Neurochemistry International 97 (2016) 172–180

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

A high fat diet alters metabolic and bioenergetic function in the brain: A magnetic resonance spectroscopy study

Kayla Raider [a], Delin Ma [a], Janna L. Harris [b, e], Isabella Fuentes [b], Robert S. Rogers [a], Joshua L. Wheatley [a], Paige C. Geiger [a], Hung-Wen Yeh [c], In-Young Choi [a, d, e], William M. Brooks [a, d, e, f, g], John A. Stanford [a, f, *]

[a] Department of Molecular & Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, USA
[b] Department of Anatomy & Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160, USA
[c] Department of Biostatistics, University of Kansas Medical Center, Kansas City, KS 66160, USA
[d] Department of Neurology, University of Kansas Medical Center, Kansas City, KS 66160, USA
[e] Hoglund Brain Imaging Center, University of Kansas Medical Center, Kansas City, KS 66160, USA
[f] Kansas Intellectual and Developmental Disabilities Research Center, University of Kansas Medical Center, Kansas City, KS 66160, USA
[g] University of Kansas Alzheimer's Disease Center, University of Kansas Medical Center, Kansas City, KS 66160, USA

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form
18 April 2016
Accepted 20 April 2016
Available online 26 April 2016

Keywords:
Brain metabolism
Diet-induced obesity
High-fat
Magnetic resonance spectroscopy
Imaging

ABSTRACT

Diet-induced obesity and associated metabolic effects can lead to neurological dysfunction and increase the risk of developing Alzheimer's disease (AD) and Parkinson's disease (PD). Despite these risks, the effects of a high-fat diet on the central nervous system are not well understood. To better understand the mechanisms underlying the effects of high fat consumption on brain regions affected by AD and PD, we used proton magnetic resonance spectroscopy (^{1}H-MRS) to measure neurochemicals in the hippocampus and striatum of rats fed a high fat diet vs. normal low fat chow. We detected lower concentrations of total creatine (tCr) and a lower glutamate-to-glutamine ratio in the hippocampus of high fat rats. Additional effects observed in the hippocampus of high fat rats included higher N-acetylaspartylglutamic acid (NAAG), and lower myo-inositol (mIns) and serine (Ser) concentrations. Post-mortem tissue analyses revealed lower phosphorylated AMP-activated protein kinase (pAMPK) in the striatum but not in the hippocampus of high fat rats. Hippocampal pAMPK levels correlated significantly with tCr, aspartate (Asp), phosphoethanolamine (PE), and taurine (Tau), indicating beneficial effects of AMPK activation on brain metabolic and energetic function, membrane turnover, and edema. A negative correlation between pAMPK and glucose (Glc) indicates a detrimental effect of brain Glc on cellular energy response. Overall, these changes indicate alterations in neurotransmission and in metabolic and bioenergetic function in the hippocampus and in the striatum of rats fed a high fat diet.

1. Introduction

A high fat diet contributes to obesity, insulin resistance, and type 2 diabetes (Marshall and Bessesen, 2002). A high fat diet is also associated with oxidative stress, chronic neuroinflammation, altered mitochondrial function, and decreased hippocampal neurogenesis and plasticity (Lindqvist et al., 2006; Pipatpiboon et al., 2012) in the central nervous system (CNS). Thus, diet-induced obesity may accelerate age-related neural pathology and disease, and increase the brain's vulnerability to insults that contribute to cognitive decline and dementia (Uranga et al., 2010; Bruce-Keller et al., 2009). In fact, greater caloric and fat intake in late middle age increases the risk of dementia and Alzheimer's disease (AD) (Kalmijn et al., 1997; Luchsinger et al., 2002). Being obese is associated with a greater chance of developing dementia (Whitmer et al., 2005) and metabolic syndrome, and type 2 diabetes increases an individual's relative risk for developing AD (Leibson et al., 1997).

A high fat diet can also affect neural pathways associated with Parkinson's disease (PD). In studies examining the effects of a high-fat diet on nigrostriatal function and vulnerability, we found that a

* Corresponding author. Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Mail Stop 3051, 3901 Rainbow Blvd., Kansas City, KS 66160, USA.
E-mail address: jstanford@kumc.edu (J.A. Stanford).

http://dx.doi.org/10.1016/j.neuint.2016.04.008
0197-0186/

high fat diet in young adult rats increases dopamine depletion in the 6-hydroxydopamine (6-OHDA)-lesioned model of PD (Morris et al., 2010; Ma et al., 2015). We also reported that rats fed a high fat diet exhibit attenuated dopamine release in the striatum and increased iron levels and markers of oxidative stress in the substantia nigra (Morris et al., 2011). These effects parallel findings reported for normal aging (Drayer et al., 1986; Hebert and Gerhardt, 1998; Ke et al., 2005; Venkateshappa et al., 2012a, 2012b), which is the greatest contributor to PD and AD in humans. Overall, increasing evidence supports the hypothesis that a high fat diet may accelerate mechanisms related to neural aging.

The goal of the current study was to determine the extent to which markers of metabolic and bioenergetic function are altered in brain regions affected by AD and PD in high fat-fed rats. We used high-field proton magnetic resonance spectroscopy (^{1}H-MRS) to measure a neurochemical profile. ^{1}H-MRS is a non-invasive chemical assay technique that affords quantification of multiple neurochemicals within a given region of interest in the brain. Although we did not conduct repeated measures in our animals, this technique has the added advantage of identifying individual differences in neural responses to a high fat diet in the absence of group differences in translational studies. Our previous ^{1}H-MRS studies have reported altered neurochemical markers of bioenergetics and metabolic function in rodent models of aging, diabetes, and brain injury (Choi et al., 2014; Wang et al., 2012; Harris et al., 2012, 2014). In the current study, we compared rats fed a high fat diet or standard chow. We measured neurochemical markers in the hippocampus and striatum in living animals *in situ* as well as proteins related to bioenergetic function (peroxisome proliferator-activated receptor gamma coactivator 1a (PGC-1a), mitochondrial transcription factor A (TFAM), nuclear respiratory factor 1 (NRF-1), and phosphorylation of AMP-activated protein kinase (AMPK)) and astrocyte function (glial fibrillary acidic protein (GFAP)) in hippocampal and striatal tissue collected from the same animals *post mortem*.

