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Diet-induced elevations in serum cholesterol are associated with alterations in hippocampal lipid metabolism and increased oxidative stress

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Abstract

The structure and function of the hippocampus, a brain region critical for learning and memory, is impaired by obesity and hyperlipidemia. Peripheral cholesterol and sphingolipids increase progressively with aging and are associated with a range of age-related diseases. However, the mechanisms linking peripheral cholesterol metabolism to hippocampal neuroplasticity remain poorly understood. To determine whether diets that elevate serum cholesterol influence lipid metabolism in the hippocampus, we maintained rats on a diet with high amounts of saturated fat and simple sugars for three months and then analyzed hippocampal lipid species using tandem mass spectrometry. The high fat diet was associated with increased serum and liver cholesterol and triglyceride levels, and also promoted cholesterol accumulation in the hippocampus. Increases in hippocampal cholesterol were associated with elevated galactosyl ceramide and sphingomyelin. To determine whether changes in lipid composition exerted biological effects, we measured levels of the lipid peroxidation products 4-hydroxynonenal-lysine and 4-hydroxynonenal-histidine; both were increased locally in the hippocampus, indicative of cell membrane-associated oxidative stress. Taken together, these observations support the existence of a potentially pathogenic relationship between dietary fat intake, peripheral cholesterol and triglyceride levels, brain cell sphingolipid metabolism, and oxidative stress.

Keywords

hippocampus; high fat diet; sphingomyelin; ceramide; cholesterol

Simple carbohydrates and saturated fats are the major components of the Western diet that promote obesity and insulin resistance (Gross et al., 2004). Data from clinical, epidemiological and animal studies indicate that the excessive energy intake adversely affects the brain, particularly during aging. Epidemiological studies of large populations suggest that individuals with a high caloric intake are at increased risk of Alzheimer's disease (Luchsinger et al., 2002). Animal studies have shown that the structure and function of the hippocampus, a brain region critical for certain forms of cognition, is adversely affected by obesity and hyperlipidemia (Molteni et al., 2002; Farr et al., 2008). The mechanism(s) by which dyslipidemia influences brain structure and function are not known, but may involve increased oxidative stress and perturbed lipid metabolism. Oxidative

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damage to proteins and lipids occurs in cardiovascular and other tissues of obese and diabetic subjects (Fridlyand and Philipson, 2006). Accumulation of membrane sphingolipids (e.g ceramides, gangliosides and sphingomyelins) in skeletal muscle, pancreatic cells and vascular endothelial cells occurs in diabetes and atherosclerotic heart disease (Summers, 2006). However, the effects of excessive caloric intake on sphingolipid metabolism in neurons remain unexplored.

To understand the relationship between diet-induced perturbations in peripheral and central lipid metabolism, we maintained rats on a diet high in saturated fats and simple sugars for three months, then analyzed lipid profiles in the hippocampus, serum, and liver. We observed that peripheral elevations in cholesterol levels are accompanied by perturbation of central lipid metabolism. Specifically, diet-induced elevations in cholesterol and triglycerides were associated with increased cholesterol, galactosyl ceramide, sulfatide, and sphingomyelin accumulation in the hippocampus. This profile was accompanied by increased oxidative stress, suggesting that there is crosstalk between central and peripheral lipid metabolism pathways, with consequences for neuronal function.

Materials and Methods

Animals and Diet Conditions

Two-month-old male Sprague-Dawley rats were purchased from Charles River Laboratories and maintained on a 12 hour light-dark schedule (lights on at 6 am). Controls were fed standard NIH chow and water, and hyperlipidemia was induced by feeding a high-fat, high-sugar chow (Dyets #101842; Dyets Inc., Bethlehem, PA), with water containing 20% high-fructose corn syrup, as described (Stranahan et al., 2008). Rats were weighed once per week. After three months on the diet, rats were euthanized by decapitation under light Isoflurane anesthesia. Hippocampi were dissected out, frozen, and stored at -80°C prior to lipid extraction and analysis. All procedures were approved by the Animal Care and Use Committee at the National Institute on Aging and followed the NIH *Guide for the Care and Use of Laboratory Animals*.

