Effect of high-fat diet on metabolic indices, cognition, and neuronal physiology in aging F344 rats

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1. Introduction

Multiple components of metabolic syndrome, including obesity and diabetes correlate with, and even predict a higher incidence of Alzheimer’s disease (Frisardi et al., 2010; Luchsinger et al., 2012; Whitmer et al., 2008). Importantly, considerable evidence also indicates that metabolic syndrome plays a critical role in cognitive decline during normal aging (Biessels et al., 2008; Launer, 2005; McNay, 2005; Morley, 2004). Although metabolic dysregulation and cognitive decline appear to be distinct pathological processes, some common aspects of both conditions include brain insulin resistance, vascular disease, and/or inflammation (McNay and Recknagel, 2011; Talbot et al., 2012). Still, little is known about how or whether changes in peripheral glucose or lipid metabolism affect neuronal function and brain aging.

Despite considerable attention focusing on the links between the periphery and the brain regarding food intake and energy metabolism, it is not yet clear how peripheral hormones/peptides (e.g., insulin, adiponectin) that regulate these processes change with age. It is also unclear how or to what extent peripheral metabolic dysregulation influences cognitive decline or neuronal vulnerability in disease states (Stranahan and Mattson, 2012). Interestingly, caloric restriction and exercise, two manipulations that slow aging and associated cognitive decline (Keenan et al., 1995), are also able to enhance adiponectin levels, which improve insulin sensitivity (Fruebis et al., 2001; Gustafson, 2010).

Animal models have been used extensively for studies of diabetes and obesity, but have some limitations for studies of brain aging and cognitive decline. Genetic models of diabetes (Zucker diabetic fatty rat and the db/db mouse) show decreased learning and altered synaptic plasticity (Li et al., 2002), although this is not always the case (Belanger et al., 2004). Irrespective of the results seen with these and other genetic models, one critical limitation is their short lifespan, which precludes studies of aging. Experimentally induced diabetes using streptozotocin (STZ) decreases hippocampal-dependent learning in young animals (Dou et al., 2005; Popovic et al., 2001), but only a few studies have been conducted in aged animals using this model (Kamal et al., 2000). Furthermore, the STZ model, although valuable for studies of the impact of type 1 diabetes on learning and memory, does not recapitulate the condition most commonly seen in the aging population.
including the clinically silent period of hyperinsulinemia that precedes type 2 diabetes (T2DM).

An alternative approach to study how peripheral metabolic dysregulation might influence cognitive decline in aging is to use a high-fat diet (HFD). Numerous rodent studies indicate that HFD decreases insulin sensitivity while increasing cholesterol levels and body weight (Buettert et al., 2007). HFD increases visceral fat mass and circulating free fatty acids (FFA), resulting in widespread inflammation via cytokine/adipokine secretion (Xu et al., 2003). An FFA-mediated reduction in insulin receptor signaling is a recognized pathway linking obesity to insulin resistance in liver, muscle, and fat. Indeed, several reports indicate that elevated plasma FFAs induce insulin resistance through inhibition of glucose transport, mediated, in part, by a decrease in phosphatidyl inositol 3–kinase (PI3K) and its interaction with insulin receptor substrate 1 (IRS1) (Curtis et al., 2005; Furuhashi et al., 2007).

Among the most commonly used strains for aging research is the F344 rat. Although this model has been characterized and is routinely used in studies of brain aging, the response to HFD has not been studied as extensively as in other strains. This is important, because the impact of dietary manipulations is clearly sensitive to rat strain (Barzilai and Rossetti, 1995; Narimiya et al., 1984; Reaven et al., 1983). Furthermore, most studies of HFD in aging rodents focus solely on effects in the periphery and largely ignore the impact of the diet on the brain (Bracho-Romero and Reaven, 1977; Keenan et al., 1995; Mooradian et al., 1997). To address this gap, we compared long-term HFD in young and middle-aged F344s. Middle-aged rats were used to parallel the age at which the initial rise in peripheral metabolic dysregulation is typically observed in the human population. We assessed effects of HFD on peripheral metabolic variables and cognitive acuity in the Morris Water Maze (MWM). At the cellular level, we measured an electrophysiological marker of age-related cognitive decline, the Ca$^{2+}$-dependent after-hyperpolarization (AHP), in area CA1 pyramidal neurons (Blalock et al., 2010; Gant et al., 2006; Moyer Jr et al., 1996; Thibault and Landfield, 1996). In the same brain area, we also measured insulin sensitivity, insulin signaling, and adiponectin. In both age groups, HFD was associated with robust dyslipidemia and mild obesity but, surprisingly, did not induce diabetes or alter spatial memory. This is unlike other models and perhaps is related to the comparatively higher peripheral levels of adiponectin that we observed in F344s. Electrophysiological measures in the hippocampus show, for the first time, that the AHP is sensitive to insulin, and that this sensitivity is reduced by HFD. Together, these results suggest the intriguing possibility that in F344 rats, the brain may be more sensitive to the effects of HFD than the periphery.

