 min at 5000 rpm. Supernatants were diluted with 400 μl trichloroacetic acid (20%) and then centrifuged for 15 min at 13,600 rpm. Supernatants were then neutralized with sodium hydroxide (NaOH), and fluorescence was measured in duplicate with an ELISA scanner at an excitation wavelength of 480 nm and emission wavelength of 525 nm. Tissue samples were processed under minimal lighting conditions. Each of the three brain areas was processed and analyzed separately allowing comparison of the two dietary groups within each brain area. A negative control sample lacking brain tissue was used with each brain area scan; the fluorescence value of the negative control was subtracted from the fluorescence intensity value for each sample. Absolute intensity values were normalized by the tissue weight for each separate sample.

2.1 Results

Body weight

Within each diet group, rats that were in the top 50% of the weight distribution at the time of the 90-day probe test were classified as DIO, and rats in the bottom 50% of the distribution were classified as DR. In all diet groups, mean body weight increased for both DIO and DR classifications from the ad libitum chow baseline test through the 90-day probe test (see Figure 1). However, amount of weight gain was not the same in all groups and classifications. By the end of testing, mean weight for DIO rats in both Groups WD and KETO were higher compared to DR rats in both of these groups and compared to both DIO and DR rats in Group CHOW. Mean body weight for the DIO and DR rats in Group CHOW differed little from each other. This pattern of results yielded a significant main effect of Probe Test ($F(5, 150) = 1258.03, p < .01$) and a significant Probe Test $\times$ Group ($F(25, 150) = 1081, p < .01$) interaction. Post-hoc tests with Bonferroni corrections for experimentwise error found no differences among any of the groups when they were tested at baseline. However, both the WD-DIO and KETO-DIO groups weighed significantly more than their WD-DR and KETO-DR counterparts and also significantly more than both the CHOW-DIO and CHOW-DR controls (all $ps < .05$) by day 60 of probe testing. Mean body weight did not differ significantly among the CHOW-DIO and CHOW-DR groups on any probe test day.

Body Adiposity

For groups given the WD and KETO diets, percent body fat increased sharply during the 10-day period after the diets were introduced, regardless of whether the rats in those groups were classified as DIO or DR (see Figure 2). Further increases in percent body fat were observed for all of these groups after the 10-day test, with the highest percent body fat exhibited by the KETO-DIO rats. In contrast, percent body fat for the CHOW-DIO and CHOW-DR rats increased very little relative to baseline across probe testing and was lower compared to all other groups. Statistical analysis of these findings revealed that the main effects of Probe Test ($F(5, 150) = 110.73$) and Diet Group ($F(5, 30) = 10.81$) and the Probe Test $\times$ Diet Group interaction ($F(25, 150) = 11.23$) were each significant (all $ps < .01$). Post hoc tests with Bonferroni corrections showed that percent body fat for the CHOW-DIO and CHOW-DR did not differ from baseline or from each other on any test. In addition, percent body fat was significantly less for both the CHOW-DIO and CHOW-DR groups compared to KETO-DIO on Days 60 and 90 and compared to WD-DIO and KETO-DR on Day 90 (all $ps < .05$). There were no significant differences in mean percent body fat between the DR and DIO classifications for any other diet group on any probe test day.

Simple discrimination performance

Because there were no significant differences between CHOW-DIO and CHOW-DR groups in mean body weight or mean percent body fat at baseline or on any probe test day, the CHOW-DIO and CHOW-DR groups were combined and labeled “CHOW” for all subsequent analyses. The top panel of Figure 3 shows performance on the simple discrimination problem for each diet group during the last two-session block of training (before diet manipulations began) when the rats were food deprived, during the baseline test when all rats had been fed ad libitum chow for 7 days, and for each probe test after the WD and KETO diets had been introduced. All groups responded more on rewarded CS+ trials than on nonrewarded CS− trials on the last block of training. An analysis of variance (ANOVA) that compared simple discrimination performance on the last block of training as a function of Diet Group (dummy variable) yielded a significant main effect of CS+ vs. CS− ($F(1, 31) = 115.09, p < .01$) but no significant effect of Diet group and no significant CS+ vs. CS− $\times$ Diet Group interaction. Thus, groups that received WD and KETO maintenance diets and had different obesity phenotypes (DIO or DR) during testing did not differ from CHOW controls at the end of discrimination training.

