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Research article

Chronic sucrose intake decreases concentrations of n6 fatty acids, but not docosahexaenoic acid in the rat brain phospholipids

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HIGHLIGHTS

- Brain total lipid and fatty acid content is increased in sucrose treated rats.
- Sucrose treatment decreases the brain content of n6 fatty acids including arachidonic acid.
- In the sucrose treated rats the content of docosahexaenoic acid remains unchanged.

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ABSTRACT

We investigated the influence of high sucrose intake, administered in drinking water, on the lipid profile of the brain and on the expression of SREBP1c and Δ-desaturase genes. Adult male rats received 30% sucrose solution for 20 weeks (Sucrose group), or plain water (Control group). After the 20th week of sucrose treatment, the Sucrose group showed permanent hyperglycemia. Sucrose treatment also increased the amount of total lipids and fatty acids in the brain. The brain fatty acid profile of total lipids as well as phosphatidylethanolamine, phosphatidylcholine and cardiolipin of the Sucrose group was extensively changed. The most interesting change was a significant decrease in n6 fatty acids, including the important arachidonic acid, whereas the content of oleic and docosahexaenoic acid remained unchanged. RT-qPCR revealed an increase in Δ-5-desaturase and SREBP1c gene expression. In conclusion, high sucrose intake via drinking water extensively changes rat brain fatty acid profile by decreasing n6 fatty acids, including arachidonic acid. In contrast, the content of docosahexaenoic acid remains constant in the brain total lipids as well as in phospholipids. Changes in the brain fatty acid profile reflect changes in the lipid metabolism of the rat lipogenic tissues and concentrations in the circulation.

1. Introduction

The brain is an organ with high lipid content consisting of complex lipids: glycerophospholipids, sphingolipids (sphingomyelin and cerebrosides), gangliosides and cholesterol. Moreover, the brain possesses a unique polyunsaturated fatty acid (PUFA) profile with the highest proportion belonging to two important fatty acids: arachidonic acid (ARA, 20:4n6) and docosahexaenoic acid (DHA, 22:6n3). These fatty acids, together with their bioactive derivatives (DHA and ARA-derived mediators) regulate important processes such as neurotransmission, cell survival and neuroinflammation [2] and endocannabinoid system [20].

The fatty acids present in the living organism can originate from food or they can be *de novo* synthesized in lipogenic tissues (mainly liver and adipose tissue, depending on the species). Additionally, fatty acids, by a series of steps of desaturation and elongation, can be bioconverted to longer-chain fatty acids with more double bonds, or shortened by β-oxidation steps and recycled between peroxisomes and the endoplasmatic reticulum [25,29]. Regulation of these steps involves different desaturation and elongation enzymes, and transcriptional factors, as well as substrate availability, competition for enzymes and partitioning into oxidation [15,27,28].

The enzymes necessary for fatty acid synthesis are expressed in the brain and saturated and monounsaturated fatty acids can be synthesized *de novo* within the brain. In contrast, only a small amount of 18:2n6 and 18:3n3 are converted to 20:4n6 and 22:6n3 in the brain [5,6]. In n3 deprived rats, conversion of 18:3n3 to 22:6n3 is low in rat brains and is similar in normal and n3 deprived

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Table 1
The specific primers used in this study.

Gene	Sense	Sequence
Sterol regulatory element binding protein 1c (SREBP1c)	Forward	GATTGCACATTTGAAGACATGCTT
	Reverse	GTCCCAGGAAGGCTTCCAGAGA
Delta-5-desaturase (Δ5D)	Forward	TGGAGAGCAACTGGTTTGTG
	Reverse	GTTGAAGGCTGACTGGTGAA
Delta-6-desaturase (Δ6D)	Forward	TGTCCACAAGTTTGTCATTGG
	Reverse	ACACGTGCAGGCTCTTTATG
Delta-9-desaturase (Δ9D)	Forward	ACATTCAATCTCGGGAGAACA
	Reverse	CCATGCAGTCGATGAAGAAC
β-actin	Forward	ACTATTGGCAACGAGCGGTT
	Reverse	TGTCAGCAATGCCTGGGTACA
Cyclophilin	Forward	CTTCTTGCTGGTCTTGCCATTCCT
	Reverse	GGATGGCAAGCATGTGGTCTTTG

rats [14], which means that PUFA synthesis in the brain is not responsive to low dietary n3 supply, in contrast to the liver. These data suggest that the brain depends on a constant supply of 20:4n6 and 22:6n3 from the blood [2]. Consequently, altered dietary intake of PUFAs have been connected with many neurological disorders [4,9].

During the last decades, the relation between the consumption of sugar and particularly sugar-sweetened beverages and the development of obesity gained significant interest [3,17]. Several investigations have found a decrease in 20:4n6 and 18:2n6 in liver tissue and plasma after long term sucrose intake [7,18,23]. Therefore, we were interested whether chronic sucrose intake via the drinking water could change the brain fatty acid profile, with specific emphasis on 20:4n6 and 22:6n3 concentrations.