2. Methods

2.1. Subjects

All experiments presented here were conducted under an approved Institutional Animal Care and Use Committee (IACUC) protocol granted by the University of Kentucky. A total of 66 male F344/NIA rats were maintained single-housed on a control diet (CD) for 3 weeks, and baseline values for the glucose tolerance test (GTT), insulin tolerance test (ITT) and glycated hemoglobin (HbA1c) were obtained (see section 2.5). After 3 weeks on CD, animals were separated into 4 groups as follows: young-adult (2.5–4 months old) on CD (n = 13); young-adult on HFD (n = 13); middle-aged (12.5–13.5 months old) on CD (n = 20); and middle-aged on HFD (n = 20). HFD was initiated on the 4th week of the study. GTT, ITT, and HbA1c tests were repeated after 4.5 months. Food consumption and body weight were monitored 2 to 3 times per week for the duration of the study and were averaged per week per animal. Four middle-aged animals on HFD, 3 middle-aged animals on CD, and 1 young-adult on CD were excluded because of poor health.

2.2. Diets

Purified diets were provided ad libitum (Harlan Teklad, Madison, WI). The CD consisted of the following (in % Kcal): 19.2 protein, 67.9 carbohydrate, and 13 fat (TD. 08485). The HFD, a “Western-style” diet, consisted of the following (in % Kcal): 15.2 protein, 42.7 carbohydrate, and 42 fat (TD. 88137).

2.3. Glucose and insulin tolerance tests

To test for differences between weight-adjusted and standard single-dose glucose or insulin tolerance tests (GTT, ITT, respectively), we monitored blood glucose levels in a series of pilot animals (n = 5–6 per group; 2 months and 13 months old). These animals were exposed to a weight-adjusted glucose (2 g/Kg body weight of 60% glucose), a weight-adjusted insulin dose (2 mU/g body weight of Humalog, Eli Lilly, Indianapolis, IN, USA), and a standard glucose/insulin dose (670 mg glucose per animal, or 450 mU Humalog per animal). The data are presented in supplemental Figure 1 and supplemental Table 1, and show that in the weight-adjusted condition, glucose levels were significantly increased in older animals (F1,26 = 21.6, p < 0.0001), an effect mediated mostly by greater glucose levels seen at the 30-minute time point (post hoc). This result was likely due to the greater mean group weight seen in older animals, and disappeared entirely under standard single-dose glucose injection conditions (supplemental Fig. 1B1). No significant differences were found in measures of area-under-the-curve (AUC) for both glucose and insulin tolerance tests using either approach (supplemental Table 1). Following a 6-hour fast, glucose levels were monitored (mg/dL using a FreeStyle Lite glucometer; Abbott Diabetes Care Inc, Alameda, CA) from tail prick blood samples taken at 30-minute intervals over a 2-hour period in response to an intraperitoneal glucose or insulin injection (Blalock et al., 2010). For some analyses, glucose responses over time were integrated to determine the area-under-the-curve (AUC) and normalized to the initial time-point value. Tests of glucose or insulin sensitivity were separated by one week to limit stress and carry-over effects. Animals that did not show at least a 5% change in glucose levels at the 30-minute time point after glucose or insulin injection were removed from the analysis.

2.4. Tissue collection

Twenty-four animals were anesthetized with pentobarbital (80 mg/Kg body weight), and blood was collected from the right ventricle immediately before perfusion for 10 minutes with ice-cold saline solution (0.9%). Brains were post-fixed in 4% paraformaldehyde overnight and cryoprotected in 30% sucrose solution at −20 °C until sectioning for immunohistochemistry. Heart, liver, and epididymal and retroperitoneal fat pads were collected and weighed (Table 1).

