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.

Keywords: Diet, Resveratrol, Lipogenesis, Adipogenesis, Cholesterol

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 (3,5,4-
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-polysaturated 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 BX100 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 HFD (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/BW) compared to other treatments (Fig. 1H). The mass of perigonadal adipose tissue was significantly lower in HFD + 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)
and HPD + RSV (0.01 ± 0.01), as well as HPD that showed higher expression than HFD (0.24 ± 0.29), HFD + RSV (0.12 ± 0.23), HPD (0.58 ± 0.26) and HD + RSV (0.08 ± 0.11) (Fig. 3B). HDL cholesterol was lower in HPD (66 ± 12 mg/dL) than in ST (100 ± 30 mg/dL) and HD + RSV (93 ± 11 mg/dL) (Fig. 3D). Glucose and triglyceride levels were not significantly different between the treatments.

3.2. Resveratrol, diets and adipogenic gene expression

Real-time PCR was performed in order to analyze the expression of adipogenic genes, such as Peroxisome proliferator-activated receptor gamma (PPARγ), Sterol regulatory element-binding transcription factor 1c (SREBP-1c), Acetyl-CoA carboxylase (ACC) and Fatty acid synthase (FAS). PPARγ expression was higher in ST (1.00 ± 0.00) than in HFD (0.66 ± 0.10), HFD + RSV (0.03 ± 0.03), HD (0.25 ± 0.33) and HD + RSV (0.08 ± 0.13) (Fig. 4A and B). SREBP-1c expression was also higher in ST (1.00 ± 0.00) than in HFD (0.12 ± 0.11), HD + RSV (0.21 ± 0.39), HD (0.20 ± 0.16) and HD + RSV (0.06 ± 0.05) (Fig. 4C and D).

The expression of ACC (Fig. 4E and F), was higher in ST (1.00 ± 0.00) than in HFD (0.24 ± 0.29), HD (0.12 ± 0.23), HD + RSV (0.58 ± 0.26) and HD + RSV (0.01 ± 0.01), as well as HPD that showed higher expression than HPD + RSV (Fig. 4F). Finally, FAS expression was higher in HPD (1.79 ± 0.66) when compared with HD + RSV (0.55 ± 0.68) (Fig. 4H).

4. Discussion

The main findings of the present study showed distinct metabolic effects of resveratrol under different dietary compositions. In the literature, experimental studies with animals, involving resveratrol administered along with a high saturated fat diet are more easily found. However, there are only a few studies that show the association between resveratrol and high-protein diet. Our study showed that animals treated with a high-protein diet and resveratrol had a decrease in BW, body adiposity, perigonadal adipose tissue, adipocyte area, total cholesterol, ACC and FAS expression, and had an increase in HDL cholesterol. Additionally, the effects of resveratrol in high-polyunsaturated fat diet included a decrease in total cholesterol levels.

The macronutrient profile of a diet, in the treatment of MS, is an important consideration that may potentiate weight loss, and a decrease in cardiometabolic risk, thus the ideal diet should combine all of the dietary components that influence these factors. High-protein and low-carbohydrate diets, low-glycemic index carbohydrates, and adequate omega-3 fatty acid intake are nutritional factors currently proposed for the treatment of MS. Saturated fat and fructose are more likely to stimulate hepatic lipid accumulation, whereas unsaturated fat, antioxidants and high-protein diets seem to have a more preventive effect.

Our study shows that resveratrol decreased body weight and body adiposity. Animals on a high-protein diet plus resveratrol had lower body weight average, perigonadal adipose tissue weight and body adiposity than animals on high-protein diet, which can be explained by the resveratrol anti-obesity effect, showing the important role of resveratrol in weight and fat loss. Resveratrol exerts its positive effects on MS by activating Sirt1, which regulates proteins that play important roles in the pathophysiology of metabolic diseases, such as the reduction of body fat adiposity.

In contrast with other studies of our group, based on a high saturated fat diet, our findings showed that mice on a high-fat diet and high-fat diet plus resveratrol presented with lower body weight than mice on the standard diet, which can be explained by the composition of our high-fat diet that is based on soybean oil, known to be rich in polyunsaturated fatty acids (PUFA). PUFA is found in foods such as seeds, nuts, and nontropical vegetable oils. Studies show that PUFA is effective in increasing lean mass and in reducing body fat or the fat:lean ratio when compared with the high-fat lard control diet. Increased intake of PUFA has been associated with cardiometabolic benefits including lowering blood lipids and reduced risk of both type 2 diabetes and cardiovascular disease.

Regarding the histological analysis of the perigonadal adipose tissue, the animals in the HPD and HPD + RSV groups showed shorter adipocyte area when compared to ST group. In addition, more importantly, resveratrol is capable of decreasing the average area of adipocytes in HPD group, contributing to an improvement in overall adiposity. This result confirms that resveratrol is effective in reducing the size of adipose tissue.

Resveratrol has demonstrated an important role in lipid metabolism. In our study, total cholesterol levels were lower in the groups HFD + RSV and HPD + RSV when compared with the HFD and HPD groups, respectively. Resveratrol reduces cholesterol synthesis by downregulating HMG-CoA reductase and enhances reverse cholesterol excretion and cholesterol transport through increasing HDL levels and the capacity of HDL to mediate cholesterol efflux from macrophages in arterial walls. Another study by our group found the same result, in which low dose resveratrol (30 mg/kg/day) decreased total cholesterol in the setting of a high-fat diet. The same result was found in the study of Kim et al. using 0.4% of resveratrol, the same dose of our study. Additionally, HDL-cholesterol levels were higher in HFD + RSV than in HFD. Jeon and Choi in a study with animals fed with an atherogenic diet and resveratrol, found that resveratrol significantly increased the plasma HDL-C concentration compared with the control.
The expression of adipogenic genes, PPAR\(\gamma\) and SREBP-1c, was lower in all diets (HFD and HPD) than in ST and there was no difference with resveratrol treatment. Thus, the decreased expression of these genes was due to the effect of both diets (high-protein and high polyunsaturated fat). However, a study showed that resveratrol decreased PPAR\(\gamma\) and SREBP-1c expression in high-fat diet group\[4\]. Kim et al.\[22\] showed that resveratrol (0.4%) significantly reversed the HFD-induced up-regulation of key adipogenic genes (PPAR\(\gamma\)2, C/EBP\(\alpha\), SREBP-1c, LPL, ap2, and leptin) in the epididymal adipose tissues of mice. PPAR\(\gamma\) is the master regulator of adipogenesis, and plays an important role in lipid metabolism and glucose homeostasis\[25\], while also being decreased by resveratrol\[8\]. SREBP-1c is the most important transcription factor regulating de novo lipogenesis, and is also inhibited by resveratrol via the Sirt1–FOXO1 signaling pathway\[36\].

On the other hand, expression of lipogenic enzymes, ACC and FAS, were reduced in HPD + RSV group, which lead us to see the effects of resveratrol on decreasing lipogenesis in high-protein diet. Though our results showed that high-protein diet decreased the ACC expression in comparison to ST, the association of resveratrol to this diet decreased even more than this expression and additionally decreased the expression of FAS. Thus, resveratrol causes a decrease in lipogenesis in the setting of a high-protein diet. Another study of our group showed that resveratrol (30 mg/kg/day) decreased ACC expression in the setting of a high-fat diet\[4\]. In the study of Kim et al.\[22\], resveratrol (0.4%) decreased the expression of FAS in mice treated with a high-fat diet.

Lipogenesis involves the synthesis of fatty acids, from acetyl CoA, used as substrates in the synthesis of triacylglycerols\[2\]. High-protein diets rich in branched-chain amino acids can increase lipogenesis by converting amino acid into acetyl-CoA\[7\], and high-fat diets increase lipogenesis through fatty acid conversion to acetyl-CoA\[33\]. Acetyl-CoA undergoes action of ACC and FAS, and increases fatty acids synthesis\[2\] (Fig. 4I). In the literature, resveratrol is described as acting by decreasing adipogenic gene expression, such as PPAR\(\gamma\) and SREBP-1c, which control lipogenic enzymatic activity\[2\]. In our study, resveratrol decreased ACC and FAS expression in high-protein diet, decreasing lipogenesis\[34,38\] (Fig. 4I). This decrease in lipogenesis should be considered as a main contributor to the reduction in body weight and body adiposity of resveratrol in high-protein diets.

Many studies show the role of resveratrol in decreasing lipogenesis in association with a high-fat diet\[2,4,38\], but in our study this result was not found. This absence of effect of resveratrol in the high-fat diet is related to the composition of our diet, rich in polyunsaturated fat (PUFA), while in other studies the source of high-fat diet is the lard, rich in saturated fat (SFA). This difference in the effect of PUFA and SFA can be attributed to their fatty acid compositions\[31\].

On the other hand, the literature has no studies showing this effect on high-protein diet. Given this, to our knowledge, our study is the first to show the role of resveratrol in decreasing lipogenesis in animals treated with a high-protein diet.

This study also has potential limitations. The current study used healthy and lean mice, so our results may not apply to obese mice who might show a different response to the treatment with diets and resveratrol. The data thus need confirmation in mice with obesity and metabolic syndrome. Also, the lack of differences between standard and standard plus resveratrol groups does not allow us to analyze in depth the effect of diet and resveratrol on body composition and metabolism. Finally, this study was not designed to compare differences between diets, so we did not use the high saturated fat diet. Future studies with this aim can be made to improve the present results.

5. Conclusion

In conclusion, the obtained results show that resveratrol decreases markers of lipogenesis and metabolic parameters in the setting of a high-protein diet. Moreover, resveratrol decreased the total cholesterol in both diets. These results indicate the increased potential of resveratrol use in prevention and treatment of metabolic syndrome, acting in different dietary compositions.

Abbreviations

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<td>ST</td>
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**Authors and contributors**

*Keila Lopes Mendes, Lucinéia de Pinho, João Marcus Oliveira Andrade, Sérgio Henrique Sousa Santos:* contributed to the drafting, conception and designing of the project; acquisition, analysis, and interpretation of the data.

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*Keila Lopes Mendes:* manuscript writing.

*Sérgio Henrique Sousa Santos:* critical revision for important intellectual content, final approval of the version to be published and agreement in order to ensure the accuracy and integrity of all aspects of the work and if the questions proposed were appropriately investigated and resolved. All authors have approved the final article.

**Competing interests**

There are no conflicts of interests.

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