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Research report

Detrimental effects of a high fat/high cholesterol diet on memory and hippocampal markers in aged rats

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HIGHLIGHTS

- Young and Aged rats were exposed for 6 months to a high fat high cholesterol diet (HFHC).
- Cognitive performance, neuroinflammation markers and phosphorylated Tau were examined.
- Young and Aged rats on HFHC diet exhibited worse performance on spatial memory task.
- Aged HFHC rats showed higher levels of p-Tau compared to Aged control and Young HFHC rats.
- This work demonstrates HFHC diet-induced cognitive impairment with aging.

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ABSTRACT

High fat diets have detrimental effects on cognitive performance, and can increase oxidative stress and inflammation in the brain. The aging brain provides a vulnerable environment to which a high fat diet could cause more damage. We investigated the effects of a high fat/high cholesterol (HFHC) diet on cognitive performance, neuroinflammation markers, and phosphorylated Tau (p-Tau) pathological markers in the hippocampus of Young (4-month old) *versus* Aged (14-month old) male rats. Young and Aged male Fisher 344 rats were fed a HFHC diet or a normal control diet for 6 months. All animals underwent cognitive testing for 12 days in a water radial arm maze to assess spatial and working reference memory. Hippocampal tissue was analyzed by immunohistochemistry for structural changes and inflammation, and Western blot analysis. Young and Aged rats fed the HFHC diet exhibited worse performance on a spatial working memory task. They also exhibited significant reduction of NeuN and calbindin-D28k immunoreactivity as well as an increased activation of microglial cells in the hippocampal formation. Western blot analysis of the hippocampus showed higher levels of p-Tau S202/T205 and T231 in Aged HFHC rats, suggesting abnormal phosphorylation of Tau protein following the HFHC diet exposure. This work demonstrates HFHC diet-induced cognitive impairment with aging and a link between high fat diet consumption and pathological markers of Alzheimer's disease.

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1. Introduction

The incidence of obesity is growing and now includes at least one-third of the adult population in the United States, with another third of the population characterized as overweight [1]. One of the greatest factors contributing to the incidence of obesity is alteration in the diet, including both content and amount of fat intake. Obesity is considered a significant risk factor for other conditions such as cardiovascular disease, type 2 diabetes, hypertension, osteoarthritis, various forms of cancer, and depression [2,3]. Studies have shown that type 2 diabetes and obesity increase the risk of cognitive dysfunction and dementia [4–6]. Indeed, reduced cognitive performance following consumption of a high fat (HF) diet has been observed in humans [7–10] and animals [11–14]. Mechanisms likely involved in diet-induced damage to the brain include impaired glucoregulation [15], increased oxidative stress,

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Table 1
Mean number of the sum of errors ± SEM made by each group for the learning and asymptotic phases during Trial 4. Slopes of the linear regression are also presented. WMC: Working Memory Correct, WMI: Working Memory Incorrect and RM: Reference Memory.

Measure n=		Young Control 8	Young HFHC 11	Aged Control 7	Aged HFHC 8
WMC	Learning	9.4 ± 1.0	13.2 ± 0.8	8.8 ± 1.1	11.4 ± 0.8
	Asymptotic	6.4 ± 0.9	12.0 ± 1.4	10.5 ± 1.5	11.4 ± 1.2
	Slope	−0.50 ± 0.23	−0.19 ± 0.27	0.28 ± 0.31	0.01 ± 0.23
WMI	Learning	1.2 ± 0.5	1.6 ± 0.6	0.7 ± 0.2	4.0 ± 1.2
	Asymptotic	0.2 ± 0.1	0.6 ± 0.3	0.7 ± 0.6	1.4 ± 0.6
	Slope	−0.17 ± 0.09	−0.18 ± 0.12	0.01 ± 0.11	−0.36 ± 0.17
RM	Learning	7.4 ± 1.2	9.4 ± 0.5	7.0 ± 1.0	9.9 ± 1.1
	Asymptotic	5.2 ± 0.8	6.6 ± 0.8	7.3 ± 1.4	8.1 ± 1.0
	Slope	−0.36 ± 0.23	−0.47 ± 0.14	0.05 ± 0.28	−0.29 ± 0.25

and increased inflammation in brain tissue [16–19]. Previous work from our laboratory provided evidence for impairment of cognitive function in rats fed a high fat high cholesterol (HFHC) diet, and suggested that alterations in the blood-brain barrier (BBB) and in microglial activation may contribute to the reduced spatial and working memory observed [12,20,21]. However, in depth analysis of biological mechanisms for observed neuroinflammation and memory loss with the HFHC diets has not been undertaken so far.