2.5. Blood serum analyses

Serum was isolated from whole blood and frozen for chemical analyses (Comparative Pathology Laboratory, University of California, Davis, CA, USA). Adiponectin levels were determined by the Vanderbilt Hormone Assay Core Lab (Vanderbilt University, Nashville, TN, USA). Because glycated hemoglobin levels are considered to be the "gold standard" for clinical monitoring of diabetes, before blood separation, a drop was used for HbA1c
measurement using a DCA Vantage Analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Serum insulin levels were determined using Millipore rat/mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Billerica, MA, USA) according to the manufacturer's instructions.

2.6. Western blotting

Hippocampal dorsal and ventral quarters were combined and used to quantify protein levels. Insulin receptor (IR) and insulin receptor substrate 1 (IRS-1) were quantified with a standard Western blot protocol. Subcellular fractions (membrane and cytosolic) were obtained from homogenates following a protocol adapted from Dou et al. (2005), and quantified for proteins using a Bradford assay. Samples were run on 10% Mini-PROTEAN TGX gels (Bio-Rad, Hercules, CA, USA). After transfer to nitrocellulose membranes, samples were immunostained overnight at 4 °C (anti-IRS-1 antibody, Santa Cruz Biotechnology, sc-81466, 1:800; anti-IR β antibody, Santa Cruz Biotechnology, sc-560, 1:600; and anti-β-actin antibody, Sigma-Aldrich, A1978, 1:2000). After secondary antibody exposure, membranes were developed with ECL plus (GE Healthcare, Pittsburgh, PA, USA) and visualized using a phosphoimager (Storm 840; Molecular Dynamics, Sunnyvale, CA, USA). ImageJ was used for signal intensity measures (National Institutes of Health, Bethesda, MD, USA; http://rsb.info.nih.gov/ij/), and each sample was normalized to β-actin. All experiments were run in duplicate.

2.7. Morris Water Maze (MWM)

As in prior work (Blalock et al., 2010), temperature was maintained at 25° to 26 °C, and 1 quadrant contained a 15-cm diameter escape platform. VideoMex-V (Columbus Instrument, Columbus, OH, USA) was used to record animal movements. For all training days, 3 trials were run (each with a different start location). Animals were placed in the pool and given 60 seconds to find the platform, otherwise, they were guided to the platform. All animals were allowed to stay on the platform for 30 seconds. After this 30-second period, animals were taken outside the MWM enclosure for 2 minutes and returned to the MWM for a second and then a third trial. On day 1, 3 cue trials were run with a hanging white cup positioned over the partially visible platform. The next 3 days of training/learning (days 4, 5, and 6) animals continued to receive 3 trials per day (without cue and a submerged platform). Twenty-four hours after the last training day, a 1- minute probe test with the platform removed was conducted. The next day, animals were trained to learn a new platform location (reversal training—opposite quadrant from previous position) also using 3 trials per day. Next, 72 hours later a reversal probe was conducted. Performance was evaluated using measures of path length to platform and a cumulative search error index. The index is representative of how far each animal deviated from an ideal path to goal (Blalock et al., 2010; Gallagher et al., 1993).

2.8. Immunohistochemistry

Coronal sections (30 μm) cut on a freezing-sliding microtome were exposed to the following primary antibodies: adiponectin (rabbit, 1:500; Abbiotec, San Diego, CA, USA), insulin receptor-α antibody (rabbit IR-α, 1:200, Abbiotec, San Diego, CA, USA), and insulin receptor subtype-1 antibody (rabbit IRS-1, 1:200, LSbio, Seattle, WA, USA). For adiponectin and IRS-1 staining, sections were pretreated 30 minutes at 85 °C in sodium citrate (pH = 6.5). All tissues were pretreated 30 minutes in 2% HzO2. Following incubation with primary antibody in TBS-T overnight at room temperature, tissues were rinsed 3 times and incubated with the appropriate biotinylated secondary antibody for 2 hours at room temperature. Sections were rinsed 3 times, incubated 2 hours with the tertiary antibody Extra-Avidinperoxidase, rinsed 3 times, and exposed to nickel-enhanced DAB staining for visualization. Slides were air dried, dehydrated in xylene, and coverslipped with DPX. For each animal, 2 sections were measured using an area drawn around the pyramidal layer of area CA1 (NIH Scion Image program) and averaged. Images of hippocampal areas were acquired with an Olympus DP70 digital camera (Olympus, Tokyo, Japan). For each antibody, all sections were processed in parallel.

