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Distinct metabolic effects of resveratrol on lipogenesis markers in mice adipose tissue treated with high-polyunsaturated fat and high-protein diets

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ABSTRACT

Objective: A healthy diet is essential for the prevention of metabolic syndrome. The present study evaluated the effect of resveratrol associated with high-polyunsaturated fat and high-protein diets on expression of adipogenic and lipogenic genes.
Research methods & procedures: FVB/N mice were divided into 6 groups (n = 7 each) and fed with experimental diets for 60 days: standard (ST), high-fat diet (HFD), and high-protein diet (HPD), with and without resveratrol (RSV) (4 g/kg diet). The body weight, food intake, energy intake (kcal), and blood parameters (HDL-C, total cholesterol, glucose, and triglyceride levels) were assessed. Real-time PCR was performed to analyze the expression of adipogenesis and lipogenesis markers: PPARγ, SREBP-1c, ACC and FAS in samples from perigonadal adipose tissue.
Results: In the HPD + RSV group, resveratrol decreased body weight, body adiposity, adipose tissue weight, adipocyte area, total cholesterol, ACC and FAS expression, and increased HDL-cholesterol in comparison to HPD. In the HPD group there was a decrease in adipocyte area, as well as PPARγ, SREBP-1c and ACC expression in comparison to ST. While in HFD + RSV, resveratrol decreased levels of total cholesterol in comparison to HFD. In the HFD group there was decrease in body weight, and PPARγ, SREBP-1c and ACC expression in comparison to ST.
Conclusions: The obtained results show that resveratrol decreases lipogenesis markers and metabolic parameters in the setting of a high-protein diet. Moreover, resveratrol decreased total cholesterol in both diets. These results point to the increased potential of resveratrol use in prevention of metabolic syndrome, acting on different dietary compositions.

1. Introduction

Metabolic syndrome (MS) is a public health problem, and its consistent increase in prevalence is a worldwide phenomenon [16], which is closely associated with the increase in prevalence of obesity [16] and sedentary lifestyles [3]. MS is a complex of interrelated risk factors, that includes raised blood pressure, dysglycemia, low high-density lipoprotein cholesterol levels, elevated triglyceride levels, and obesity, contributing to the development of cardiovascular disease and diabetes [3].

More attention must be given to lifestyle changes based on a healthy diet and an increase in physical activities in order to prevent and treat obesity and MS [3]. Although general recommendations for adults of Dietary Reference Intakes (DRI) include a total fat intake of 20–35% of daily caloric consumption, 10–35% of total calories as protein, and carbohydrates oscillating from 45% to 65% [18], the world population is increasing its consumption of diets rich in sugars, refined carbohydrates, proteins, fats and animal-source foods, while diets rich in legumes, coarse grains, and other vegetables are decreasing everywhere [28].

A good strategy for the prevention of MS development is nutraceutical intake, such as resveratrol (RSV). Resveratrol (3,5,40-

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trihydroxystilbene) is one of the natural polyphenolic compounds mainly found in grape skins and red wine [9]. RSV is well known for its anti-cancer, anti-inflammatory, anti-obesity, cardioprotective, and antioxidant properties [2,10,15,17]. RSV promotes lipolysis and fatty acid β oxidation, thus decreasing adipogenesis and lipogenesis and consequently acting as an anti-obesity compound [38].

Adipogenesis is characterized by an increase in the number of adipocytes in adipose tissue (hyperplasia), that starts with the differentiation of adipocytes from stem cells [30]. In this differentiation process, sterol regulatory element binding protein 1c (SREBP-1c) and peroxisome proliferator-activated receptor gamma (PPARγ), the latter being considered the main adipogenesis inducing regulator, are required to induce the well-known shape of the adipocytes, which is spherical, from a fibroblast cell shape [2]. During the last phase of differentiation, the adipocytes show a great increase in lipogenesis, via increased expression and activity of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). This process is controlled by SREBP-1c [2].

The literature describes experimental animal studies involving the administration of resveratrol with a high saturated fat diet, and reports improvement in health and metabolic parameters of the animals studied [4,26]. However, resveratrol may have distinct metabolic effects in other dietary compositions, besides the high saturated fat diet. There are only a few studies involving the concomitant consumption of resveratrol in other dietary patterns, such as high-polyunsaturated fat and high-protein diets. In this context, this study aims to evaluate the effect of high-dose of resveratrol associated with different dietary macronutrients on expression of adipogenic and lipogenic genes in the adipose tissue.

2. Materials and methods

2.1. Animals and experimental diets

Forty two female FVB/N mice, aged to 8 weeks, were divided into 6 groups that were fed with experimental diets for 60 days (n = 7 per treatment). The mice from the State University of Montes Claros (Montes Claros, Minas Gerais, Brazil) were housed in cages, under a 12 h:12 h light-dark cycle (lights on from 7:00 to 19:00 h) at a controlled temperature of 25.0 ± 2.0 °C. Food and water were offered ad libitum. This study was approved by Ethics Committee of Experimentation and Animal Welfare of Unimontes, Montes Claros, Brazil (process no. 064/2013).

