

# Resveratrol increases brown adipose tissue thermogenesis markers by increasing SIRT1 and energy expenditure and decreasing fat accumulation in adipose tissue of mice fed a standard diet

João Marcus Oliveira Andrade · Alessandra Caroline Montes Frade ·  
Juliana Bohnen Guimarães · Kátia Michelle Freitas · Miriam Teresa Paz Lopes ·  
André Luiz Sena Guimarães · Alfredo Maurício Batista de Paula ·  
Cândido Celso Coimbra · Sérgio Henrique Sousa Santos

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## Abstract

**Purpose** Adipose tissue is central to the regulation of energy balance. Two functionally different fat pads are present in mammals: white adipose tissue, the primary site of triglyceride storage, and brown adipose tissue (BAT), which is specialized in heat production. In this context, new strategies capable of modulating the development and function of white and BAT become relevant. In the present study, we analyzed the influence of resveratrol (sirtuin activator) on energy balance and the expression of thermogenesis markers.

**Methods** Mice were divided into two groups: standard diet (ST) and standard diet plus resveratrol (ST + RSV).

**Results** After 2 months of treatment, ST + RSV mice presented significantly decreased fat accumulation in adipose tissue, with diminished total cholesterol and glucose plasma levels. Additionally, increased oxygen consumption was observed in ST + RSV group. Analyses of mRNA of thermogenesis-related genes showed significant increase in UCP1, SIRT1, PTEN and BMP-7 expression in BAT.

**Conclusion** Our data suggest that improved metabolism produced by oral administration of resveratrol is, at least in part, associated with increased thermogenesis followed by high expression of UCP1 and SIRT1, which can mediate higher energy expenditure and decreased fat accumulation in adipose tissue.

João Marcus Oliveira Andrade and Alessandra Caroline Montes Frade equally contributed to this study.

J. M. O. Andrade · A. L. S. Guimarães ·  
A. M. B. de Paula · S. H. S. Santos

Laboratory of Health Science, Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros (UNIMONTES), Montes Claros, Minas Gerais, Brazil

A. C. M. Frade · K. M. Freitas · M. T. P. Lopes · S. H. S. Santos  
Pharmacology Department, Biological Sciences Institute,  
Federal University of Minas Gerais (UFMG), Belo Horizonte,  
Minas Gerais, Brazil

J. B. Guimarães · C. C. Coimbra  
Physiology and Biophysics Department, Biological Sciences  
Institute, Federal University of Minas Gerais (UFMG),  
Belo Horizonte, Minas Gerais, Brazil

S. H. S. Santos (✉)  
Departamento de Farmacologia, Universidade Federal de Minas  
Gerais, Av. Antônio Carlos 6627-ICB, Belo Horizonte,  
Minas Gerais 31270-901, Brazil  
e-mail: sergiosousas@hotmail.com

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## Introduction

Adipose tissue is a key organ in the regulation of energy balance. Two functionally different types of adipose tissue are present in mammals: white adipose tissue (WAT), the primary site of triglyceride storage, and brown adipose tissue (BAT), which is specialized in energy expenditure and thermogenesis [1]. These two tissues differ in anatomical localization, abundance, maintenance throughout the life of the animal, morphology and mainly in function [2].

Adaptive thermogenesis (nonshivering thermogenesis), for the maintenance of basic body temperature and heat balance energy, is an important function of BAT. It is mediated by uncoupling proteins (UCPs), which are located in the inner mitochondrial membrane. These proteins

act as uncouplers of oxidative phosphorylation by dissipating the proton gradient across the membrane and producing heat, rather than being used to drive the synthesis of ATP [3]. UCPs can, in this manner, dissipate surplus caloric energy and can consequently be important regulators of body weight.

In this context, thermogenic ingredients may be considered functional agents that could help to prevent a positive energy balance and obesity.

Resveratrol (3,5,4'-trihydroxystilbene) is a natural polyphenolic compound found in grapes and red wine, which has been shown to extend the life span in many organisms [4, 5]. It is a natural activator of the sirtuins (SIRT) family, the mammalian homolog of yeast silent information regulator 2 (Sir2), and is comprised of a highly conserved family of proteins, with one or more sirtuins present in virtually all species from bacteria to mammals [6, 7]. Previous studies have shown that resveratrol administration improves metabolic profile in mice and rats by modulating adipose tissue function [8, 9].