ANOVA was also used to compare discrimination performance for the same groups on each of the probe test sessions (i.e., ad lib chow baseline and 10, 24, 40, 60, and 90 days after the high energy maintenance diets had been introduced). This analysis revealed significant main effects of CS+ vs. CS− ($F(1, 31) = 299.47, p < .01$) and Probe Test ($F(5, 155) = 3.69, p < .01$) as well as significant CS+ vs. CS− $\times$ Probe Test ($F(5, 155) = 3.48, p < .01$) and CS+ vs. CS− $\times$ Probe Test $\times$ Diet Group ($F(20, 155) = 3.19, p < .01$) interactions. Subsequent Duncan tests showed that the effect of CS+ vs. CS− was significant for the WD-DIO, KETO-DIO, KETO-DR, and CHOW groups on all probe tests and was significant for Group WD-DR on all but the ad libitum baseline test ($ps < .05$). A one-way ANOVA compared each HE diet group with the Chow-fed group on each test session. This analysis revealed that both Group WD-DR and Group WD-DIO were significantly different from the Chow group on the ad libitum baseline test (smallest CS+ vs. CS− $\times$ Group interaction $F(1, 16) = 5.22, p < .01$ for the WD-DIO vs. Chow comparison). As Figure 3 shows, baseline discrimination performance was impaired for the WD-DR relative to the Chow group, whereas the WD-DIO group exhibited greater discrimination performance on the ad lib baseline test relative to the Chow group. The basis of this difference in performance relative to the Chow fed control group is uncertain given that all groups were under the same dietary conditions when the baseline test was conducted (i.e., following 7 days of ad lib chow). No other significant differences between any HE diet group (WD-DIO, WD-DR, KETO-DIO, KETO-DR) and the Chow group were obtained on probe test days 10–90.

FN discrimination performance

An analysis parallel to that for the simple discrimination problem was used to evaluate serial FN discrimination performance. The bottom panel of Figure 3 shows performance on the FN discrimination problem for the same diet groups and periods of training and testing that were presented for the simple discrimination problem. For the FN discrimination, all groups responded more on rewarded T+ trials than on nonrewarded L→T− trials on the last block of training. For this block, ANOVA obtained a significant main effect of T+ vs. L→T− ($F(1, 31) = 69.00, p < .01$), but no main effect of or interaction with Diet Group (dummy variable). Thus, FN discrimination performance was similar for all prospective diet groups at the end of training.

Considering the results of the subsequent test phase, discrimination performance among the diet groups varied as a function of Probe Test. ANOVA revealed significant main effects of T+ vs. L→T− ($F(1, 31) = 145.52, p < .01$) and Probe Test ($F(5, 155) = 3.94, p < .01$) as well

as a significant $T+ \text{ vs. } L \rightarrow T- \times \text{ Probe Test} \times \text{Diet Group}$ ($F(20, 155) = 2.38, p < .01$) interaction. Duncan tests showed that Groups Chow and KETO-DIO responded significantly more on $T+$ than on $L \rightarrow T-$ trials during all probe tests. As was the case for simple discrimination, Group WD-DR responded significantly more on $T+$ than on $L \rightarrow T-$ trials on all probe tests except for the baseline test, which was conducted before the WD was introduced. Only Groups WD-DIO and KETO-DR failed to show significant FN discrimination on any tests after the WD and KETO diets had been introduced. For Group WD-DIO, differences in responding on $T+$ and $L \rightarrow T-$ trials failed to achieve significance on the 10-, 24-, and 90-day probe tests. For Group KETO-DR, the difference between these trial types was nonsignificant on probe test days 10 and 90.

Separate one-way ANOVAs were used to determine if the magnitude of the compared $T+$ vs. $L \rightarrow T-$ differences for Group WD-DR on the baseline test, for Group KETO on the 10- and 90-day tests, and for Group WD-DIO on the 10-, 24-, and 90-day tests were significantly different compared to CHOW controls. These ANOVAs obtained significant $T+ \text{ vs. } L \rightarrow T- \times \text{Diet Group}$ interactions on Day 90 for the WD-DIO versus CHOW ($F(1, 16) = 5.27, p < .05$) and KETO-DR vs CHOW ($F(1, 16) = 5.52, p < .05$) comparisons. None of the other comparisons with the CHOW controls achieved significance. The results indicate that FN performance on the 90-day probe test was impaired for Groups WD-DIO and KETO-DR relative to the CHOW control group.

Probe test ketone and triglyceride levels

Figure 4 shows ketone levels for each diet group as a function of probe test day. As expected, ketone levels for groups maintained on the ketogenic diet (KETO-DIO and KETO-DR) were higher compared to all other diet groups, which differed little from each other. This pattern of results yielded significant main effects of Diet Group ($F(4, 31) = 13.44, p < .01$) and Probe Test ($F(5, 155) = 7.59, p < .01$). Figure 4 also shows that ketone levels peaked for the KETO-DIO and KETO-DR groups on the 10-day probe test and then decreased for both groups by the 90-day probe test. These results yielded a significant Diet Group $\times$ Probe Test interaction ($F(20, 155) = 1.75, p < .05$). Post-hoc Duncan tests showed that for overall probe testing, ketone levels were significantly higher for Groups KETO-DIO and KETO-DR compared to Groups CHOW, WD-DIO and WD-DR ($ps < .05$). Additional Duncan tests showed that ketone levels for Group KETO-DIO were significantly higher compared to Chow controls on probe test days 10, 24, 40, and 90, whereas this difference was significant for Group KETO-DR only on probe test days 10, 24, and 40 (all $ps < .05$). Figure 5 shows that free feeding triglyceride levels did not vary systematically during probe testing as a function of Diet Group. Although these data yielded a significant main effect of Probe Test ($F(5, 150) = 9.74, p < .01$), neither the main effect of Diet Group nor the Diet Group $\times$ Probe Test interaction achieved significance.