Serum Chemistry Analyses

Total postprandial serum cholesterol (high- and low-density lipoprotein; HDL, LDL) and triglycerides were measured using a Roche Cobas Fara II robotic chemical analyzer according to the manufacturer's specifications. Total cholesterol levels were determined using a kit, as were triglyceride levels, HDL levels, and LDL levels. All reagents for these analyses were purchased from Wako Diagnostics USA (Richmond, VA).

Lipid Extraction from Liver and Hippocampus

Rats on the high-fat diet with serum cholesterol values falling in the top third of the distribution (n=6 high-fat diet, n=6 control diet) were selected for measurement of lipids and oxidative stress markers. A modified Bligh and Dyer procedure was used for extraction of total lipids from brain and liver samples, as described (Cutler et al., 2004). Briefly, each sample was homogenized at room temperature in 10 volumes of deionized water, then in 3 volumes of 100% methanol containing 30 mM ammonium acetate, and vortexed. Four volumes of chloroform then were added, and the mixture was vortexed and then centrifuged at 1,000 g for 10 minutes. The bottom (chloroform) layer was removed and analyzed by direct injection into a tandem mass spectrometer. Lipid extractions were performed using borosilicate-coated glass tubes, pipettes, and injectors.

Mass Spectroscopy

Extracted lipids were analyzed using an electro-spray ionization API 3000 tandem mass spectrometer. The ion spray voltage (V) was 5,500 at a temperature of 80°C with a nebulizer gas of 8 psi, curtain gas of 8 psi, and the collision gas set at 4 psi. The declustering potential was 80 V, the focusing potential 400 V, the entrance potential -10 V, the collision energy 30 V, and the collision cell exit potential was 18 V. The MS/MS scanned from 300 to 2,000 atomic mass units (amu) per second at a step of 0.1 amu. Each species of sphingolipids, phospholipids, cholesterol esters, and lipid peroxides initially was identified by a Q1 mass scan, then by precursor ion scanning or neutral loss scanning of a purified standard. Samples were injected into the ES/MS/MS for 3 minutes, where the mass counts accumulated and the sum of the total counts under each peak was used to quantitate each species.

Sphingomyelins, ceramides, cholesterol, and cholesterol ester standards (C16:0, C18:0, C18:1, and cholesteryl-arachidonate C20:0) were purchased from Sigma. Ceramides dC18:1/C16:0 - C24:0, C24:1, phosphatidylcholine C16:0/C18:1, C18:0/C18:1, phosphatidylethanolamine C16:0/C18:1, phosphatidylglycerol C16:0/C18:1, phosphatidylserine C16:0/C18:1, phosphatidylinositol C16:0/C18:1, and phosphatidic acid C16:0/C18:1 were purchased from Avanti Polar Lipids (Alabaster, AL). Palmitoyl-lactosyl ceramide dC18:0/C16:0, stearoyl-lactosyl-ceramide dC18:1/C18:0, lignoceryl-glucosyl-ceramide dC18:1/C24:0, lignoceryl-galactosyl-ceramide dC18:1/C24:0, and sulfatide (stearoyl-galactosyl-ceramide-sulfate dC18:1/C24:0) were purchased from Matreya Inc. (Pleasant Gap, PA). 4-hydroxynonenol and adducts were purchased from Cayman Chemicals.

Statistical Analysis

For the serum measures, raw values for cholesterol, HDL, LDL, and triglycerides were first converted to percent of control values, then compared across diet groups using bidirectional student's t-tests. For the mass spectrometry measures, peak values were first normalized to total lipids, then compared between the diet conditions using bidirectional student's t-tests. All statistical analyses were conducted using Graphpad Prism 5.0 (La Jolla, CA) with significance set at $p < 0.05$.

Results

A diet high in fat and sugar increases levels of triglycerides and cholesterol in serum

Rats maintained on a high fat, high sugar diet supplemented with 20% high-fructose corn syrup have increased serum cholesterol levels (Figure 1A; $t_{10}=3.21$, $p = 0.004$). This increase in serum cholesterol was attributable to elevated levels of both high-density lipoprotein (HDL; $t_{10}=5.94$, $p = 0.001$) and low-density lipoprotein (LDL; $t_{10}=2.29$, $p = 0.041$). Serum triglycerides were also increased in rats on a high fat, high sugar diet, relative to control rats ($t_{10}=3.59$, $p = 0.005$). The high fat, high sugar diet also increased body weight gain at all time points examined (Figure 1B; $F_{1,11}=39.40$, $p < 0.001$).