The experimental groups were: standard diet (ST), standard diet plus resveratrol (ST + RSV), high-fat diet (HFD), HFD plus resveratrol (HFD + RSV), high-protein diet (HPD), and HPD plus resveratrol (HPD + RSV). The ST + RSV group was included in the experimental phase, but was not used in the posterior analysis (plasma parameters, histology and real-time PCR) due to the absence of statistically significant differences regarding body composition (see Fig. 1A to J).

The experimental diets (HFD and HPD) were formulated as described in previous studies [6,11,13] and were standardized and purchased from Rhoster®, Brazil. The high fat diet had the following composition: cornstarch (40.57%), casein (14%), dextrinized starch (15.5%), sucrose (10%), soybean oil (10%), cellulose - fiber (5%), mineral mix AIN-93M (3.5%), vitamin mix AIN-93 (1%), L-cysteine (0.18%), choline bitartrate (0.25%), and *tert*-butylhydroquinone (0.0008%). The high protein diet had the following composition: cornstarch (32.57%), casein (28%), dextrinized starch (15.5%), sucrose (10%), soybean oil (4%), cellulose - fiber (5%), mineral mix AIN-93M (3.5%), vitamin mix AIN-93 (1%), L-cysteine (0.18%), choline bitartrate (0.25%), and *tert*-butylhydroquinone (0.0008%). Standard diet (Labina®) was produced by Purina®, Brazil. The centesimal composition of each diet is detailed in Table 1. In RSV groups, resveratrol powder (Sigma–Aldrich Co. LLC., Saint Louis, MO, EUA) was added to diet powder in the proportion of 4 g/kg diet [29,35], which corresponds to 300 mg/kg of body weight/day.

2.2. Measurements of body weight, food intake, tissue collection and plasma parameters

Body weight (BW), food intake, and energy intake (food intake in kcal) were recorded twice weekly. At the end of the experiment, the animals were fasted overnight (12 h) and euthanized by decapitation. Samples of blood and adipose tissue (perigonadal, mesenteric and retroperitoneal) were collected, weighed, and stored immediately in liquid nitrogen and subsequently at −80 °C for posterior analysis. Body adiposity was calculated by the sum of perigonadal, mesenteric, and retroperitoneal adipose tissues. Blood samples were centrifuged (3000 rpm for 10 min) and the plasma was separated for the determination of glucose, triglycerides, high density lipoprotein (HDL), and total cholesterol levels, by enzymatic tests (Wiener Lab, Argentina).

2.3. Histology

Perigonadal adipose tissue samples were fixed in formaldehyde solution (10%) and embedded in paraffin serially sectioned at 5 mm, stained with hematoxylin and eosin (HE), and evaluated under a conventional light microscope using an Olympus FSX100 microscope (Tokyo, Japan). Images of fat tissue areas (10 ocular and 40 objective lenses) were captured with FSX-BSW software (Olympus, Tokyo, Japan). Adipocyte cell area was measured using ImageJ software (NIH, USA).

2.4. Reverse transcription and real-time PCR

Samples of perigonadal adipose tissue were prepared using Trizol reagent (Invitrogen Corp.VR, San Diego, CA, USA) and treated with DNAse (Invitrogen Corp.VR). Reverse transcription was carried out with M-MLV (Invitrogen Corp.VR) using random hexamer primers. Levels of genes of interest (Table 2) were determined by Real Time PCR (SYBR Green reagent) in Step One Plus equipment (Applied Biosystems-EUA). Gene expression was quantified using the relative comparative Ct (threshold cycle) method with GAPDH as the endogenous control [23].

2.5. Statistical analysis

Analyses were performed using GraphPad Prism software (Version 5.0, GraphPad Software Inc., San Diego, CA, USA). Data were evaluated by one-way ANOVA, followed by Tukey post test. All data are given as means ± S.D. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Resveratrol, diets, body composition and metabolic parameters

A decrease in the BW average was observed in HFD (21.12 ± 0.80 g) and HFD + RSV (21.93 ± 1.37 g) when compared to ST (25.08 ± 0.83 g) (Fig. 1A). The group HPD + RSV (19.47 ± 1.63 g) had lower BW than HPD (23.44 ± 1.00 g) and ST (25.08 ± 0.83 g) (Fig. 1B). The body weight in ST + RSV group (22.58 ± 7.14 g) was not significantly different when compared to other treatment groups (Fig. 1A and B). Average food intake (Fig. 1C and D) and energy intake (Fig. 1E and F) were not statistically different between the groups.

Body adiposity was lower in HPD + RSV (0.019 ± 0.006 g/BW) than in ST (0.040 ± 0.013 g/BW) and HPD (0.038 ± 0.006 g/BW), but there was no statistical difference in ST + RSV group (0.031 ± 0.007 g) compared to other treatments (Fig. 1H). The mass of perigonadal adipose tissue was significantly lower in HPD + RSV (0.008 ± 0.002 g/BW) than in HPD (0.015 ± 0.005 g/BW), and there was no statistical difference in ST and ST + RSV groups when compared to other treatments (Fig. 1J). Mesenteric adipose tissue was higher in HPD (0.009 ± 0.004)