Post 90-day probe test metabolic and hormonal measures

Table 1 compares the Chow group with each of the HE diet groups with respect to mean fasting levels of insulin, glucose, GLP-1, and triglycerides that were completed following the 90-day behavioral probe test. One-way ANOVAs and Dunnett tests were used to compare CHOW controls to each of the other groups. One-way ANOVA obtained a significant main effect of group for glucose levels ($F(4, 31) = 6.13, p < .01$). Dunnett tests showed that glucose levels were significantly lower for Group CHOW compared to Groups WD-DIO, KETO-DIO, and KETO-DR ($ps < .05$) but not Group WD-DR. ANOVA also obtained a significant main effect of Group ($F(4, 31) = 2.71, p < .049$) for insulin levels. Although insulin levels trended higher for both Groups WD-DIO and KETO-DIO groups, Dunnett tests failed to yield a significant difference for any group relative to CHOW

controls. ANOVAs failed to obtain significant main effects for either fasting GLP-1 or fasting triglyceride levels.

Blood-Brain Barrier (BBB) Permeability

After the conclusion of fasting measures of metabolic and hormonal activity, permeability of the BBB was assessed for the WD-DR, WD-DIO, and KETO-DIO groups as well as for the 6 rats that weighed the most in the CHOW group. Figure 6 shows mean NaFl concentration ($\mu\text{g NaFl/mg brain tissue}$) for each of these groups in the hippocampus, striatum, PFC, and cerebellum. Separate one-way ANOVAs were performed to compare NaFl concentration of each group as a function of brain region. For the hippocampus, ANOVA obtained a significant main effect of Group ($F(3, 17) = 16.18, p < .01$). Post-hoc Duncan tests showed that NaFl concentration was significantly higher for Group WD-DIO compared to each of the other groups ($ps < .01$), which did not differ from one another. No significant main effects of Group were obtained by ANOVAs that evaluated NaFl concentration for the striatum, PFC, or cerebellum.

Correlations of 90-day probe test FN performance with post 90-day metabolic and hormonal measures

Table 2 shows the degree of correlation between the difference score obtained by subtracting for each group the mean number of photobeam breaks on L→T− trials from the mean number recorded on T+ trials during the 90-day probe test and each measure of hormonal and metabolic activity that was obtained shortly after that test. The correlational analysis was conducted for all diet groups collapsed across the DIO and DR phenotypes. The results show that for the group maintained on the KETO diet, FN performance was significantly and positively correlated with level of circulating ketones ($ps < .05$). That is, higher levels of ketones were associated with higher levels of FN performance. Ketone levels were not significantly correlated with FN performance for any other group. Ad libitum triglyceride levels and levels of GLP-1 were significantly and negatively correlated with FN performance for the chow controls, but not for any of the groups that were given a HE diet. Table 2 shows that for the WD and KETO groups, negative and significant correlations were obtained between FN performance and glucose levels, whereas higher glucose levels were associated with poorer FN performance for both of these groups. Fasting triglyceride levels were not significantly correlated with FN performance for any group.

Cross-lagged panel correlations

Figure 7 presents the results of cross-lagged panel correlations for rats given the WD (left side) and KETO (right side) diet collapsed across the DIO and DR classifications. Only results for these two diets are shown because only these diets were associated with impaired FN performance. The cross-lagged panel correlations assess relationships among FN discrimination performance and ketone levels (top panels), body weight (middle panels), and percent body fat (bottom panels) on the 24-day and 90-day probe tests. The top left panel of Figure 7 shows that for the WD group, FN performance was not significantly correlated with ketone levels on Probe Test Day 24 or Day 90 (indicated by the horizontal lines in the panel). The correlation between ketone levels at Day 24 and Day 90 also failed to achieve significance (top horizontal line in the panel). However, the positive correlation between FN performance at Day 24 and Day 90 (bottom horizontal line in the panel) was significant ($p < .05$). Furthermore, neither of the cross-lagged correlations (indicated by the diagonal lines in the panel) was significant. This indicates that for Group WD, ketone levels on Day 24 were not predictive of FN performance on Day 90, nor was FN performance on Day 24 predictive of ketone levels on Day 90. For Group KETO (top right panel), while ketone levels and FN performance were not significantly correlated at Day 24, the positive correlation between these two factors was significant when they were compared on Day 90.

In addition, like Group WD, the correlation between Group KETO FN performance on Day 24 and Day 90 was positive and significant, whereas the correlation between ketone levels on those two probe test days was not. Also like Group WD, the correlation between FN performance on Day 24 and Day 90 ketone levels was nonsignificant, but unlike Group WD, for Group KETO a significant positive correlation was obtained between Ketone level at Day 24 and FN performance on Day 90. Thus, for Group KETO, higher ketone levels early in testing predicted better FN performance later on.