2.9. Electrophysiology

Electrophysiological data were collected from 31 animals at least 1 week after the last day of MWM to limit the impact of learning and arousal on transient hippocampal excitability changes (Moyer Jr et al., 1996). Hippocampal slices were obtained according to previously published protocols (Blalock et al., 2010). Animals were briefly anesthetized in a CO2 chamber and decapitated. Hippocampi were removed, and transverse slices were prepared (350 μm) in ice-cold, low-calcium, artificial cerebrospinal fluid (ACSF) composed of the following (in mmol/L): 128 NaCl, 1.25 K2HPO4, 10 glucose, 26 NaHCO3, 3 KCl, 0.1 CaCl2, 2 MgCl2, using a Vibratome (TPI, Saint Louis, MO, USA). Slices were transferred to a heated (32 °C) interface-type chamber and maintained in oxygenated (95% O2, 5% CO2) normal-calcium ACSF containing 2 mmol/L CaCl2 and 2 mmol/L MgCl2 for at least 2 hours before recording. Each hippocampal slice was placed in a recording chamber (RC22C, Warner Instruments, Hamden, CT, USA) and maintained in a continuous flow of oxygenated normal-calcium ACSF pre-heated at 32 °C using a TC2Bip/HPRE2 in-line heating system (Cell Micro Controls, Norfolk, VA, USA). The recording chamber was mounted on the stage of a Nikon E600FN inverted microscope (Nikon, Tokyo, Japan). Cells were impaled with sharp microelectrodes filled with 2 mol/L KmeSO4 and 10 mmol/L HEPES, pH 7.4, pulled from borosilicate glass capillaries (World Precision Instruments, Sarasota, FL, USA).

To generate an afterhyperpolarization (AHP), cells were held at ~65 mV (baseline) and depolarized with a 100-millisecond intracellular current injection sufficient to generate 3 Na+ action potentials. AHPs were elicited every 30 seconds, and between 2 and 10 sweeps were averaged for each cell. The medium AHP (mAHP) was measured as the peak hyperpolarization immediately after the offset of the depolarizing current injection; the slow AHP (sAHP) was measured 800 milliseconds after the end of the current injection. AHP duration was measured from the end of the depolarizing step until return to baseline. Neurons with input resistance greater than 35 MΩ, holding current less than 250 pA, and overshooting action potentials were included in this analysis. Action potential height, input resistance, current injection to elicit the AHPs, and
recording electrode resistance were quantified (Table 3). Data were acquired using pClamp 8.0 (Molecular Devices) software through a Digidata 1320A A/D converter and an Axoclamp2B (Molecular Devices).

Some hippocampal slices were exposed to acute insulin to determine its effect on the AHP. Humalog was perfused into the flow of oxygenated recording ACSF at a concentration of 4 μmol/L (diluted in ACSF from the 600 μmol/L stock) and subjected to an 8-fold dilution, resulting in a final concentration at 500 nmol/L insulin. Another set of cells were recorded (n = 5) to control for the impact of time and vehicle application. In these cells, a 15-minute perfusion with ACSF did not alter the AHP.

2.10. Statistical analysis

Body weight and food consumption were analyzed from weeks 4 to 23 using a 2-way repeated-measures (RM) analysis of variance (ANOVA; for time and diet effects) in young and middle-aged animals separately. A 2-way ANOVA across diet and age was also used at week 23. Two-way RM ANOVAs were used for all other measures with a primary focus on diet and age effects. Post hoc analyses used the Bonferroni test. For all variables measured, outliers (> 2 SD from group mean) were removed from the analysis.

The peripheral lipid index (PLI) for each animal in the study was constructed from measures of retroperitoneal fat, the HDL-to-LDL ratio, and cholesterol. Each measure obtained from each animal was ranked. The average of each animal’s rank from these 3 ratios was measured as that subject’s PLI value (higher values represent poorer lipid profiles). A similar procedure was used to obtain a spatial learning and memory index (SLMI) using measures of cumulative search errors during learning, and during the probe and reversal probe (high values represent poorer spatial memory). Correlations report the Pearson r values.