In a recent report, it was shown that brown remodeling of WAT and thermogenesis are dependent on SIRT1 [10]. Activation of NAD<sup>+</sup>-dependent deacetylase SIRT1 by natural compound, calorie restriction or exercise promotes mitochondrial biogenesis and activities [11, 12], raising the possibility that SIRT1 regulates BAT functions. The study published by Lagouge et al. [13] showed that treatment of mice with resveratrol (concentration of 4 g/kg of food) increased their aerobic capacity (increased running time and consumption of oxygen) by an induction of genes for oxidative phosphorylation and mitochondrial biogenesis, with increase in PGC-1 $\alpha$  activity mediated by SIRT1.

Alberti et al. [3] showed that resveratrol increases the level of UCP expression in two important thermogenic tissues (BAT and skeletal muscle), suggesting that resveratrol can contribute to increased whole-body energy dissipation and consequently to increased energy expenditure, thus reducing energetic efficiency.

Taking these data into account, we hypothesize that oral administration of resveratrol in mice fed with a standard diet would modulate thermogenesis by increasing thermogenic gene expression. This mechanism might contribute to alteration in energy efficiency, lipidic and glycemic profile and loss of fat mass.

## Materials and methods

### Animals and diets

The experiment was conducted with sixteen male mice (4 weeks old) from the Federal University of Minas Gerais (Belo Horizonte, Minas Gerais, Brazil) which were

randomly divided into two groups ( $n = 8$  each) and fed the following respective experimental diets for 8 weeks: standard diet (ST) and standard diet plus resveratrol (ST + RSV) (concentration of 4 g/kg of food to provide a 400-mpk dose), as described previously [3, 13].

Standard diet (Purina-Labina<sup>®</sup>), used for regular maintenance of the mice, is composed of 50.30 % of carbohydrate, 41.90 % of protein and 7.80 % of fat with a total of 2.18 kcal per 1 g of diet [14, 15].

All experimental procedures were approved by the Ethics Committee of the Universidade Federal de Minas Gerais for the Care and Use of Laboratory and were conducted in accordance with the regulations described in the Committee's Guiding Principles Manual.

### Measurements of body weight, food intake and tissue collection

Mice were individually housed, and food intake was measured twice a week during treatment to obtain food efficiency (food intake/body weight). After an overnight fast, mice were killed by decapitation. Samples of serum; epididymal, mesenteric and retroperitoneal WAT; interscapular BAT and gastrocnemius muscle were collected, weighed, immediately frozen in dry ice and stored at  $-80^{\circ}\text{C}$  for subsequent analysis.

### Oxygen consumption measurement

After 4 weeks of treatment, the animals were submitted to oxygen consumption measurement to verify thermogenic effects. Animals were transferred from their cages to an acrylic box where oxygen consumption ( $\text{VO}_2$ ), an index of metabolic rate, was measured by an open-flow indirect calorimeter (PANLAB, Apparatus LE405, Gas Analyzer). The calorimeter was calibrated before each use with a certified mixture of gases (50 %  $\text{O}_2$  and 1 %  $\text{CO}_2$ /20.2 %  $\text{O}_2$  and 0 %  $\text{CO}_2$ —White Martins). Animals were allowed to rest for at least 1 h with  $\text{VO}_2$  ( $\text{mLO}_2 \times \text{kg}^{-1} \times \text{min}^{-1}$ ) being continuously recorded online using a computerized system (Metabolism OXYLET System). Data analyses used only the last 20 min of  $\text{VO}_2$  recording, when animals had already returned to rest condition after the stress of handling. All experiments were conducted between 8:00 and 12:00 h to prevent circadian variation, and ambient temperature was controlled at  $23 \pm 1^{\circ}\text{C}$  [16, 17].

### Locomotor activity

The experiments measuring the spontaneous locomotor activity (SLA) were carried out in an open field (40 cm in diameter with a 50-cm-high Plexiglas wall) located in an isolated room, and the lighting in the room was 200 lx. The

animals were video-recorded, and the distance moved was automatically analyzed with the aid of ANYMAZE software 4.5 (Stoelting, Wood Dale, IL, USA). For evaluating the effect of treatment on spontaneous locomotor activity, the animals were placed in the center of the open field and the total distance moved in centimeters was recorded during 30 min [18].