Figure 7 shows the pattern and direction of significant correlations involving body weight (middle panels) and percent body fat (bottom panels) and FN performance for Groups WD and KETO. For both groups, body weight on Day 24 was significantly and positively correlated with Day 90 body weight, and the same significant positive correlation was obtained for percent body fat on those probe test days. However, there were differences in the cross-lagged correlations between FN performance and body weight and percent body fat. For Group WD, significant negative correlations were obtained between FN performance on Day 24 and both body weight and body fat on Day 90, indicating that those animals that had the lowest FN performance on Day 24 were heaviest and fattest on Day 90. However, the correlations between 24-day body weight and Day 90 FN performance and between 24-day percent body fat and FN performance on the 90-day test both failed to achieve significance, indicating that weight or adiposity on Day 24 did not predict FN performance on Day 90. Neither of the cross-lagged comparisons involving body weight or percent body fat was significant for Group KETO. These results show that for Group WD, but not for Group KETO, poor FN performance at the 24-day probe test was associated with elevated body weight and percent body fat 56 days later, whereas neither body weight nor percent body fat at the earlier time point predicted subsequent FN performance.

3.1 Discussion

The present findings agree with our previous reports that (a) consuming a WD significantly impairs performance on a hippocampal-dependent serial FN discrimination problem, but not on a simple discrimination problem that does not require an intact hippocampus; (b) consuming this diet increases BBB permeability and this increase leads to a greater accumulation of NaFl selectively in the hippocampus; (c) DIO rats that showed the highest body weight and adiposity after 90 days on WD were also most sensitive to the adverse effects of this diet on FN performance and BBB function.

We also obtained several novel findings: For DIO rats fed ad lib WD for 90 days, impaired FN discrimination performance was associated with elevated fasting blood glucose levels compared to control rats given standard chow for the same period. Further, for all rats fed WD, a large and significant negative correlation between fasting blood glucose level and FN performance was obtained. This confirmed that higher fasting levels of blood glucose were predictive of poorer FN discrimination performance following 90 days on the WD.

However, the effects of body weight, adiposity, and blood glucose levels were not related to FN performance in a simple way. Like the WD-DIO rats, both DIO and DR rats fed the KETO diet had higher body adiposity and fasting blood glucose levels compared to the Chow-fed controls. Yet FN performance was impaired only for the KETO-fed DR rats, even though body weight differences between this group and chow-fed controls were not significant. Furthermore, KETO-fed DIO rats did not show impaired FN performance, even though their body weight, adiposity, and blood glucose level was significantly higher compared to the control animals. This pattern was the opposite of that shown by WD-DIO rats. These findings make it difficult to attribute the FN performance deficits exhibited by

the WD-DIO and KETO-DR groups to any simple effects of diet-induced increases in body weight, body adiposity, or fasting blood glucose level.

Moreover, while body weight, body adiposity, and blood glucose levels were significantly elevated for both Groups WD-DIO and KETO-DIO, evidence of increased BBB permeability was found only in the FN impaired WD-DIO group (the opportunity to assess BBB data for Group KETO-DR was lost due to an experimental error). Thus, while the available findings link impaired FN performance to increased BBB permeability, increased BBB permeability was not linked directly to elevated levels of body weight, body fat, or blood glucose. Also, given the differences in the composition of the WD and KETO diets, it does not appear that the effects of diet on hippocampal-dependent FN performance and BBB integrity depend only on the amount of saturated fat or the source and amount of carbohydrate.

One way to approach these seemingly complex results is to ask why elevated body adiposity and fasting blood glucose levels were associated with impaired FN performance for Groups WD-DIO and KETO-DR but not for the KETO-DIO group. Previous research identified diet-induced impairment in some types of hippocampal-dependent learning memory tasks with hyperinsulinemia (e.g., Kamal et al., 2013) and elevated levels of triglycerides (Granholt et al., 2008, Freeman et al., 2011). In addition, some recent reports suggest that GLP-1 and its analogues may have effects that could improve cognitive functioning (McClellan et al., 2011, Ma et al., 2012, Parthasarathy and Holscher, 2013). However, our findings do not allow us to distinguish among FN-impaired and unimpaired groups based on fasting levels of insulin, total GLP-1, or on fasted or non-fasted levels of triglycerides because none of these factors varied systematically with FN performance.

One measure that separated the unimpaired KETO-DIO group from KETO-DR and WD-DIO groups was level of circulating ketone bodies at the time of the 90 day probe test. Compared to the CHOW controls, only Group KETO-DIO had significantly higher levels of circulating ketone bodies after 90 days of ad lib diet. This difference suggests that: (a) elevated levels of ketone bodies spared Group KETO-DIO from FN discrimination deficits induced by high adiposity and/or blood glucose; (b) Groups WD-DIO and KETO-DR showed impaired FN performance because they lacked the protection afforded by elevated ketone bodies; and (c) Group WD-DR exhibited no FN performance deficits in the absence of elevated ketone bodies because neither adiposity nor blood glucose levels were elevated for that group.