3. Results

3.1. Body weights, organ weights, and food consumption

Body weights increased significantly from weeks 4 to 23 in young (F(1,23) = 213.1; p < 0.0001) and middle-aged animals (F(1,31) = 28.0; p < 0.0001) on a control diet (CD). As shown in Fig. 1A, HFD increased body weights more robustly in young animals (F(1,23) = 9.2; p < 0.01) compared to middle-aged animals (F(1,31) = 2.0; p = 0.17). By the end of the study (week 23) significant aging (F(1,54) = 44.0; p < 0.0001) and diet effects (F(1,54) = 11.9; p = 0.001) were still present, with a greater impact of the diet seen on measures of body weight in younger animals. Table 1 provides analyses of peripheral tissue weights normalized to body weight. Epididymal and retroperitoneal fat pad weights were increased by HFD (epididymal F(1,54) = 23.8; p < 0.0001, retroperitoneal F(1,54) = 42.0; p < 0.0001) but only retroperitoneal was also increased by age (F(1,54) = 20.0; p < 0.0001). Liver weights were increased by diet (F(1,54) = 10.9; p < 0.05) and decreased by age (F(1,54) = 5.5; p < 0.05), whereas heart weights were significantly decreased by diet (F(1,54) = 4.2; p < 0.05). The decrease in normalized liver and heart weights is likely due to the increase in body weights in the middle-aged and HFD groups (analysis on non-weight-corrected data did not reveal decreases in absolute tissue weights). As shown in Fig. 1B, across weeks 4 to 23, HFD caused a significant reduction in food consumption, but only in older animals (F(1,31) = 29.9; p = 0.0001). Analysis of food consumption by the end of the study, on week 23, shows significant aging (F(1,54) = 17.6; p < 0.0001) and diet effects (F(1,54) = 6.8; p < 0.05). The greater decrease in food consumption seen in the older animals is consistent with their lower weight gain in comparison to the younger animals.

3.2. Blood measures

Consistent with prior reports in aging rats (Barzilai and Rossetti, 1995; Kalant et al., 1988; Li et al., 1996), and based on blood levels of glucose and insulin, our animals did not show signs of hyperglycemia either initially, or after 4.5 months of HFD or CD (Table 2). We complemented these static measures with assessment of dynamic glucose responses to glucose or insulin injections (measured as AUC, Table 2). Because the conventional clinical approach for the GTS uses a single dose irrespective of weight, and because of the results from our pilot study (see Methods), we report data using the standard single-dose method. Animals were subjected to the
of glucoregulation, we measured glycated hemoglobin levels as determined by a greater AUC (F \(_{1,54} = 12.8\); p = 0.0007) and HFD (F \(_{1,54} = 27.3\); p < 0.0001). Triglyceride levels were significantly increased by HFD (F \(_{1,54} = 53.2\); p < 0.0001), but middle-aged animals had lower levels compared to younger animals (F \(_{1,52} = 44.6\); p < 0.0001). Non-esterified fatty acids (NEFA) and adiponectin levels were not changed by either diet or age (Table 2).

To summarize these data, we compiled a peripheral lipid index (PLI) for each animal using measures of retroperitoneal fat, HDL-to-LDL ratio and cholesterol. This approach revealed significant differences by both age (F \(_{1,54} = 53.7\); p < 0.0001) and diet (F \(_{1,54} = 29.3\); p < 0.0001), effectively separating the 4 animal groups (Fig. 3A). The value of each animal using the PLI was then correlated with a learning and memory index (described below).

### 3.3. Behavior

We monitored learning and memory performance using the MWM spatial task. As previously reported (Frick et al., 1995; Rowe et al., 2007), middle-aged animals on CD showed a significant decrease in performance (Fig. 4). This was evidenced by age-dependent differences in time to reach the platform during the training/learning phase (F \(_{1,54} = 7.2\); p < 0.05; data not shown). Performance was also analyzed using a cumulative search error index for both the learning phase (Fig. 4B; F \(_{1,54} = 9.24\); p < 0.01) and the memory phase of the task (probe, Fig. 4C). During the probe trial a significant age-dependent decrease in memory performance was seen (F \(_{1,54} = 4.1\); p < 0.05). During the reversal probe, 72 hours after reversal training (new platform location), similar results were observed (F \(_{1,54} = 5.8\); p < 0.05; data not shown).