Accumulation of cholesterol in the liver in rats maintained on a high fat diet

In order to measure cholesterol accumulation in a known target organ for high-fat, high sugar diets, we measured total cholesterol levels in the liver using tandem mass spectrometry. Rats that were fed a high-fat, high-sugar diet had elevated cholesterol accumulation in liver samples, relative to rats maintained on the control diet (Figure 2A-B; $t_{10}=3.76$, $p=0.02$). This observation is in agreement with previous reports (Sun et al., 1979; Chanussot et al., 1988).

Changes in hippocampal oxidative stress following maintenance on a high fat diet

4-Hydroxynonenal (HNE) is a product of lipid peroxidation and an indicator of oxidative stress. During free radical propagation, HNE can bind free amino acids (e.g. lysine and histidine). We quantified signal intensity for HNE-lysine and HNE-histidine in the hippocampus of rats maintained on a high-fat, high-sugar diet using tandem mass spectrometry. Levels of HNE-lysine were significantly elevated in the hippocampus of rats with diet-induced hyperlipidemia (Figure 3A; $t_{10}=2.31$, $p=0.04$), as were levels of HNE-histidine ($t_{10}=3.17$, $p=0.01$). This profile is suggestive of increased oxidative stress in the brains of rats on the high-fat, high-sugar diet.

Alterations in hippocampal lipid metabolism with a high fat and sugar diet

Hippocampal accumulation of C18:0 sphingomyelin was elevated in rats on the high-fat, high-sugar diet (Figure 3B; $t_{10}=3.54$, $p=0.005$). Other sphingomyelin chain lengths that showed significant increases included C22:0 ($t_{10}=3.05$, $p=0.01$) and C24:0 ($t_{10}=3.45$, $p=0.006$). While a trend for increased accumulation of C20:0 sphingomyelin was detected, this did not reach statistical significance ($t_{10}=2.03$, $p=0.07$).

Free cholesterol was also significantly increased in the hippocampus of rats on the high-fat, high-sugar diet (Figure 3C; $t_{10}=4.17$, $p=0.001$). Likewise, levels of galactosyl ceramide (Figure 3D; $t_{10}=2.62$, $p=0.03$) and ceramide sulfatide (Figure 3E; $t_{10}=3.14$, $p=0.01$) were elevated in rats with diet-induced hyperlipidemia. Levels of sphingomyelin precursor phosphorylcholine, ceramide precursors, free fatty acids (C16:0, 18:0, 18:1, and 24:0), 8-epi-prostaglandin F2 α , phosphatidylethanolamine, and cholesterol esters were also examined, and showed no significant diet effect ($p>0.05$).

Discussion

Diets high in saturated fats and simple sugars compromise the expression of a number of neurotrophic factors that support and enhance hippocampal plasticity (Stranahan et al., 2008; Molteni et al., 2002). Impairment of neurotrophin expression has been shown in vitro and in vivo to promote oxidative stress in neurons (Spina et al., 1992; Mattson et al., 1995). Exposure to a high-fat diet exacerbates cognitive dysfunction in aged mice (Uranga et al., 2010), possibly through reductions in endogenous antioxidant activity (Morrison et al., 2010), further implicating oxidative stress as a mediator of neurocognitive impairment with aging. The current report describes alterations in central lipid accumulation in a subset of rats that exhibit significantly elevated serum lipids following exposure to a high-fat, high-sugar diet. Because we selected rats that showed the largest increase in serum lipids with a high-fat, high sugar diet, it is not possible to determine whether the diet itself changes hippocampal lipid profiles, or if differences in hippocampal lipid composition arise from possible genetic differences in susceptibility to diet-induced elevations in peripheral cholesterol and triglycerides. However, this is the first report that attempts to correlate hippocampal lipidomic profiles with peripheral metabolic markers.