In addition, for rats fed the KETO diet, level of ketone bodies were significantly and positively correlated with hippocampal-dependent FN performance on the 90-day probe test. This correlation was small and nonsignificant for the other diet groups. The cross-lagged panel correlations also showed that ketone levels at Day 24 of KETO diet exposure predicted FN performance at Day 90, with higher ketone levels being associated with better performance. These findings suggest that ketone bodies afford protection against the detrimental effects of diet-induced gluco-dysregulation and elevated body adiposity on hippocampal-dependent cognitive function. This conclusion is consistent with the results of other recent studies (e.g., (Kwon et al., 2008, Linard et al., 2010, Hallbook et al., 2012).

The cross-lagged panel correlations also provided information about the direction of the potential causal relationship between WD-diet induced weight gain, adiposity, and cognitive dysfunction. For rats fed the WD, weaker FN performance at Day 24 was associated with both higher body weight and higher adiposity at Day 90. However, the correlation between body weight and percent body fat at Day 24 with FN performance at Day 90 were both much lower and nonsignificant. Thus, while the adverse effect of consuming WD on

cognitive function was a good predictor of subsequent weight gain and increased adiposity, the effect of that diet on weight gain and adiposity did not reliably predict subsequent cognitive deficit. This finding is consistent with the conclusion that cognitive deficit precedes excessive body weight gain for animals maintained on WD. None of these cross-lagged correlations were significant for the KETO diet group.

Another feature of our findings deserves discussion. Deficits in FN performance for rats in Groups WD-DIO and KETO-DR were apparent after 24 days on the WD, and were absent at 45 and 60 days, before reappearing at 90 days of WD exposure. This pattern is reminiscent of results reported by Thaler et al., (2012), which showed that inflammatory signaling and reactive gliosis in the rat hypothalamus within 3–7 days after initiation of a diet similar to our WD. These effects temporarily subsided following 7–14 days before re-emerging after 28 days of diet exposure. Thus, although occurring over different time frames, the pattern of early hypothalamic pathology, recovery, and subsequent return of markers of hypothalamic damage reported by Thaler et al, parallels the effects of WD on hippocampal-dependent FN performance for DIO rats in the present study. It is unclear if impairments that are observed after short periods of diet exposure involve the same mechanisms as those that appear following more prolonged exposure. Definitive answers to this question will require comparisons of BBB and hippocampal pathology at several time points following initiation of WD.

3.1.1 Conclusions

In summary, our results confirmed that for rats maintained on the WD, deficits in hippocampal-dependent cognitive functioning and in the structural integrity of the BBB are linked to the capacity of that diet to promote obesity. However, our findings also suggest that elevated ketone bodies may protect against the adverse effects of high adiposity and/or high blood glucose levels on hippocampal-dependent cognition. The question of whether obesity leads to cognitive impairments or cognitive impairments lead to obesity was also addressed by the present research. We found that for rats fed a WD, poorer cognitive performance after 24 days on the diet was significantly associated with higher body weight and adiposity after 90 days on that diet. In contrast, neither body weight or body adiposity following 24 days of diet exposure predicted cognitive performance after 90 days on the diet. This pattern suggests that cognitive deficits are more likely to precede obesity rather than vice-versa.

The results of human studies suggest that obesity is associated with cognitive decline across the lifespan, from relatively mild deficiencies early in life to severe dementia in old age (Smith et al., 2011). Furthermore, the hippocampus and structures comprising the larger hippocampal formation have been identified as early targets of brain pathologies (Didic et al., 2011) that give rise to progressive deterioration of cognitive function as such pathologies spread to additional medial temporal lobe structures and then to other areas of the brain (Smith, 2002). It may be that consuming diets high in saturated fat and carbohydrate produces changes in the brain that impair hippocampal-dependent cognitive functioning; these changes precede and perhaps contribute to overeating, leading not only to obesity and metabolic disease, but also to further interference with the hippocampus and perhaps other substrates for cognitive functioning. From this perspective, early detection of mild forms of hippocampal-dependent cognitive dysfunction and early therapeutic interventions that effectively treat these forms of cognitive disorder should also help to prevent subsequent weight gain and stop the initiation of a vicious-cycle of obesity and cognitive decline (Davidson et al., 2005, Kanoski and Davidson, 2011).

Acknowledgments

The preparation of this manuscript and the research that is described herein was supported by grants P01 HD052112 and R01 HD29792 from the National Institutes of Child Health and Development and grants ES008146 and ES017055 from the National Institutes of Environmental Health Sciences.