#### Determination of circulating biochemistry parameters

Serum was obtained after centrifugation (3,200 rpm for 10 min at 4 °C). Total serum cholesterol, triglycerides, high-density protein (HDL) and glucose were assayed using enzymatic kits (Wiener®, Argentina). Measurements were taken in Wiener BT-3000 plus Chemistry Analyzer (Wiener®, Argentina).

#### Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Total RNA from the BAT was prepared using Trizol reagent (Invitrogen Corp., San Diego, California, USA), treated with DNase and reverse transcribed with M-MLV (Invitrogen Corp.) using random hexamer primers. Levels of UCP1, UCP3, Cidea, adiponectin, PTEN, BMP-7, PRDM16 and SIRT1 mRNA were determined by qRT-PCR using SYBR Green reagent (Applied Biosystems®, USA) in a PlusOne platform (Applied Biosystems®, USA). Gene expression was normalized to the endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

#### Applied biosystems

##### Statistical analysis

All data were transferred to GraphPad Prism software (Version 5.0®, San Diego, California, USA) and analyzed with 95 % confidence ( $P < 0.05$ ). Data are expressed as mean  $\pm$  SEM. The statistical significance of differences in mean values between mice groups was assessed by Student's *t* test.

#### Results

ST + RSV mice did not present reduced final body weight ( $P = 0.72$ ) and food intake ( $P = 0.62$ ) (Fig. 1a, b). However, we observed significant decreases in epididymal (ST  $0.0075 \pm 0.0051$  vs. ST + RSV  $0.0022 \pm 0.0019$  g/body weight,  $P < 0.05$ ) and retroperitoneal (ST  $0.0012 \pm 0.0006$  vs. ST + RSV  $0.0006 \pm 0.0003$  g/body weight,  $P < 0.05$ ) adipose tissue in the group treated with resveratrol (Fig. 1c,

e). No significant differences were found between ST and ST + RSV group to mesenteric adipose tissue, skeletal muscle mass and BAT (Fig. 1e–g).

ST + RSV mice showed diminished total cholesterol (ST  $139.6 \pm 9.39$  vs. ST + RSV  $110.8 \pm 11.52$  mg/dL,  $P < 0.01$ ) and glucose levels (ST  $133.4 \pm 22.19$  vs. ST + RSV  $96.4 \pm 15.13$  mg/dL,  $P < 0.05$ ) (Fig. 2a, b). Regarding HDL and triglycerides, no significant differences were observed between ST and ST + RSV groups (Fig. 2c, d).

The oxygen consumption was increased in ST + RSV mice (ST  $36.59 \pm 2.77$  mL O<sub>2</sub>  $\times$  Kg<sup>-1</sup>  $\times$  min<sup>-1</sup> vs. ST + RSV  $42.33 \pm 1.35$  mL O<sub>2</sub>  $\times$  Kg<sup>-1</sup>  $\times$  min<sup>-1</sup>;  $P < 0.001$ ) (Fig. 3a, b). Locomotor activity did not show difference between the groups (ST  $104.9 \pm 32.54$  cm/30 min. vs. ST + RSV  $80.32 \pm 32.76$  cm/30 min.) (Fig. 3c).