2. Material and methods

All procedures were performed in accordance with the Croatian Animal Welfare Act and approved by the Croatian National Ethics Committee and the Veterinary Directorate, Ministry of Agriculture, Republic of Croatia (authorization EP 13/2015).

Twenty-two Wistar rats (male, approximate body weight 200 g) were used in this study, over a period of 20 weeks. Before the exposure trial began, the animals passed through a week-long accommodation period. The rats were placed in pairs in polycarbonate cages and kept in steady-state microenvironment conditions (temperature: 22 ± 1 °C; light schedule: 12 h of light and 12 h of darkness). We assigned the animals into two experimental groups: the control rats were given tap water for drinking (Control, n = 12) and the metabolic syndrome rats were given 30% sucrose in their drinking water for 20 weeks (Sucrose, n = 12) as previously described [7]. The drinking solutions were prepared daily. All animals were fed ad libitum with rodent feed containing 18% crude protein, 5% crude fat and 18% crude fiber. The rats were weighed weekly at 8.00 h using an electronic balance, and at the same time non-fasting blood glucose levels were determined using an Accu-Chek Go [12]. Last glucose measurement was performed additionally by semi-automatic analyser (SABA 18, AMS, Italy). At the end of the experiment (20th week), the rats were sacrificed under Narketan/Xylapan anesthesia (Narketan®, 80 mg kg^{-1} b.m. + Xylapan®, 12 mg kg^{-1} b.m., i.p., Vétoquinol, Bern, Switzerland).

Immediately after the animals were sacrificed, the brains were removed, weighed and separated into two pools: one for lipid extraction, isolation of phospholipid classes and analysis of fatty acids, and the other for extraction and quantification of mRNA. All samples were individually wrapped in aluminum foil and stored at −80 °C until analysis.

Lipids were extracted from homogenized brain tissue by the method of Folch et al. [25] using chloroform/methanol (2:1, v/v) in the presence of the antioxidant butylated hydroxytoluene (30 mg/100 ml, Sigma-Aldrich, St. Louis, MO). Phospholipids were separated by 1-D TLC using H_3BO_3 impregnated (2,3%) 20 × 20 cm silica gel 60 plates (Merck KGaA, Darmstadt, Germany) with chloroform, ethanol, triethylamine and water (30:35:35:7, v/v) in the first development, and hexane and diethyl-ether (50:50, v/v) in the second development. Spots corresponding to phosphatidylethanolamine (PE), phosphatidylcholine (PC) and cardiolipin (CL) were immediately scraped from the plate, and extracted twice using 5 ml chloroform-methanol-water (5:5:1, v/v). The separated chloroform layer was transferred to a tube, evaporated to dryness under nitrogen, redissolved in 100 μl of chloroform-methanol (2:1, v/v) and stored at −80 °C.

Fatty acids from total lipids, PE, PC and CL were transmethylated using 2 M KOH in methanol. The analysis of fatty acid composition was performed using a Shimadzu GC–MS QP2010 Ultra Gas Chromatograph Mass Spectrometer (Shimadzu, Kyoto, Japan), equipped with a capillary column BPX70 (0.25 mm internal diameter, 0.25 μm film thickness, 30 m long, SGE, Austin, TX, USA). The injector temperature was set to 250 °C, and 1 μl of each sample was injected with a split ratio of 1:80. Helium was used as the carrier gas, and the linear velocity was 35 cm/s. The oven program was set as follows: temperature set at 40 °C for 3 min, then increased at the rate of 20 °C/min up to 130 °C, then increased at 1.5 °C/min up to 200 °C, and then increased at 45 °C/min up to 250 °C, and held for 10 °C. All the experimental measurements were repeated three times and the average values reported. Nonadecanoic fatty acid (C19:0) was used as an internal standard. The results of fatty composition were expressed as the mole percentage of total fatty acids.

Total RNA was isolated from 50 mg of brain tissue using the SV Total RNA Isolation System (Promega GMBH, Mannheim, Germany), according to the manufacturer's instructions. RNA concentration and purity were verified by A260/A280 and A260/A230 ratios (BioDrop μLITE, BioDrop, Cambridge, UK). Only samples displaying 260/280 > 1.8 were retained. The RT-qPCR was performed using a One-Step SYBR PrimeScript RT-PCR Kit II, according to the manufacturer's manual (Perfect Real Time, TaKaRa Bio Inc. Shiga, Japan) on a Stratagene MxPro3005 termocycler (Agilent Technologies, US and Canada). The fluorescence intensity of SybrGreen dye was detected after each amplification step. The primers used in this study are listed in Table 2. Relative expression ratios were calculated as normalized ratios to rat *ß*-actin and cyclophilin genes. The final relative gene expression ratios were calculated using ΔΔCt method ($2^{\Delta\Delta Ct}$) and the results were expressed as fold change relative to control. A list of specific primers is presented in Table 1.

3. Results

From the 16th week the Sucrose group had higher non-fasting mean blood glucose values than the Control ($P < 0.05$) (Fig. 1a). The wet brain weight, brain weight index, concentrations of brain total