5.1 References

- Benoit SC, Davis JF, Davidson TL. Learned and cognitive controls of food intake. *Brain Res.* 2010; 1350:71–76. [PubMed: 20561510]
- Bielohuby M, Menhofer D, Kirchner H, Stoeck BJM, Muller TD, Stock P, Hempel M, Stemmer K, Pfluger PT, Kienzle E, Christ B, Tschop MH, Bidlingmaier M. Induction of ketosis in rats fed low-carbohydrate, high-fat diets depends on the relative abundance of dietary fat and protein. *American Journal of Physiology-Endocrinology and Metabolism.* 2011; 300:E65–E76. [PubMed: 20943751]
- Carmant L. Assessing ketosis: approaches and pitfalls. *Epilepsia.* 2008; 49(Suppl 8):20–22. [PubMed: 19049579]
- Davidson TL, Kanoski SE, Schier LA, Clegg DJ, Benoit SC, Davidson TL, Kanoski SE, Schier LA, Clegg DJ, Benoit SC. A potential role for the hippocampus in energy intake and body weight regulation. *Current Opinion in Pharmacology.* 2007; 7:613–616. [PubMed: 18032108]
- Davidson TL, Kanoski SE, Walls EK, Jarrard LE, Kanoski SE, Walls EK, Jarrard LE. Memory inhibition and energy regulation. *Physiology & behavior.* 2005; 86:731–746.
- Davidson TL, Monnot A, Neal AU, Martin AA, Horton JJ, Zheng W. The effects of a high-energy diet on hippocampal-dependent discrimination performance and blood-brain barrier integrity differ for diet-induced obese and diet-resistant rats. *Physiology & behavior.* 2012; 107:26–33. [PubMed: 22634281]
- Didic M, Barbeau EJ, Felician O, Tramon E, Guedj E, Poncet M, Ceccaldi M. Which Memory System is Impaired First in Alzheimer's Disease? *Journal of Alzheimers Disease.* 2011; 27:11–22.
- Farr SA, Yamada KA, Butterfield DA, Abdul HM, Xu L, Miller NE, Banks WA, Morley JE. Obesity and Hypertriglyceridemia produce cognitive impairment. *Endocrinology.* 2008; 149:2628–2636. [PubMed: 18276751]
- Freeman LR, Granholm ACE. Vascular changes in rat hippocampus following a high saturated fat and cholesterol diet. *Journal of Cerebral Blood Flow and Metabolism.* 2012; 32:643–653. [PubMed: 22108721]
- Freeman LR, Haley-Zitlin V, Stevens C, Granholm AC. Diet-induced effects on neuronal and glial elements in the middle-aged rat hippocampus. *Nutritional Neuroscience.* 2011; 14:32–44. [PubMed: 21535919]
- Granholm AC, Bimonte-Nelson HA, Moore AB, Nelson ME, Freeman LR, Sambamurti K. Effects of a saturated fat and high cholesterol diet on memory and hippocampal morphology in the middle-aged rat. *Journal of Alzheimers Disease.* 2008; 14:133–145.
- Hallbook T, Ji SG, Maudsley S, Martin B. The effects of the ketogenic diet on behavior and cognition. *Epilepsy Research.* 2012; 100:304–309. [PubMed: 21872440]
- Herculano B, Tamura M, Ohba A, Shimatani M, Kutsuna N, Hisatsune T. beta-Alanyl-L-Histidine Rescues Cognitive Deficits Caused by Feeding a High Fat Diet in a Transgenic Mouse Model of Alzheimer's Disease. *Journal of Alzheimers Disease.* 2013; 33:983–997.
- Holland PEC, Lamoureux JA, Han JS, Gallagher M. Hippocampal lesions interfere with Pavlovian negative occasion setting. *Hippocampus.* 1999; 9:143–157. [PubMed: 10226775]
- Izacson R, Nielsen E, Dannaeus K, Bertilsson G, Patrone C, Zachrisson O, Wikstrom L. The glucagon-like peptide 1 receptor agonist exendin-4 improves reference memory performance and decreases immobility in the forced swim test. *European Journal of Pharmacology.* 2011; 650:249–255. [PubMed: 20951130]
- Jarrard LE, Davidson TL. On the hippocampus and learned conditional responding: effects of aspiration versus ibotenate lesions. *Hippocampus.* 1991; 1:107–117.
- Kamal A, Ramakers GMJ, Gispen WH, Biessels GJ, Al Ansari A. Hyperinsulinemia in rats causes impairment of spatial memory and learning with defects in hippocampal synaptic plasticity by involvement of postsynaptic mechanisms. *Experimental Brain Research.* 2013; 226:45–51.