Human epidemiological studies suggest that elevated intake of saturated fat in middle age may increase the risk to develop Alzheimer's disease (AD) [22] as well as age-related milder forms of cognitive impairment [23–26]. A randomized controlled trial demonstrated that a 4-week consumption of a high saturated fat/high glycemic index meal significantly increased the levels of $A_{\beta 42}$ in the cerebrospinal fluid (CSF) [27]. Animal studies utilizing transgenic mouse models of AD have shown that a diet rich in saturated fat and cholesterol can increase AD-pathology hallmarks, such as amyloid levels, Tau phosphorylation, and behavioral deficits [28–30]. Furthermore, diet-induced obesity has been shown to potentiate Tau-pathology in mice [31,32], but it is not known whether these effects are exacerbated with aging, nor is it known whether the Tau phosphorylation cascade is involved in HFHC-diet induced cognitive impairment. Therefore, the aims of this study were to investigate the consequences of long-term consumption of a HFHC diet on cognitive performance, hippocampal morphology, and expression of phosphorylated Tau in young *versus* aged rats.

2. Materials and methods

2.1. Animals and diets

Male Fischer 344 rats (Harlan, Indianapolis, IN) were given one week to acclimate to the vivarium, housed two to a cage and kept on a controlled 12 h light/12 h dark cycle and *ad libitum* access to food and water. Young and Aged rats were randomly assigned to the dietary groups: Young Control (4-month old, n = 8), Young HFHC (4-month old, n = 11), Aged Control (14-month old, n = 7), and Aged HFHC (14-month old, n = 8). The control diet consisted of standard rat chow (Harlan Teklad 8656, Indianapolis, IN) and provided 34% protein, 13% fat, and 53% carbohydrate (in kcal). The treatment diet (HFHC) consisted of (by weight): 10% hydrogenated coconut oil and 2% cholesterol ("Custom Diet D2-AIN93 without choline bitartrate and with 2% cholesterol", MP Biomedicals, Solon, OH) and provided (in kcal) 15% protein, 36% fat and 49% carbohydrate. Rats were fed the control or HFHC diet for six months. Young rats were 10 months old and aged rats were 20 months old at the end of the study. Animal protocols were approved by the Medical University of South Carolina Institutional Care and Use Committee and carried out according to guidelines from the National Institutes of Health. Body weights (measured in grams) were evaluated every other week throughout the study in order to monitor effects of the diet. Food consumption was measured weekly per cage (two rats per cage) throughout the study.

2.2. Cognitive assessment

The 8-arm water radial arm maze (WRAM) was used in order to assess working and reference spatial memory according to previously published protocols from our laboratory [12,33,34]. Four escape platforms were placed in the maze; the four baited arms were assigned randomly and kept consistent over the 12 days of testing. Every day, four trials were administered (maximum of 3 min each). After each trial, the platform found was removed, allowing for a win-shift paradigm of this task [35,36]. Three types of errors were quantified: Working Memory Correct (WMC), Reference Memory (RM), and Working Memory Incorrect (WMI) [35]. WMC errors were first and repeat entries into an arm that previously contained a platform. RM errors were first entries into any arm that never contained a platform. WMI errors were repeat entries into an arm that never contained a platform, *i.e.* repeat entries into a reference memory arm [12,33,37]. Data were blocked into two phases: an initial learning phase (days 2–6), and a latter phase, or asymptotic (days 7–12). The first day of testing was considered a training day and was not included in the analyses. For WMC errors, trial 1 was not included in the analysis because it is not possible to make a WMC error on the first trial of each day.

2.3. Immunohistological evaluation of the hippocampus

Rats were anesthetized deeply with isoflurane gas (Novaplus) and the brain was rapidly removed. The right hemisphere was fixed in 4% paraformaldehyde for 48 h and transferred to 30% sucrose in phosphate buffered saline at 4 °C. Sections of hippocampus (40 μm) were processed for immunohistochemistry. Tissue from the left hemisphere was dissected, frozen and processed for biochemical analysis as described previously [38,39]. Immunohistochemistry was performed according to our previously published protocols [12,20], using the following antibodies: NeuN (Millipore, 1:1000), Calbindin-D28k (Millipore, 1:1000), MAP2 (microtubule-associated protein, Chemicon, 1:1000), and OX-6 (major histocompatibility complex, MHC, Class II marker, Serotec, 1:1000). Free-floating sections were incubated with primary antibodies for 48 h at 4 °C, washed, and then incubated with secondary antibodies directed against the appropriate species for 1 h at room temperature. Sections were washed and placed in the Elite ABC reagent (Vector, Burlingame, CA), washed and incubated with 3,3′-diaminobenzidine (DAB; 0.02%) with nickel ammonium sulfate (NAS) (Fisher Scientific, Pittsburgh, PA; 25 mM). Sections were washed, mounted on subbed slides, air dried overnight, dehydrated, coverslipped with Permount (Fisher Scientific, Pittsburgh, PA, USA) and examined with a light microscope (Nikon Optiphot).