In comparison to the effects of aging on learning and memory, the HFD had no impact on maze performance (Fig. 4B). Nevertheless, we tested for the presence of an association between the peripheral lipid index (PLI) and learning/memory. For each animal, individual values obtained from the cumulative search errors during learning, the probe and the reversal probe, were ranked and averaged to yield a single index. This provided us with a spatial learning and memory index (SLMI) for each animal. We correlated PLI data with SLMI data for all 58 animals and obtained a weak, albeit significant, correlation (r = 0.3; p < 0.05; Fig. 3B). We interpret this as supporting evidence for a periphery-brain link; however, the association appears to be mediated mostly by strong changes in peripheral variables, as opposed to behavioral variables (HFD had no significant impact on learning or memory).

### 3.4. Physiology

We also evaluated the potential effects of HFD on hippocampal physiology in area CA1 neurons ex vivo. We focused on the after-hyperpolarization (AHP) because of its sensitivity to the aging process and learning (Song et al., 2012; Thibault and Landfield, 1996). Electrophysiological cell properties and electrode characteristics were not affected by age or diet (Table 3). As previously reported, the AHP was larger in middle-aged animals (Fig. 5C) (Disterhoft and Oh, 2007; Gant et al., 2006). The medium AHP (mAHP) was significantly increased with age (F \(_{1,31} = 6.81\); p < 0.05), and trends were seen for increases in the slow AHP amplitude (F \(_{1,31} = 2.9\); p < 0.1) and duration (F \(_{1,31} = 3.0\); p < 0.1). HFD, however, was unable to significantly alter the AHP; albeit a strong trend for an increase in the mAHP was seen in young animals.
Given the relationship between the AHP and learning, the lack of a diet effect on the AHP is consistent with the lack of a diet effect on spatial learning and memory.

Because prior clinical evidence shows that aging and diabetes can downregulate insulin transporters at the blood–brain barrier (Duelli et al., 2000; McNay, 2005) and may be responsible for decreased insulin sensitivity in the aged brain (Gasparini et al., 2002; Steen et al., 2005), we hypothesized that aging and/or HFD may alter insulin sensitivity in the hippocampus. In a subset of cells (Fig. 5D and E), we quantified insulin sensitivity by measuring the AHP before and after a 15-minute insulin perfusion (500 nmol/L Humalog). Irrespective of age (Fig. 5D), the sAHP from animals on CD showed a significant reduction in response to acute insulin (F_1,10 = 7.1; p < 0.05). Although there was no significant interaction term in the 2-way RM ANOVA (p = 0.14), the degree of insulin-mediated reduction in the sAHP appears to be larger in aged, compared to younger, animals (Fig. 5D). Importantly, in HFD animals, this reduction was eliminated (Fig. 5E), suggesting the HFD caused a decrease in insulin sensitivity.

### 3.5. Western blot and immunohistochemistry analysis from hippocampal tissue

As an additional measure of insulin signaling in the brain, and because insulin signaling is dependent on IRS-1 in its early stage (Talbot et al., 2012), we quantified protein levels for IR and IRS-1 using Western and immunohistochemical techniques. Prior studies have reported decreases in brain IR signaling in aging and AD, as well as in response to dietary manipulations and AD (Pratchayasakul et al., 2011; Talbot et al., 2012; Zhao et al., 2004). However, these effects may be dependent on animal strain, treatment duration (Mielke et al., 2006; Ross et al., 2012), and cognitive status (Dou et al., 2005; Zhao et al., 2004). Western blot analysis showed no significant age or diet effects on the upstream portions of the insulin pathway (IR and IRS-1 levels; Fig. 6A); however, IRS-1 staining showed a small, yet significant, age-dependent reduction in area CA1 immunoreactivity (F_1,20 = 11.5; p < 0.005; Fig. 6B middle panel). A robust age-dependent reduction in adiponectin staining was seen in the pyramidal layer of area CA1 (F_1,20 = 165.1; p < 0.0001; Fig. 6B and C). Given that this reduction was seen in the same animals that displayed decreases in cognitive function (Fig. 4C), this suggested that CNS adiponectin may modulate cognition with age. To test this possible association, we correlated the SLMI index for each animal to ranked area CA1 adiponectin staining and found that a significant association was indeed present (Fig. 3C; r = 0.5, p < 0.05).