2. Materials & methods

2.1. Animals and diet

Two-month-old male Fischer 344 (F344; Harlan) rats were given access *ad libitum* to a high fat diet (D12492 from Research Diets, New Brunswick, NJ; 60% calories from fat, 20% calories from carbohydrate, 20% calories from protein; n = 6) or standard rat chow (Harlan Teklad rodent diet 8604 from Teklad Diets, Madison, WI; 14% calories from fat, 54% calories from carbohydrate, 32% calories from protein; n = 6) for five months prior to MRI/MRS measurements. Rats were randomly assigned to diet groups. Body weights for the control and high fat groups prior to diet implementation were 265 ± 3 and 266 ± 4 g, respectively. We did not collect blood for glucose or insulin measures in these rats because we wanted to avoid multiple anesthesia episodes and because our previous studies have documented insulin resistance and glucose intolerance following 60% high fat diets lasting from 5-weeks to 6-months (Morris et al., 2010, 2011; Ma et al., 2015). Procedures conformed to the National Research Council's *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Kansas Institutional Animal Care and Use Committee. Experiments were in compliance with the ARRIVE guidelines.

2.2. Magnetic resonance imaging and spectroscopy

Animals were fasted for 12 h prior to MR scans to maintain consistency with our previous studies measuring insulin resistance. Isoflurane was administered for 4 min at 4% prior to placing the animal in the magnet cradle where anesthesia was maintained at 1.5–3.5% during imaging. Throughout the *in vivo* experiments, respiration was maintained at 40–80 cycles/min and body temperature was maintained at 37 °C via a feedback control system. We collected water-suppressed MR spectra using a STEAM sequence (TE = 2 ms, TR = 4000 ms, Varian 9.4T spectrometer) from two regions of interest (ROI) over the hippocampus and striatum (Fig. 1A–D). First- and second-order shims were adjusted using FASTMAP (Gruetter, 1993), and MR spectra (Fig. 1E) were analyzed with LCModel software as described previously (Harris et al., 2012, 2014). LCModel uses a basis set of spectra acquired from *in vitro* samples of pure chemicals to estimate the *in vivo* neurochemical concentrations, and the unsuppressed water signal from the ROI as a reference for each scan (Pfeuffer et al., 1999). Peak assignments for individual metabolites in the neurochemical profile have been previously validated (Pfeuffer et al., 1999; Tkác et al., 2003). We measured the following neurochemicals: alanine (Ala), ascorbate (Asc), aspartate (Asp), creatine (Cr), γ-aminobutyric acid (GABA); glucose (Glc), glutamine (Gln), glutamate (Glu), glycerophosphocholine (GPC), glutathione (GSH), myo-inositol (mIns), lactate (Lac), macromolecules (Mac), N-acetylaspartate (NAA), N-acetylaspartyl glutamate (NAAG), phosphocholine (PCho), phosphocreatine (PCr), phosphoethanolamine (PE), serine (Ser), and taurine (Tau). In addition, total choline (tCho: GPC + PCho), total creatine (tCr: Cr + PCr), and the ratios of PCr/Cr and Glu/Gln were evaluated. ^{1}H-MRS does not distinguish between intracellular and extracellular compartments. However, since the extracellular volume and the extracellular concentrations of neurochemicals measured by ^{1}H-MRS are each small, the extracellular contribution is generally considered to be negligible.

The duration of anesthesia did not differ between high fat and chow groups. However, the average concentration of isoflurane that was required to maintain respiration within the target range (40–80/min) was higher in the high fat group (average high fat = 3.1%, average C = 2.7%; p = 0.007), likely due to the greater body weights and fat absorption. Although it is possible that this small difference could have influenced brain metabolism, we found no correlations between anesthesia concentrations and levels of Glc, Glu, NAA, or Lac measured in the striatum or hippocampus (R^2 ranged from 0 to 0.26). Thus, we think the relatively small group variations in anesthesia are unlikely to have significantly influenced the study conclusions.

2.3. Western blot

We measured GFAP as a marker of astroglial responses in the striatum and hippocampus of rats using western blot. We also measured protein markers of bioenergetic function, including PGC1α, NRF-1, and TFAM, and assessed the activation of AMPK using pAMPK/total AMPK. Antibodies against GFAP, TFAM, phospho-AMPK, and total AMPK were obtained from Cell Signaling Technology (Beverly, MA), and antibodies against PGC1α were obtained from Calbiochem (San Diego, CA). Antibodies against Actin were obtained from Abcam (Cambridge, MA). Goat-anti-rabbit HRP-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Enhanced chemiluminescence reagents were purchased from Thermo Scientific (Waltham, MA). All other reagents were obtained from Sigma (St. Louis, MO).

Following MRI/MRS scans, brains were extracted and hippocampus and striatal tissue was dissected freehand using a stainless steel adult rat brain slicer matrix with 1.0 mm coronal slice intervals. Hippocampal and striatal sections corresponded with the ROI analyzed with MRS. Brain tissue was frozen until processed as described previously (Ma et al., 2015; Morris et al., 2008). Specifically, frozen samples were diluted in cell extraction buffer and the