Ceramides are generated when sphingomyelin is cleaved by the enzyme sphingomyelinase, which is activated following oxidative stress (Filosto et al., 2010). We have observed increased levels of galactosyl ceramide, a glycosylated form of ceramide, and sulfatide, a sulfated form of ceramide, in the hippocampus of rats with diet-induced hyperlipidemia. Accumulation of ceramides directly contributes to apoptosis in neurons (Arboleda et al., 2009). In Alzheimer's disease patient tissue, oxidative stress is elevated, and levels of cholesterol and ceramides are increased (Cutler et al., 2004; He et al., 2010). This is interesting in light of the epidemiological correlation between the prevalence of insulin resistant diabetes – a condition that arises, in part, from dietary factors – and the incidence

of Alzheimer's disease (Stranahan and Mattson, 2011). Both diabetes and Alzheimer's disease are associated with cognitive impairment in human populations (Stranahan and Mattson, 2008) and both diseases increase oxidative stress in the brain in animal models (Maiese et al., 2007; Kapogiannis and Mattson, 2011). In the current report, diet-induced hyperlipidemia, which often presages insulin resistance, altered hippocampal lipidomic profiles in a manner that is reminiscent of Alzheimer's disease (Cutler et al., 2004). In this regard, parallel mechanisms are recruited in diabetes and dementia, and understanding these mechanisms has the potential to uncover population-specific treatments to prevent cognitive decline among individuals with diabetes.

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References

- Arboleda G, Morales LC, Benítez B, Arboleda H. Regulation of ceramide-induced neuronal death: cell metabolism meets neurodegeneration. *Brain Res Rev.* 2009; 59:333–46. [PubMed: 18996148]
- Chanussot F, Esnault-Dupuy C, Martigne M, Portugal H, Lairon D, Quignard A, Alcindor LG, Pauli AM, Lafont H, Houton JC. Metabolism of low- and high-density-lipoprotein-free cholesterol in rats fed high-fat diets. *Ann Nutr Metab.* 1988; 32:271–81. [PubMed: 3254685]
- Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, Troncoso JC, Mattson MP. Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proc Natl Acad Sci U S A.* 2004; 101:2070–5. [PubMed: 14970312]
- Farr SA, Yamada KA, Butterfield DA, Abdul HM, Xu L, Miller NE, Banks WA, Morley JE. Obesity and hypertriglyceridemia produce cognitive impairment. *Endocrinology.* 2008; 149:2628–36. [PubMed: 18276751]
- Filosto S, Fry W, Knowlton AA, Goldkorn T. Neutral sphingomyelinase 2 (nSMase2) is a phosphoprotein regulated by calcineurin (PP2B). *J Biol Chem.* 2010; 285:10213–22. [PubMed: 20106976]
- Fridlyand LE, Philipson LH. Reactive species, cellular repair and risk factors in the onset of type 2 diabetes mellitus: review and hypothesis. *Curr Diabetes Rev.* 2006; 2:241–259. [PubMed: 18220630]
- Gross LS, Li L, Ford ES, Liu S. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. *Am J Clin Nutr.* 2004; 79:774–779. [PubMed: 15113714]
- He X, Huang Y, Li B, Gong CX, Schuchman EH. Deregulation of sphingolipid metabolism in Alzheimer's disease. *Neurobiol Aging.* 2010; 31:398–408. [PubMed: 18547682]
- Kapogiannis D, Mattson MP. Disrupted energy metabolism and neuronal circuit dysfunction in cognitive impairment and Alzheimer's disease. *Lancet Neurol.* 2011; 10:187–98. [PubMed: 21147038]
- Luchsinger JA, Tang MX, Shea S, Mayeux R. Caloric intake and the risk of Alzheimer disease. *Arch Neurol.* 2002; 59:1258–1263. [PubMed: 12164721]
- Maiese K, Chong ZZ, Shang YC. Mechanistic insights into diabetes mellitus and oxidative stress. *Curr Med Chem.* 2007; 14:1729–38. [PubMed: 17627510]
- Mattson MP, Lovell MA, Furukawa K, Markesbery WR. Neurotrophic factors attenuate glutamate-induced accumulation of peroxides, elevation of intracellular Ca²⁺ concentration, and neurotoxicity and increase antioxidant enzyme activities in hippocampal neurons. *J Neurochem.* 1995; 65:1740–1751. [PubMed: 7561872]
- Molteni R, Barnard RJ, Ying Z, Roberts CK, Gómez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience.* 2002; 112:803–14. [PubMed: 12088740]

