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Original article

Dietary supplementation of grape seed and skin flour mitigates brain oxidative damage induced by a high-fat diet in rat: Gender dependency

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ABSTRACT

It is unknown whether gender has an impact on brain injury in obesity, and, if so, whether treatment with grape seed and skin flour could exert a protective effect. Both male and female rats were fed a standard diet (SD) or a high fat diet (HFD) during eight weeks and treated with high dosage grape seed and skin flour (GSSF). Fat-induced oxidative stress was evaluated into the brain with a special emphasis on transition metals determination. HFD induced male-cholesterol overload (+78.12%) and an oxidative stress status characterized by increased lipoperoxidation (+68.97%), carbonylation (+40.28%), decreased antioxidant enzyme activities as glutathione peroxidase (−61.07%) and manganese-superoxide dismutase (−35.47%) but not catalase. Additionally HFD depleted the brain from manganese (−71.31%) and dropped glutamine synthetase activity (−36.16%), without affecting copper nor iron nor their associated enzymes. HFD also altered intracellular mediators as superoxide anion (+36.12%), calcium (+44.41%) and also calpain (+76.54%) a calcium dependent protease. Importantly all these alterations were detected exclusively in male brain and were efficiently corrected upon GSSF treatment. In conclusion, GSSF has the potential to alleviate the deleterious lipotoxic effect of HFD treatment that occurred in male brain and perhaps in post-menauposal female brain.

1. Introduction

Hypercaloric diet is one of the most important contributors associated with obesity, and a wide variety of high-fat diet has been used to induce obesity in rodents. Obesity-related excessive adiposity is associated with increased systemic inflammation and cardiovascular risk [1,2] partly due to the secretion of several adipokines that influence inflammation and insulin resistance. Studies in rodent have reported gender differences in the development of obesity- induced organ lipotoxicity and dysfunction in which females seem to be more protected owing to the presence of estrogen. Gender dimorphism has also been described in fat-induced oxidative stress of various tissues such as liver and heart [3].

Up to now, many bioactive food components like those found in grape have been shown to prevent from a wide array of chronic disorders linked to metabolic syndrome [4]. Grape seed and skin flour (GSSF), a polyphenol rich mixture containing flavonoîds, non flavonoîds, oligomeric proanthocyanidins, is commonly used as a nutritional supplement [5]. Although the effect of grape polyphenols on experimentally-induced obesity has been largely approached [6], their preventive effect on ectopic fat deposition and particularly into the brain are scarce. In this respect, grape seed catechins were recently shown to protect rat cortical astrocytes against palmitic acid-induced lipotoxicity [7]. The present work reported the potential anti-lipotoxic effect of high dosage GSSF into the brain of either female or male HFD fed rats, with an emphasis on the putative protection against lipotoxicity-induced oxidative stress and inflammation.

2. Materials and methods

2.1. Diets

Standard rodent chow in pellet form was from ALMAS, Bizerte Tunisia and contained (as energy contribution) 5% fat, 70% carbohydrate, and 25% protein. High-fat diet (HFD) was prepared by soaking commercial food pellets into warmed (100 °C) and

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liquefied fat (peri-renal) of animal origin (sheep) for 15 min and allowed to dry at room temperature. HFD consisted of 39% fat, 45% carbohydrate, and 16% protein and fatty acid composition is given elsewhere [2].

GSSF was processed from the grape cultivar Carignan of *Vitis vinifera* from northern Tunisia. Seeds were manually separated from skins, air dried, and grounded separately with a coffee grinder (FP 3121 Moulinex) until a fine powder was obtained and both powders were then mixed (50:50) on a dry mass basis to obtain GSSF. HFD containing 5% (w/w) GSSF was prepared by mixing grape seed and skin powder to already grounded HFD and air dried. Total phenolic content of GSSF (Table 1) was determined according to [8].

2.2. Animals and experimental design

All experiments were conducted in agreement with the requirements of the local ethic committee of Carthage University and with the NIH guidelines for the care and use of laboratory animals [9]. Eighteen male and female Wistar rats (180–200 g) were maintained in animal facility at a controlled temperature (22 ± 2 °C), a 12 h (light−dark) cycle, and divided into 6 groups of 6 animals each, fed either a standard diet (SD), HFD or HFD + GSSF (5%) for 8 weeks. At the end of the treatment period, rats were anesthetized with urethane (40 mg mL^{-1}), sacrificed by decapitation. Blood was collected using heparin as anticoagulant, and plasma processed for lipid, oxidative stress parameters and adipokines determination. Brain was dissected, weighed, and homogenized in phosphate buffered saline pH 7.4 with an ultrathurax homogenizator. Homogenates were centrifuged at 10 000*g* for 15 min at 4 °C, and the resulting supernatant used for the determination of lipid content, oxidative stress, intracellular mediators, transition metals and associated enzymes. Brain index was expressed as follows: [brain mass (g)/body mass on day 60 (g)].

2.3. Brain analyses

Lipids were extracted from whole brain according to Folch et al. [10]. Triglycerides were determined using commercially available kit from Biomaghreb (Tunisia). LDL-cholesterol (LDL-C) was determined using a commercial kit from Biolabo, SA (France), and HDL-cholesterol (HDL-C) using a kit from Biomaghreb.

Brain lipoperoxidation was evaluated using a malondialdehyde (MDA) assay [11]. Briefly brain homogenates were precipitated with trichloroacetic acid, and MDA from supernatant was allowed to react with thiobarbituric acid (TBA). Spectrophotometric measurement was done at 532 nm, and MDA concentration was calculated using the absorbance coefficient of the MDA–TBA complex: 1.56×10^5 cm^{-1} $(mol\,L^{-1})^{-1}$.

Oxidative damage to proteins was evaluated by quantifying protein carbonylation in brain homogenates according to Levine et al. [12]. Briefly, after protein precipitation with 20% TCA and dissolution in 2,4 dinitrophenylhydrazine (DNPH)-containing buffer, absorbance was measured at 366 nm and results expressed as nmol carbonyl protein/mg protein. Total protein was determined according to Ohnishi and Barr [13] and non protein thiol (NPSH) determined according to Ellman [14].

Brain homogenates were also used to evaluate endogenous antioxidant enzyme activities as glutathione peroxidase (GPx; EC 1.11.1.9) [15], catalase (CAT; EC 1.11.1.6) [16] and superoxide dismutase (SOD; EC 1.15.1.1) [17]. Characterization of the SOD isoforms was performed using KCN (3 mmol L^{-1}) as Cu–Zn inhibitor, whereas Mn-SOD is insensitive to this inhibitor.

Tissue samples were also wet ashed in nitric acid (15.5 mol L^{-1}), diluted, and filtered for copper, and manganese measurements by atomic absorption spectroscopy. Glutamine synthetase (GS) activity (EC 6.3.1.2) a Mn containing enzyme was determined according to Santoro et al. [18]. Tyrosinase activity (EC 1.14.18.1) a Cu dependent enzyme was determined using L- tyrosine as substrate in 50 mM sodium phosphate buffer pH 6.5 at 25 °C [19].

Brain ionizable calcium, H_2O_2 and free iron were measured using commercial kits from Biomaghreb, according to Stern and Lewis [20], Kakinuma et al. [21] and Leardi et al. [22] respectively.

Calpain activity was measured according to Sasaki et al. [23] using the fluorogenic substrate N-succinyl-Leu–Tyr-amido 4-methyl coumarin. Briefly, brain homogenates (100 μg protein) were pre-incubated at 37 °C in a buffer containing 63 mmol imidazole–Hcl at pH 7.3, 10 mmol L^{-1} β mercaptoethanol, and 5 mmol L^{-1} $Cacl_2$ for 10 min. The fluorogenic substrate (200 μmol L^{-1}) was added, and the change in fluorescence (380 nm excitation, 460 nm emission) was monitored for 40 min using a Bio-Tek FLx-800 microplate reader. The non calcium dependent fluorescence was measured under the same conditions using a calcium-free buffer containing 1 mmol L^{-1} EDTA and 10 mmol L^{-1} EGTA. Calcium dependent calpain activity was expressed as change in fluorescence per minute per milligram of protein.

Brain acetylcholinesterase activity (AChE; EC 3.1.1.7) was determined according to [24]. Activity of the electron transport chain complex 1 (NADH dehydrogenase) was measured following the procedure of Hatefi [25] in which the rate of NADH oxidation was evaluated by measuring the decrease in absorbance at 340 nm.

Lipase activity was determined according to Humbert et al. [26]. Before running the analysis, a 100 mmol L^{-1} solution of p-nitrophenol dodecanoate in dimethylsulfoxide (DMSO) and ethanol was prepared. The reaction mixture contained 5 mmol L^{-1} p-nitrophenol dodecanoate, 50 mmol L^{-1} Tris–HCl buffer (pH = 8.5), and 50 μL of sample. The mixture was incubated at 37 °C for 1 h and the reaction stopped with 60 mmol L^{-1} EDTA. After centrifugation at 10 000*g* for 5 min, absorbance was measured at 412 nm. One unit (U) is defined as the amount of the enzyme catalyzing the release of 1 μmol of p-nitrophenol (ε = 18.3 $(mmol\,L^{-1})^{-1}cm^{-1}$). Brain superoxide anion ($O2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet}$) were determined according to Marklund and Marklund [27] and Payà et al. [28], respectively.

Plasma prolactin was determined using the Demeditec prolactin rat ELISA kit (Demeditec Diagnostics GmbH Germany) according to Beach et al. [29]. Plasma adiponectin was measured using the Assay Max rat adiponectin ELISA Kit (Assayparo).

2.4. Oil-Red-O staining

The whole brain was quickly dissected, embedded in Tissue-Tek OCT compound and stored at − 80 °C. 10 μm-thickness cryosections were fixed and Oil-Red-O stained for lipid detection, and visualized at magnification ×400.

Table 1
Phenolics levels in diet and faeces.

	SD	HFD	HFD + GSSF
Diet total phenolics (mg/g)	0.20 ± 0.04	0.17 ± 0.01	0.54 ± 0.12
Fecal total phenolics (mg/g)	0.15 ± 0.01	0.13 ± 0.01	0.36 ± 0.03

SD, standard diet; GSSF, grape seed and skin flour; HFD, high fat diet. Data are presented as mean ± SEM. $p < 0.05$ was considered significant. HFD or HFD + GSSF vs. SD: $^{*}p < 0.05$.