- Kanoski SE, Davidson TL. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiology & behavior*. 2011; 103:59–68. [PubMed: 21167850]
- Kanoski SE, Meisel RL, Mullins AJ, Davidson TL, Kanoski SE, Meisel RL, Mullins AJ, Davidson TL. The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat. *Behavioural Brain Research*. 2007; 182:57–66. [PubMed: 17590450]
- Kanoski SE, Zhang Y, Zheng W, Davidson TL. The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat. *Journal of Alzheimer's disease : JAD*. 2010; 21:207–219.
- Kenny DA. Cross-lagged panel correlation - test for spuriousness. *Psychological Bulletin*. 1975; 82:887–903.
- Kinzig KP, Scott KA, Hyun J, Bi S, Moran TH. Altered hypothalamic signaling and responses to food deprivation in rats fed a low-carbohydrate diet. *Obesity Research*. 2005; 13:1672–1682. [PubMed: 16286514]
- Krikorian R, Shidler MD, Dangelo K, Couch SC, Benoit SC, Clegg DJ. Dietary ketosis enhances memory in mild cognitive impairment. *Neurobiology of Aging*. 2012:33.
- Kwon YS, Jeong SW, Kim DW, Choi ES, Son BK. Effects of the ketogenic diet on neurogenesis after kainic acid-induced seizures in mice. *Epilepsy Res*. 2008; 78:186–194.
- Linard B, Ferrandon A, Koning E, Nehlig A, Raffo E. Ketogenic diet exhibits neuroprotective effects in hippocampus but fails to prevent epileptogenesis in the lithium-pilocarpine model of mesial temporal lobe epilepsy in adult rats. *Epilepsia*. 2010; 51:1829–1836. [PubMed: 20633040]
- Lutas A, Yellen G. The ketogenic diet: metabolic influences on brain excitability and epilepsy. *Trends in Neurosciences*. 2013; 36:32–40. [PubMed: 23228828]
- Ma T, Du XL, Pick JE, Sui GZ, Brownlee M, Klann E. Glucagon-Like Peptide-1 Cleavage Product GLP-1(9-36) Amide Rescues Synaptic Plasticity and Memory Deficits in Alzheimer's Disease Model Mice. *Journal of Neuroscience*. 2012; 32:13701–13708.
- Madsen AN, Hansen G, Paulsen SJ, Lykkegaard K, Tang-Christensen M, Hansen HS, Levin BE, Larsen PJ, Knudsen LB, Fosgerau K, Vrang N. Long-term characterization of the diet-induced obese and diet-resistant rat model: a polygenetic rat model mimicking the human obesity syndrome. *J Endocrinol*. 2010; 206:287–296. [PubMed: 20508079]
- Malone JI, Hanna S, Saporta S, Mervis RF, Park CR, Chong L, Diamond DM. Hyperglycemia not hypoglycemia alters neuronal dendrites and impairs spatial memory. *Pediatric Diabetes*. 2008; 9:531–539.
- McClellan PL, Parthasarathy V, Faivre E, Holscher C. The Diabetes Drug Liraglutide Prevents Degenerative Processes in a Mouse Model of Alzheimer's Disease. *Journal of Neuroscience*. 2011; 31:6587–6594. [PubMed: 21525299]
- Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience*. 2002; 112:803–814. [PubMed: 12088740]
- Park HR, Park M, Choi J, Park KY, Chung HY, Lee J. A high-fat diet impairs neurogenesis: involvement of lipid peroxidation and brain-derived neurotrophic factor. *Neuroscience letters*. 2010; 482:235–239. [PubMed: 20670674]
- Parthasarathy V, Holscher C. The type 2 diabetes drug liraglutide reduces chronic inflammation induced by irradiation in the mouse brain. *European Journal of Pharmacology*. 2013; 700:42–50. [PubMed: 23276669]
- Puig KL, Floden AM, Adhikari R, Golovko MY, Combs CK. Amyloid Precursor Protein and Proinflammatory Changes Are Regulated in Brain and Adipose Tissue in a Murine Model of High Fat Diet-Induced Obesity. *Plos One*. 2012:7.
- Samoilova M, Weisspapir M, Abdelmalik P, Velumian AA, Carlen PL. Chronic in vitro ketosis is neuroprotective but not anti-convulsant. *Journal of Neurochemistry*. 2010; 113:826–835. [PubMed: 20163521]
- Schioth HB, Craft S, Brooks SJ, Frey WH, Benedict C. Brain Insulin Signaling and Alzheimer's Disease: Current Evidence and Future Directions. *Molecular Neurobiology*. 2012; 46:4–10.

- Smith AD. Imaging the progression of Alzheimer pathology through the brain. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:4135–4137. [PubMed: 11929987]
- Smith E, Hay P, Campbell L, Trollor JN. A review of the association between obesity and cognitive function across the lifespan: implications for novel approaches to prevention and treatment. *Obesity Reviews*. 2011; 12:740–755. [PubMed: 21991597]
- Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao XL, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschop MH, Schwartz MW. Obesity is associated with hypothalamic injury in rodents and humans (vol 122, pg 153, 2012). *Journal of Clinical Investigation*. 2012; 122:778–778.

Highlights

Western diet (WD) impairs hippocampal-dependent cognitive function in rats

WD is linked to blood-brain barrier (BBB) and glucoregulatory dysfunction

Effects of WD on hippocampal cognitive and BBB function depend on obesity phenotype

Diet-induced ketosis may have protective effects on the hippocampus and BBB

Hippocampal-dependent cognitive dysfunction may precede and promote weight gain

Figure 1.

Mean body weight (g) for rats fed WD (WD-DR and WD-DIO), ketogenic diet (KETO-DR and KETO-DIO), or standard chow (CHOW-DR and CHOW-DIO) at baseline and throughout the 90-day diet exposure. Alphabetic indicators (a, b, c, d,) shown in the legend are used to denote groups that differed significantly ($p < .05$) from CHOW-DIO and CHOW-DR groups on a given probe test day. Differences between CHOW-DIO and CHOW-DR groups were not significant on any day of probe testing. Vertical bars depict standard error of the mean (SEM).