### 4. Discussion

In recent years, increasing attention has focused on determining whether peripheral metabolic dysregulation and associated weight gain and diabetes pose a risk for accelerated brain aging. The F344 rat has been used extensively to study aging; however, few studies have examined the impact of peripheral metabolic dysregulation and associated weight gain and diabetes on brain aging. The F344 rat has been used extensively to study aging; however, few studies have examined the impact of peripheral metabolic dysregulation and associated weight gain and diabetes on brain aging.

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the brain, as shown by electrophysiological analyses of the AHP. Importantly, these results provide the first demonstration of the sensitivity of the AHP to insulin, and suggest that the consequences of HFD can be observed in the brain even in the absence of overt peripheral metabolic dysregulation.

4.1. Peripheral effects

The HFD had some of the intended effects in the periphery, at least on measures of lipid homeostasis, increasing triglycerides and...
lowering the HDL/LDL ratio (Table 1). This was supported by other peripheral variables including increased fat pads and liver weight (Fig. 1 and Table 1). Interestingly, the diet-induced reduction in food consumption (Fig. 1B) was larger in older animals and mirrors the reduced weight gain seen in this group (Fig. 1A), suggesting greater age-dependent accommodation to the higher caloric density of the HFD. Nevertheless, the effects of the HFD on peripheral lipids as well as in 3 key metabolic hormones (insulin, glucagon, and adiponectin), were similar in both young and aging rats (Table 2).

Sustained blood glucose levels during a 2-hour glucose tolerance test (GTT) are reflective of poor glucose uptake into cells (i.e., insulin resistance) and, together with high insulin levels between meals, are indicative of a prediabetic/diabetic condition. Even though we show evidence for a small diet-mediated increase in the area-under-curve of the glucose response during the GTT (Table 2), it does not appear that F344s on HFD were prediabetic, because insulin levels were not significantly changed and even tended to decrease with HFD. Furthermore, analyses of insulin sensitivity using the insulin tolerance test (ITT) showed lower glucose levels at 30 min in middle-aged animals (Fig. 2B2). Although this suggests that older animals are more sensitive to insulin, reductions in glucagon levels and glycogenolysis (altered counter regulation) could also have accounted for lower glucose levels. Serum glucagon levels, however, were not significantly different by either age or diet (Table 2). Finally, even though HFD increased HbA1c slightly, it is likely that such small changes have little physiological impact, given the absence of elevated fasting glucose levels (Fig. 2A1 and B1). Together, these results highlight a clear effect of HFD on peripheral lipids, supportive of the adipogenic nature of this diet. However, in the F344, neither frank diabetes nor prediabetes appear to develop. In fact, we present some evidence for an increase in insulin sensitivity in older animals (Fig. 2B2).

Compared to other rodent models, the level of circulating adiponectin in the F344 appears to be elevated. Reported total adiponectin levels typically range between 10 ng/mL and approximately 7 μg/mL (Garekani et al., 2011; Malloy et al., 2006; Zhang et al., 2010; Zhu et al., 2004). Here we found adiponectin levels that are higher and reaching approximately 20 μg/mL (Table 2). These levels are comparable to those induced by 12 weeks of medium-intensity exercise seen in young Wistar rats (Garekani et al., 2011). Increased adiponectin levels have been shown to protect from metabolic dysregulation associated with HFD (Asterholm and Scherer, 2010) and are inversely correlated with insulin resistance (Gustafson, 2010). Thus, it seems reasonable to propose that the increased levels of adiponectin in the F344 may have protected against some of the impact of HFD in the periphery, thereby maintaining glucoregulation. Consistent with this possibility, one of the strongest predictors of insulin sensitivity is the circulating adiponectin level, with insulin-sensitive obese subjects showing greater adiponectin levels when compared to insulin-resistant and diabetic obese patients (Kloting et al., 2010).
4.2. Brain effects

As previously reported (Blalock et al., 2010), we observed impaired learning and memory performance in aging F344s (Fig. 4). However, the peripheral lipid dysregulation caused by the HFD was only weakly correlated with memory performance in young or aged animals (Fig. 3B). There is considerable inconsistency in the literature regarding the impact of HFD on cognition. In young Long-Evans rats, increased fat intake has been shown to impair spatial learning and memory (Greenwood and Winocur, 1990), particularly when a lard-type diet is used (Jurdak et al., 2008). Although a high-fat/high-sucrose diet causes insulin resistance and reduction in learning and memory in Sprague-Dawley rats (Stranahan et al., 2008) and CD1 mice (Farr et al., 2008), this is not the case for C57BL/6 mice (Mielke et al., 2006; Pistell et al., 2010). In our study, HFD did not impair learning and memory in young rats or exacerbate decrements in performance in the older rats. Perhaps earlier or longer HFD exposure may be required to negatively alter learning or memory in this model. Alternatively, the age-dependent decline in cognition may have been too extensive and therefore less likely to be affected by HFD. Importantly, the F344s appears to be resistant to the potential negative consequences of HFD on cognition, and could well serve as a valid model to identify factors that confer protection.