- Morrison CD, Pistell PJ, Ingram DK, Johnson WD, Liu Y, et al. High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: implications for decreased Nrf2 signaling. *J Neurochem.* 2010; 114:1581–9. [PubMed: 20557430]
- Spina MB, Squinto SP, Miller J, Lindsay RM, Hyman C. Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and N-methyl-4-phenylpyridinium ion toxicity: involvement of the glutathione system. *J Neurochem.* 1992; 59:99–106. [PubMed: 1613515]
- Stranahan AM, Mattson MP. Bidirectional metabolic regulation of neurocognitive function. *Neurobiol Learn Mem.* 2011 in press. Jan 12. [Epub ahead of print].
- Stranahan AM, Mattson MP. Impact of energy intake and expenditure on neuronal plasticity. *Neuromolecular Med.* 2008; 10:209–18. [PubMed: 18543119]
- Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, Mattson MP. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus.* 2008; 18:1085–8. [PubMed: 18651634]
- Summers SA. Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res.* 2006; 45:42–72. [PubMed: 16445986]
- Sun JV, Tepperman HM, Tepperman J. Lipid composition of liver plasma membranes isolated from rats fed a high glucose or a high fat diet. *J Nutr.* 1979; 109:193–201. [PubMed: 430221]
- Uranga RM, Bruce-Keller AJ, Morrison CD, Fernandez-Kim SO, Ebenezer PJ, et al. Intersection between metabolic dysfunction, high fat diet consumption, and brain aging. *J Neurochem.* 2010; 114:344–61. [PubMed: 20477933]

Figure 1. A diet high in fat and sugar results in increased serum cholesterol and triglycerides and accelerated weight gain

(A), Serum cholesterol levels are elevated following three months on a high-fat diet (HFD). Increases in serum cholesterol are attributable to both increased high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Triglyceride levels in serum also increase with three months of maintenance on the HFD. Asterisk (*) indicates significance at $p < 0.05$ following bidirectional student's t-tests. (B), Rats on a high-fat, high-sugar diet gained more weight than rats on the control diet. Asterisk (*) indicated significance at $p < 0.05$ following repeated measures ANOVA. For both graphs, error bars represent the standard error of the mean.

Figure 2. Lipid extraction methods used in the present study detect cholesterol accumulation the liver, a known target organ for high-fat diets

(A-B), Representative tandem mass spectra of cholesterol in liver samples from a rat on the control diet (A) and a rat on the high-fat diet (B). (C), Quantitative comparison of normalized cholesterol content in livers from rats on the control or high-fat, high-sugar diet (HFD). Asterisks (**) indicate significance at $p < 0.01$ following bidirectional student's t-test. Error bars represent the standard error of the mean.

Figure 3. The hippocampus responds to diet-induced hyperlipidemia with increased oxidative stress and altered lipid metabolism

(A), Peaks corresponding to the lipid peroxidation products 4-hydroxynonenal-lysine (HNE-lysine) and 4-hydroxynonenal-histidine (HNE-histidine) are higher in rats maintained on a high-fat, high-sugar diet (HFD). (B), The intensity of the signal corresponding to C18:0, C22:0, and C24:0 sphingomyelin in hippocampal lipid extracts is stronger among rats maintained on a high fat diet, relative to control rats. (C), Total cholesterol levels in the hippocampus are higher in rats with diet-induced hyperlipidemia. (D), Signal intensity for galactosyl ceramide C16:0 – C24:0 is greater in rats maintained on a high-fat diet, relative to rats on the control diet. (E), The peak representing signal intensity for sulfatide (C18:1 - C24:0) was elevated following maintenance on a high-fat diet. For all graphs, asterisk (*) indicate significance at $p < 0.05$ following bidirectional student's t-test. Error bars represent the standard error of the mean.