Figure 2.

Mean percent body fat for rats fed WD (WD-DR and WD-DIO), ketogenic diet (KETO-DR and KETO-DIO), or standard chow (CHOW-DR and CHOW-DIO) at baseline and throughout the 90-day diet exposure. Alphabetic indicators (a, b, c, d,) shown in the legend are used to denote groups that differed significantly ($p < .05$) from CHOW-DIO and CHOW-DR groups on a given probe test day. Differences between CHOW-DIO and CHOW-DR groups were not significant on any day of probe testing. Vertical bars depict standard error of the mean (SEM).

Figure 3.

Simple (CS+ and CS-; top panels) and serial FN (T+, L→T-; bottom panels) discrimination performance, as indexed by mean beam breaks, on the last 2 blocks of training when food deprived (Train), after 7 days on ad lib standard chow (BL), and 10, 24, 40, 60, and 90 days after the introduction of WD (WD-DR and WD-DIO), ketogenic diet (KETO-DR and KETO-DIO), or continuation on standard chow (CHOW). * denote probe tests in which responding on rewarded trials was significantly greater than on nonrewarded trials. Brackets with a corresponding * denote contiguous probe test days in which the difference in responding on rewarded compared to nonrewarded trials was significant. Error bars depict SEM.

Figure 4.

Mean beta-hydroxybutyrate levels for rats fed WD (WD-DR and WD-DIO), ketogenic diet (KETO-DR and KETO-DIO), or standard chow (CHOW) at baseline when all groups were fed ad libitum standard chow and on each probe test day (10, 24, 40, 60 and 90) after the experimental diets were introduced. Alphabetic indicators (a, b, c, d,) shown in the legend are used to denote groups that differed significantly ($p < .05$) from CHOW controls on a given test day. Error bars depict SEM.

Figure 5.

Mean triglyceride levels for rats fed WD (WD-DR and WD-DIO), ketogenic diet (KETO-DR and KETO-DIO), or standard chow (CHOW) at baseline test when all groups were fed ad libitum standard chow and on each probe test day (10, 24, 40, 60 and 90) after the experimental diets were introduced. Alphabetic indicators (a, b, c, d,) shown in the legend are used to denote groups that differed significantly ($p < .05$) from CHOW controls on a given test day. No significant differences were observed on any test. Error bars depict SEM.

Figure 6.

NaFl concentration ($\mu\text{g NaFl/mg brain tissue}$), a measure of BBB permeability, in hippocampus, striatum, PFC, and cerebellum for rats maintained on WD (WD-DIO and WD-DR), ketogenic diet (KETO-DIO), and standard chow (CHOW) for 90 days. * denote significant difference ($p < .05$) from CHOW control. Error bars depict SEM.

Figure 7.

Cross-lagged panel correlations depicting the correlations the degree and direction of relationship between FN discrimination performance and ketone levels (top two panels), body weight (middle two panels), and percent body fat (bottom two panels) on probes test days 24 and 90 for rats fed WD- (left panels) and KETO diet (right panels), respectively. Significant correlations ($p < .05$) are indicated by numbers that are in italicized, bold-face font, with an accompanying *.

Table 1

Chow versus HE diet groups Post 90-day Probe Test: Fasting Metabolic and Hormonal Measures

	CHOW	WD-DIO	WD-DR	KETO-DIO	KETO-DR
Insulin (ng/mL)	0.58 (+/- .04)	0.84 (+/- .04)	0.57 (+/- .09)	0.84 (+/- .19)	0.54 (+/- .07)
Total GLP-1 (pmol/L)	13.01 (+/- 0.84)	9.29 (+/- 1.11)	11.68 (+/- 1.62)	9.96 (+/- 0.96)	9.59 (+/- 1.10)
Glucose (mg/dL)	71.88 (+/- 2.71)	81.5 (+/- 1.99)	70.5 (+/- 2.58)	85.0 (+/- 3.90)	84.67 (+/- 1.22)
Triglycerides (mg/dL)	18.67 (+/- 3.82)	17.26 (+/- 2.97)	19.70 (+/- 4.48)	14.16 (+/- 4.65)	13.86 (+/- 3.67)

Bold-faced and italicized font = significant ($p < .05$) difference from control

Numbers in parentheses indicate standard errors of the mean (SEM)

Table 2

Correlations of 90-day Probe Test Serial FN performance with Post 90-day Metabolic and Hormonal Measures

		CHOW	WD	KETO
Ad libitum	BHB (mmol/L)	−0.26	−0.27	<i>0.76</i>
	Triglycerides (mg/dL)	<i>−0.61</i>	−0.01	−0.09
Fasting	Insulin (ng/mL)	−0.17	−0.34	−0.01
	Total GLP-1 (pmol/L)	<i>−0.73</i>	−0.10	0.15
	Glucose (mg/dL)	−0.35	<i>−0.76</i>	<i>−0.68</i>
	Triglycerides (mg/dL)	−0.52	0.47	−0.10

Bold-faced and italicized font = significant ($p < .05$) correlation