Neuroprotective effects of polyunsaturated fatty acids in an in vitro hypothalamic model

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Introduction

Obesity and its comorbidities are thought to be due in part to high fat diets (HFD), which are rich in saturated fatty acids (SFAs) such as palmitic acid (PA;14:0). In rodent models increased PA can induce hypothalamic insulin resistance, inflammation and neurodegeneration (NGD) [2, 5, 6]. Ceramides, metabolites of PUFAs, are thought to elicit the onset of apolitical NGD, yet these events are not fully understood in the development of obesity. Additionally, ceramides adversely affect metabolism, increase inflammation that contributes to NGD-associated disorders [7-10].

Conversely, diets rich in polyunsaturated fatty acids (PUFAs) improve insulin sensitivity and lipid metabolism in several tissues [11]. Diets rich in n-3 and n-6 PUFAs such as α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and linoleic acid (LA) have several health benefits such as cardiovascular improvement and potentially the reduction of NGD related to obesity [8, 9, 11, 12].

The specific role of dietary SFAs-induced pathological ceramide synthesis has not been fully evaluated in the brain, but increased dietary PUFAs have been linked to the prevention of NDG disorders such as Alzheimer’s disease [13]. In rodent NDG models, n-3 and n-6 PUFAs can decrease oxidative damage (such as lipid peroxidation) and contribute to hypothalamic integrity, an important site for regulating energy balance, has yet to be defined [4, 14, 15].

Previously, we demonstrated that HFD up to 11-weeks in multiple SD rat models can either increase 1) hypothalamic apoptotic markers of NDG in PVN neurons and ARC-POMC neurons as measured by IHC, caspase-3 and cell death [16] or 2) increase both total and long chain (C18 and C20) ceramides [17]. These data agree with literature showing that HFD increases ceramides in several tissues, including hypothalamic [10, 15, 16].

Further studies are critical for determining the potential neuroprotective effect of PUFAs and their underlying mechanisms. We have begun to profile the effect of SFAs and PUFAs on hypothalamic integrity and NGD using an in vitro model. These studies are ongoing and we hypothesize that PUFAs protect against PA-induced NGD.

Methods

Cell line and Assays: Differentiated non-tumor derived, immortalized adult mouse hypothalamic (A130) cells (CellLines-Derived, North Carolina) were maintained in DMEM medium supplemented with 10% FBS at 37°C 5% CO2. Cells were plated overnight in a 96 well plate (3,500/well), pretreated with either: soy oil (SO), DHA, EPA, LA and then challenged with PA (Sigma, St. Louis, MO), Fig 1. Cell viability (ViostarBlue, Invitrogen) and caspase-3 (Caspase-Glo 3/7, Promega) were determined by either changes in relative fluorescent unit (RFU) or luminescence units (RLU), which were analyzed using a microplate spectrophotometer (Spectramax-M5, Molecular Devices).

Statistical Methods: Significant differences were determined by unpaired, two-tailed Student’s t-test using GraphPad Prism 5 (GraphPad, San Diego, CA) for either raw RFU or RLU values. Data represented was normalized against control values and graphed as percent change relative to control.

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Experimental outline

Table 1: Composition and molar concentrations per 100 g standard soy oil (Sigma, St. Louis MO).

Discussion

It is well known that HDs can promote obesity through purely energetic effects. New evidence shows that HDs are associated with hypothalamic inflammation and NGD, which implies effects of HD on brain function. Ceramides, a class of lipid molecules containing sphingoid chains generated from PA provide a potential mechanistic link between HFD consumption and outcomes such as insulin resistance, diabetes mellitus, and NGD. The development of normal brain structure and function is critical for the central regulation of energy metabolism, and thus hypothalamic NGD may result in disorders energy metabolism, including obesity (Fig 5).

Our preliminary data and ongoing studies are supported by a recent publication demonstrating that PUFAs can directly alter hypothalamic function [12]. There have been many health benefits attributed to PUFAs, yet Americans do not consume the amounts of 0.05-1.0 g per day recommended by both the American Heart Association (AHA) and the American Diabetic Association (ADA) [21].

References: