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Grape skin extract protects against programmed changes in the adult rat offspring caused by maternal high-fat diet during lactation☆,☆☆

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Abstract

Maternal overnutrition during suckling period is associated with increased risk of metabolic disorders in the offspring. We aimed to assess the effect of *Vitis vinifera* L. grape skin extract (ACH09) on cardiovascular and metabolic disorders in adult male offspring of rats fed a high-fat (HF) diet during lactation. Four groups of female rats were fed: control diet (7% fat), ACH09 (7% fat plus 200 mg kg^{-1} d^{-1} ACH09 orally), HF (24% fat), and HF+ACH09 (24% fat plus 200 mg kg^{-1} d^{-1} ACH09 orally) during lactation. After weaning, all male offspring were fed a control diet and sacrificed at 90 or 180 days old. Systolic blood pressure was increased in adult offspring of HF-fed dams and ACH09 prevented the hypertension. Increased adiposity, plasma triglyceride, glucose levels and insulin resistance were observed in offspring from both ages, and those changes were reversed by ACH09. Expression of insulin cascade proteins IRS-1, AKT and GLUT4 in the *soleus* muscle was reduced in the HF group of both ages and increased by ACH09. The plasma oxidative damage assessed by malondialdehyde levels was increased, and nitrite levels decreased in the HF group of both ages, which were reversed by ACH09. In addition, ACH09 restored the decreased plasma and mesenteric arteries antioxidant activities of superoxide dismutase, catalase and glutathione peroxidase in the HF group. In conclusion, the treatment of HF-fed dams during lactation with ACH09 provides protection from later-life hypertension, body weight gain, insulin resistance and oxidative stress. The protective effect ACH09 may involve NO synthesis, antioxidant action and activation of insulin-signaling pathways.

Keywords: Grape skin extract; Hypertension; Insulin resistance; Oxidative stress; Developmental programming

1. Introduction

Metabolic syndrome (MS) including the presence of obesity, hypertension, dyslipidemia and insulin resistance that predisposes type 2 diabetes is becoming more prevalent in recent years [1,2]. Epidemiological and experimental studies have described that the programming of energy balance already begins in very early development. Indeed, particular conditions in the nutritional environment during the perinatal and or postnatal periods may lead to adjustments in the physiology of humans and animals, with lasting effects in adulthood [3–5].

Experimental studies have shown that adult offspring of rodents fed fat (lard) or highly palatable (lard and sugar enriched) diets during gestation develop the MS phenotype, despite being reared on a standard chow diet [6–8]. Likewise, early postnatal nutrition may also cause differential programming of energy homeostasis. However, few have investigated the role of the suckling period in inducing cardiovascular and metabolic disorders in offspring of dams fed a fat-rich diet [9]. In this sense, we have recently described that normally fed adult female offspring of high fat-fed dams during lactation developed hypertension, dyslipidemia and gain of weight. In addition, our findings have also suggested the oxidative stress programming in the adult female offspring [10]. This may reinforces the hypothesis that oxidative stress, the imbalance between prooxidant and antioxidants, can be an early event in the development of chronic diseases related to metabolic and cardiovascular disorders associated with MS [11–14].

Numerous studies have demonstrated a potential role for bioactive food components as an adjunct to the treatment of obesity and MS [15–17]. Natural polyphenols, obtained from many plants,

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have been shown to exert important actions on the cardiovascular system [18].

Previously, we have demonstrated that an alcohol-free grape skin extract obtained from skins of *Vitis labrusca* (ACH09), a vinifera grape largely used in Brazil to produce red wine, induced an endothelium-dependent vasodilation, antihypertensive and antioxidant effects [19,20]. The activation of the insulin-signaling cascade and reduction of hyperglycemia in alloxan-induced diabetic mice by the grape skin extract have also been demonstrated [21]. However, there is no report about the activity of ACH09 on cardiovascular and metabolic disorders and oxidative stress of male offspring of an experimental model of developmental programming. We have now further investigated the changes occurring at proteins expression of the insulin-signaling cascade as a consequence of maternal food over-nutrition during suckling period that may be determinant of the insulin resistance. Hence, this study aimed to test the hypothesis that the beneficial effects of ACH09 on metabolic control of dams would pass on to the male offspring as adults; that is, the metabolic derangements in offspring of high-fat (HF) diet fed dams could be ameliorated if dams are supplemented with ACH09.

Therefore, experiments were undertaken to determine the action of ACH09 on cardiovascular and metabolic programming with focus on insulin resistance and changes on oxidative status observed in adult male offspring of dams fed an HF diet during lactation.

2. Methods and materials

2.1. Preparation of the grape skin ACH09 extract

The dried and powdered skin fruits of *Vitis vinifera* L. (Vitaceae) were extracted in an aqueous solution at 100°C with occasional shaking for about 120 min. The solution was then introduced into an ion-exchange resin column (cationic) and washed sequentially with ethanol, ethanol/H_2O (1:1) and H_2O. The H_2O fraction was discarded. The ethanolic and hydroalcoholic fractions were placed together and evaporated under vacuum at 60°C, followed by spray drying of the concentrated solution (inlet temperature 190°C and outlet temperature of 85°C). The extract obtained in the process was a fine powder, soluble in H_2O, with about 30% of total polyphenols according to the Folin–Ciocalteau procedure [22].

2.2. Analysis of grape skin ACH09 extract

To identify the active principles in grape skin, the extract was analyzed by LC/UV/MS with an atmospheric pressure chemical ionization interface [21]. LC/UV analysis of the dried hydroalcoholic grape skin extract was performed on a Hewlett-Packard series 1100 photodiode array detector (DAD) liquid chromatography system (Hewlett-Packard, Waldbronn, Germany). HPLC/UV/DAD analysis was performed with a Symmetry RP-18 column (4 mm; 250 ¥ 3.9 mm i.d.; Waters, Milford, MA, USA), solvent system: A, MeOH with 0.5% formic acid; B, H_2O with 0.5% formic acid; gradient mode 20% of A to 100% of A in 25 min; flow rate 1 ml/min; injection volume 10 ml; sample concentration 10 mg/ml in MeOH. DAD conditions were 210, 254 and 540 nm; UV data were recorded at 190–600 nm (step 2 nm). LC/MSn was performed directly after UV-DAD measurements. A Finningan LCQ ion trap (Finningan MAT, San Jose, CA, USA) was used with an atmospheric pressure chemical ionization interface. MSn experiments were completed by programming-dependent scan events. The first event was a full MS scan Mr (150.0–1500.0) (MS1); during the second event, the main ion recorded was isolated and selectively fragmented in the ion trap (MS2).The collision energy was set to 15 eV. HPLC analysis of the dried hydroalcoholic grape skin extract involved dissolving 10 mg of the extract in 1 ml methanol/H_2O (1:1) with 0.5% formic acid (HPLC quality). A total of 20 ml was analyzed by HPLC. Standards of peonidin-3-*O*-glucoside (**1**), petunidin-3-*O*-glucoside (**2**), malvidin-3-*O*-glucoside (**3**) and malvidin-3-(6-*O*-*trans*-*p*-coumaryl)-5-*O*-diglicoside (**4**) were purchased from Polyphenols Laboratories (Sandnes, Norway).

2.3. Animals and diet

All procedures were carried out in accordance with The Ethics Committee for Experimental Animals Use and Care (CEA) of Instituto de Biologia Roberto Alcântara Gomes/Universidade do Estado do Rio de Janeiro. The CEA follow guidelines from Intramural Animal Care and Use program of the National Institutes of Health (NIH). Virgin female Wistar rats 3 months aged were caged with one male rat at a proportion of 3:1. After mating, determined by the presence of a vaginal plug, each female was placed in an individual cage with free access to water and food until delivery. Within 24 h of birth, excess pups were removed so that only eight offspring were kept per cage to standardize milk availability during lactation. During the weaning period (21 days), the dams had free access to standard diet (AIN 93) or HF diet 24% (20% animal lard and 4% corn oil) in a temperature-controlled room with 12-h light/dark cycle and allocated into four groups. The control group corresponding to male offspring from dams fed the standard diet and allowed access to water (control group: 7% fat; 90.9±1.8 kcal) or ACH09 (ACH09 group, 200 mg kg^{-1} d^{-1}) during weaning. Two other groups were fed an HF diet with access to water (HF group: 24 % fat; 106.0±1.9 kcal) or ACH09 (HF+ACH09 group: 24 % fat; 106±2.1 kcal) during weaning (Table 1). The dose of ACH09 was based on previous studies that showed antihypertensive and antioxidant effects of the extract [10,19,23]. From weaning onward, all offspring were fed *ad libitum* the standard maintenance diet (AIN 93). Maternal food intake (g) was recorded daily in dams during weaning, and no changes were observed between groups (control: 25±1.8; control + ACH09: 24.3±2; HF: 24±1.9; HF+ACH09: 23.1± 2.1). Food consumption of dams was estimated by subtracting the amount of food left on the grid and amount of spilled food from the initial weight of food supplied. During lactation, offspring body weight (BW) was weekly recorded from 1 week of age (to avoid maternal rejection of the pups) until animals were fully grown at 90 or 180 days old.

The diets were elaborated by the Department of Experimental Nutrition, Federal Fluminense University (RJ, Brazil) in accordance with the standard recommendations for rodents in the maintenance state of American Institute of Nutrition (AIN-93M) [24].

2.4. Arterial pressure measurement and vascular perfusion study

Systolic blood pressure (SBP) was measured in conscious rats by use of tail-cuff plethysmopraphy (Letica 5000 device) once a week during 90 or 180 days.

Mesenteric arterial beds (MABs) of the rats were isolated in accordance with the method previously described [25]. Briefly, rats were anesthetized, and the MAB was rapidly removed, cannulated and perfused at a flow rate of 4 ml/min with physiological salt solution (PSS), bubbled with 95% O_2/5% CO_2, using a peristaltic pump (Lifecare model 4; Abbott/Shaw, Chicago, IL, USA). The PSS (composition, mmol/l: NaCl 118, KCl 4.7, CaCl2 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, EDTA 0.026 and glucose 6.0) was bubbled with 95% O_2/5% CO_2 at 37°C. Perfusion pressure was measured using a pressure transducer (HP 1280) connected to a preamplifier (HP 8805B), and the chart was recorded (7754A; Hewlett-Packard, Lexington, MA, USA). The preparations were left to equilibrate for 30 min. Then injections of 120 μmol of KCl were administered every 10 min until consistent responses were obtained. After the equilibration period, basal pressure (24±2 mm Hg; n=48) was elevated (80–100 mm Hg) by adding norepinephrine (NE; 10–30 μmol/l) to the perfusion solution. When the pressor effect of NE reached a plateau, dose–response curves to bolus injections of acetylcholine (ACh, 1–100 pmol) were injected as single doses in order to assess the endothelium-dependent vasodilator response. The vasodilator effect of ACh was expressed as a percentage decrease of the pressor effect of NE.

2.5. Plasma assays

Blood was collected from male rats with 90 or 180 days. The animals were fasted for 6 h, and blood samples were then collected by cardiac punction in anesthetized animals. Glicemia was determined with a glucometer (Accu-Chek Active, Roche, Manhein, Germany), and insulin concentrations were determined with the Insulin 125I radioimmunoassay Kit (MP Biomedicals, LLC-Orangeburg, NY, USA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from the real-time fasting serum glucose and fasting insulin concentrations of different groups of rats using the mathematical HOMA-IR formula: HOMA-IR=(fasting serum insulin in μU/ml×fasting serum glucose in mg/dl)/22.5. Plasma total cholesterol and triglyceride levels were measured by a colorimetric assay (Analisa, Belo Horizonte, Brazil).

2.6. Malondialdehyde assay

As an index of lipid peroxidation, we used the thiobarbituric acid reactive substances method for analyzing malondialdehyde (MDA), as previously described

Table 1
Composition of the experimental diet

Nutrient (U/kg diet)	Diets	
	Standard	HF
Casein (g)	251.16	226.74
Cornstarch (g)	478.5	330
Sucrose (g)	100	100
Corn oil (g)	70	43
Lard (g)	–	200
Fiber (g)	50	50
Mineral mix* (g)	35	35
Vitamin mix* (g)	10	10
L-Cystine (g)	3	3
Choline (g)	2.5	2.5
Energy (kcal)	3636.8	4614.9
% as Carbohydrate	58.9	35.7
% as Protein	23.8	16.9
% as Fat	17.3	47.4

* Vitamins and minerals mix following the AIN-93M recommendation for rodents.