At the cellular level, and to complement data from Western blots and immunohistochemistry, we used electrophysiological analyses and monitored brain insulin response and sensitivity directly. This allowed us to determine the impact of HFD on a key biomarker of brain aging associated with impaired cognition, namely the Ca\(_{\text{2+}}\)-dependent AHP, which is larger with aging. Importantly, reductions in the AHP have been shown to improve memory (Moyer Jr et al., 1996; Song et al., 2012). We show for the first time that the AHP was significantly reduced by acute insulin exposure to hippocampal slices. Furthermore, the insulin-mediated inhibition appeared to be greater in older compared to younger animals on the control diet (Fig. 5D), providing some support for increased insulin sensitivity with age in this animal model. Notably, the sensitivity of the AHP to insulin was lost by exposure to HFD in both age groups (Fig. 5E). These results may have relevance for clinical studies examining the potential benefits of intranasal insulin for cognitive decline in AD (Schioth et al., 2012). Thus, in addition to other beneficial effects of insulin, including those on metabolism, vascularization, neurotransmission, and A\(_\beta\) clearance (Kim and Feldman, 2012; McNay and Recknagel, 2011), the reduction in the AHP may represent
a novel neuroprotective mechanism. Our results also demonstrate that neurons can respond to acute insulin relatively quickly. It remains to be determined whether the impact of insulin in the brain represents acute or chronic effects of the hormone (Kamal et al., 2012). Finally, it is interesting to note that hormones that oppose insulin actions such as glucocorticoids, increase the AHP (Kerr et al., 1989), and drugs that restore insulin sensitivity (e.g., thiazolidinediones) reduce the AHP (Blalock et al., 2010). Thus, the hippocampal AHP appears to be an insulin-sensitive target, the modulation of which could well represent a mechanism by which insulin improves cognition in aging and AD.

Adiponectin staining in the brain revealed a robust age-dependent decrease in the hippocampus that did not show sensitivity to HFD (Fig. 6B and C). Given that peripheral adiponectin levels did not change with age, this result suggests reduced adiponectin transport into the brain with aging. There is clear evidence for adiponectin transport into the CNS (Qi et al., 2004; Une et al., 2011), where it is able to increase peripheral energy metabolism (Qi et al., 2004) and to stimulate neurogenesis (Zhang et al., 2011). Because a decrease in adiponectin levels is associated with mild cognitive impairment (Teixeira et al., 2013), this led us to examine the relationship between hippocampal adiponectin staining and the spatial learning and memory index (SLMI) for each animal. The correlation was significant (Fig. 3C), and provides further support for the potential role of CNS adiponectin in modulating learning and memory processes. Future studies directly manipulating adiponectin levels in the aged brain should clarify this relationship.

4.3. Conclusion

Human studies are implicating the peripheral metabolic dysregulation that occurs with type 2 diabetes mellitus as a risk factor for accelerated aging-related cognitive decline; yet, little is known regarding possible underlying mechanisms, as the corresponding studies in aging animal models have not been performed. We treated young and aging F344s with a HFD and, surprisingly, found that they did not display peripheral signs of glucose/insulin dysregulation or overt diabetes, unlike what is seen in other animal models. However, the effects in the brain appeared to differ from those in the periphery, and we found that: (1) in rats fed the control diet, the AHP, which determines the extent of neuronal excitability, was modulated by acute insulin; and (2) the response to insulin was lost in F344s on the HFD. As we show, for the first time, that the AHP is sensitive to insulin, our results appear to have important implications for neuronal function, irrespective of age, and show that a common Western-style diet can have an impact on the brain even in the absence of overt diabetes-like changes in the periphery. Because of the rather resilient nature of the F344 to diabetes, our studies also suggest that the F344 may represent a unique model to better understand mechanisms that may help to prevent diabetes and associated complications.

Disclosure statement

None of the authors on the manuscript has an actual or potential conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.02.019.

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