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Dietary whey protein stimulates mitochondrial activity and decreases oxidative stress in mouse female brain

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HIGHLIGHTS

- Dietary whey protein isolate (WPI) decreases oxidative stress in mouse brain.
- Dietary WPI increases mitochondrial activity in mouse brain.
- Dietary WPI increases gene expression associated with mitochondrial biogenesis.
- Clinical evaluation for WPI treating certain neurological disorders is warranted.

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ABSTRACT

In humans and experimental animals, protein-enriched diets are beneficial for weight management, muscle development, managing early stage insulin resistance and overall health. Previous studies have shown that in mice consuming a high fat diet, whey protein isolate (WPI) reduced hepatosteatosis and insulin resistance due in part to an increase in basal metabolic rate. In the current study, we examined the ability of WPI to increase energy metabolism in mouse brain. Female C57BL/6J mice were fed a normal AIN-93M diet for 12 weeks, with (WPI group) or without (Control group) 100 g WPI/L drinking water. In WPI mice compared to controls, the oxidative stress biomarkers malondialdehyde and 4-hydroxyalkenals were 40% lower in brain homogenates, and the production of hydrogen peroxide and superoxide were 25–35% less in brain mitochondria. Brain mitochondria from WPI mice remained coupled, and exhibited higher rates of respiration with proportionately greater levels of cytochromes $a+a_3$ and $c+c_1$. These results suggested that WPI treatment increased the number or improved the function of brain mitochondria. qRT-PCR revealed that the gene encoding a master regulator of mitochondrial activity and biogenesis, *Pgc-1alpha* (peroxisome proliferator-activated receptor-gamma coactivator-1alpha) was elevated 2.2-fold, as were the PGC-1alpha downstream genes, *Tfam* (mitochondrial transcription factor A), *Gabpa/Nrf-2a* (GA-binding protein alpha/nuclear respiratory factor-2a), and *Cox-6a1* (cytochrome oxidase-6a1). Each of these genes had twice the levels of transcript in brain tissue from WPI mice, relative to controls. There was no change in the expression of the housekeeping gene *B2mg* (beta-2 microglobulin). We conclude that dietary whey protein decreases oxidative stress and increases mitochondrial activity in mouse brain. Dietary supplementation with WPI may be a useful clinical intervention to treat conditions associated with oxidative stress or diminished mitochondrial activity in the brain.

1. Introduction

Due to the high energy demand in brain tissue, neurons have a high mitochondrial density, and central nervous system (CNS) activities depend heavily on proper mitochondrial function. CNS mitochondrial dysfunction results in lower energy production, increased oxidative stress and apoptosis, factors related to neurodegenerative diseases, such as Parkinson's, Alzheimer's, Huntington's diseases, as well as amyotrophic lateral sclerosis [7,8]. Impaired mitochondrial function has also been linked to psychotic illnesses, including schizophrenia [17] and bipolar disorder [1], as well as senile dementia and diminished longevity [3,4].

Abbreviations: B2MG, beta-2 microglobulin; CNS, central nervous system; COX-6A1, cytochrome oxidase-6a1; ERR, estrogen-related receptor; G/M, glutamate + malate; GABPA/NRF-2A, GA-binding protein alpha (mouse)/nuclear respiratory factor-2a (human); MFN2, mitofusin-2; PGC-1a, peroxisome proliferator-activated receptor-gamma coactivator-1alpha; PPAR, peroxisome proliferator-activated receptor; qRT-PCR, quantitative real-time polymerase chain reaction; ROS, reactive oxygen species; Succ, succinate; TFAM, mitochondrial transcription factor A; WPI, whey protein isolate.

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Clinical symptoms in various types of mitochondrial dysfunction are often very general, such that identifying mitochondrial dysfunction in the etiology of such disorders may be quite difficult [7]. Since mitochondria are important in maintaining CNS health, strategies to improve mitochondrial function would have clinical relevance.

Although protein-deficient diets result in impaired brain development and activity [10], there is little information on how a protein-enriched diet may influence biochemical and physiological parameters that impact on CNS function. We have previously shown that dairy protein dietary supplementation, in the form of whey protein isolate (WPI) increased mitochondrial activity in mouse female liver [28]. We extended that study herein, to determine whether dietary WPI might increase mitochondrial activity in other tissues, such as brain. In the current study, we focused on biochemical and genetic parameters related to oxidative stress, respiration and the expression of genes associated with mitochondrial function and biogenesis.

2. Materials and methods

2.1. Animals and treatment

All experiments involving mice were conducted in accordance with the National Institutes of Health standards for care and use of experimental animals as stated in Principles of Laboratory Animal Care (NIH Publication No. 85-23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm), and the University of Cincinnati Institutional Animal Care and Use Committee. Female C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA), 10 weeks of age, were group-housed, maintained on a 12-h light/dark cycle, and had *ad libitum* access to water and chow (AIN-93M, Research Diets, New Brunswick, NJ). After 10 d acclimation, mice were assigned groups (4 mice/group) such that the average initial body weights for groups were as close as possible. Mice were allowed either water (Control group) or water supplemented with 100 g WPI/L (WPI group). The estrous cycle was not monitored. The precise compositions of the control and WPI diets, as well as body weights, food consumption, fluid intake and energy balance during experimental period have been described [28]. A formulation of WPI (Bioplex Nutrition, Blaine, WA) was chosen due to its high purity, containing over 90% protein with no lipids, sugars or additives, and low salt content. Body weight, food consumption and fluid intake were measured weekly. After 12 weeks, mice were fasted for 4 h, killed by CO_2 asphyxiation, and brain tissue harvested.

2.2. Mitochondrial preparation and respiration

Each brain was homogenized in ice-cold isolation buffer consisting of 70 mM sucrose, 225 mM mannitol, 1 mM EGTA and 5 mM HEPES, pH 7.4. Nonsynaptic brain mitochondria were prepared by centrifugation through a Percoll (Amersham Biosciences, Piscataway, NJ) gradient, as described [5]. The mitochondrial pellet was suspended in respiratory buffer consisting of 70 mM sucrose, 220 mM mannitol, 0.5 mM EDTA, 2.5 mM KH_2PO_4, 2.5 mM $MgCl_2$, 0.1% recrystallized bovine serum albumin, and 2 mM HEPES, pH 7.4 [24]. Respiration was measured polarographically with a Clark-type oxygen electrode (Oxytherm electrode, Hansatech Instruments, Norfolk, England). After determining state 2 respiration in the presence of 0.4 mM ADP, state 3 respiration was determined following the addition of 6 mM succinate (Succ), or 3 mM glutamate + 3 mM malate (G/M).

2.3. Products of lipid peroxidation and reactive oxygen

The tissue content of malondialdehyde and 4-hydroxyalkenals, products of lipid peroxidation, were estimated using the colorimetric probe 1-methyl-2-phenylindole (BIOXYTECH LPO-586™, OXIS Health Products, Inc., Portland, OR), following the procedure described by the manufacturer. H_2O_2 was monitored at 37 °C with freshly prepared mitochondria as 5 μM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) chemiluminescence that was inhibited by 500 U/ml catalase, utilizing the substrates G/M or Succ, under substrate-limited conditions (state 2) [24]. Superoxide production was monitored as 20 μM lucigenin (bis-*N*-methylacridinium) chemiluminescence that was inhibited by 5 μM of the superoxide dismutase mimetic Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin pentachloride [24].

2.4. Other assays

Brain reduced glutathione (GSH) levels [23] and cytochromes $a + a_3$ and $c + c_1$ [26] were quantified as described. Protein was measured by the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL).

2.5. RNA isolation, cDNA synthesis and quantitative real-time PCR (qRT-PCR)

Each entire brain was removed and quickly rinsed in ice-cold RNase-free PBS, flash-frozen in liquid N_2 and stored at −80 °C. Frozen tissue was pulverized and ~50 mg was homogenized in Tri Reagent™ (Molecular Research Center Inc., Cincinnati, OH) and RNA was isolated from the homogenate per the manufacturer's protocol. Total RNA quantity and quality were assessed by 260 nm absorbance and absorbance ratios of 260/280 nm and 260/230 nm ratios, respectively, utilizing Agilent Bioanalyzer/Nanodrop analysis (Agilent 2100 Bioanalyzer). RNA samples ($n = 4$ per treatment group) with 260/280 nm absorbance ratios ≥2.0 were used for qRT-PCR. cDNA synthesis was performed using an RNA-to-cDNA kit (Verso cDNA synthesis kit, #AB1453, Thermo Fisher Scientific, Waltham, MA). Synthesis of cDNA was carried out using 0.5 μg total RNA. qRT-PCR was performed on an MJ Opticon (Bio-Rad, Hercules, CA) using SYBR Green 2X RT-PCR master mix (Bio-Rad) with a total reaction volume of 20 μL. Thermocycling parameters were: 95 °C for 3 min followed by 40 cycles of 95 °C for 30 s, 55 °C for 60 s, 72 °C for 60 s. Primer sequences and amplicon size are given in Table 1. Primer sequences were confirmed using a basic local alignment search tool (PRIMER-BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). All reactions were performed in duplicate, and gene expression values were calculated using the difference in target gene expression relative to 18S rRNA utilizing the $2^{-\Delta\Delta ct}$ method [12].

2.6. Statistics

Data obtained from 4 brains were used for data analysis. Statistical significance of the differences between group sample mean values was determined by a one-way ANOVA to determine that the data was normally distributed, followed by the Student–Newman–Keuls test for pair-wise comparison of means, utilizing SPSS Statistical Analysis software (SPSS Inc., Chicago, IL).

3. Results

3.1. Oxidative stress and GSH status

After 12 weeks of treatment, brain tissue from WPI contained lower levels of the products of lipid peroxidation, malondialdehyde