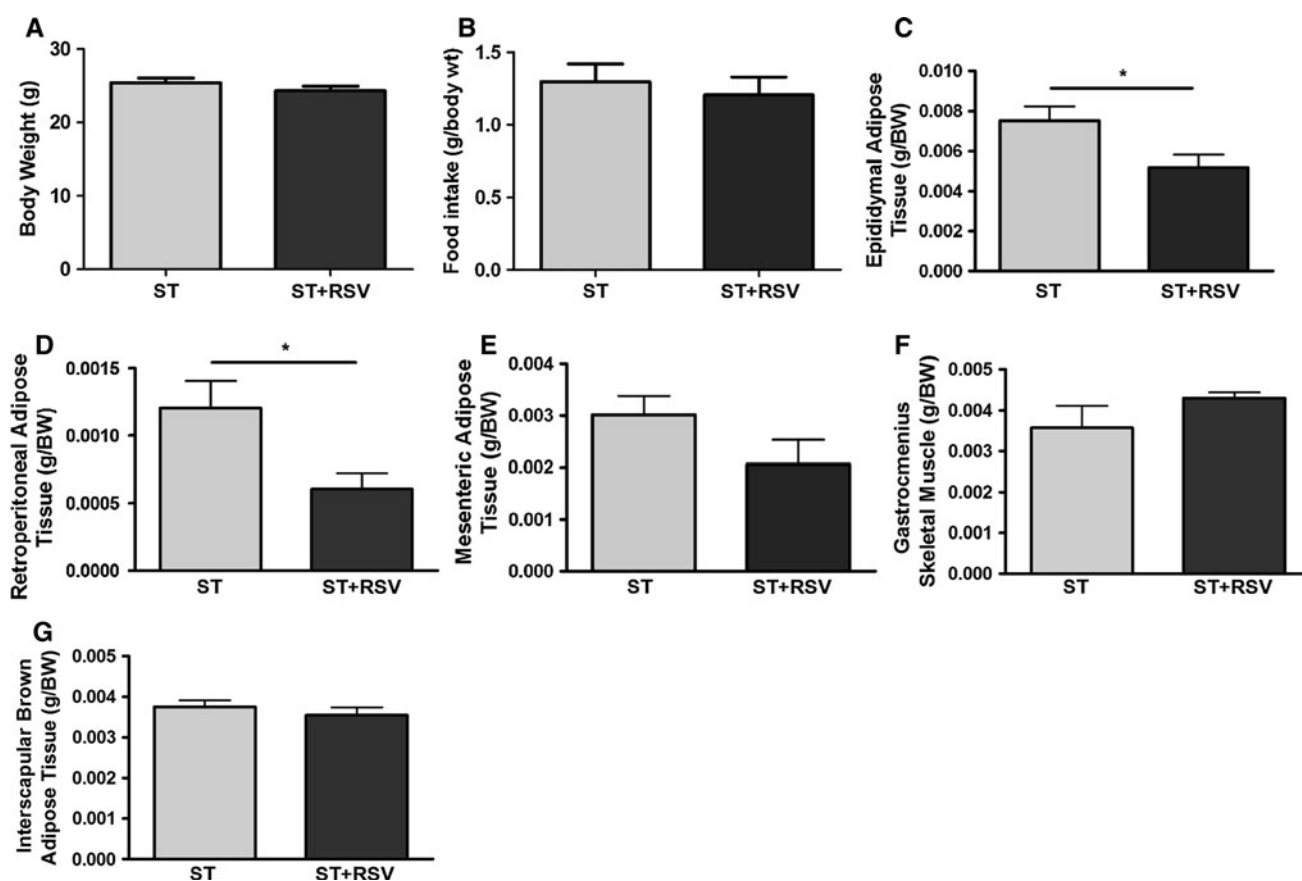
Analyses of mRNA expression of thermogenesis-related genes showed significant increase in UCP1 (ST  $0.448 \pm 0.15$  vs. ST + RSV  $0.85 \pm 0.22$ ,  $P < 0.01$ ) (Fig. 4a). Other targets such as UCP3 (ST  $0.73 \pm 0.10$  vs. ST + RSV  $0.79 \pm 0.24$ ), Cidea (ST  $0.805 \pm 0.183$  vs. ST + RSV  $1.003 \pm 0.492$ ), PRDM16 (ST  $0.59 \pm 0.13$  vs. ST + RSV  $0.69 \pm 0.28$ ) and adiponectin (ST  $0.933 \pm 0.17$  vs. ST + RSV  $0.895 \pm 0.15$ ) mRNA expression in the interscapular BAT did not show significant difference between the groups (Fig. 4b, e). Furthermore, an increase was observed in UCP1/UCP3 ratio in the same tissue (Fig. 4f).

Additionally, we observed a significant increase in SIRT1 (ST  $0.65 \pm 0.19$  vs. ST + RSV  $1.07 \pm 0.19$ ,  $P < 0.05$ ), PTEN (ST  $0.30 \pm 0.12$  vs. ST + RSV  $0.51 \pm 0.08$ ,  $P < 0.05$ ) and BMP-7 (ST  $0.32 \pm 0.08$  vs. ST + RSV  $0.51 \pm 0.074$ ,  $P < 0.05$ ) expression (Fig. 4g, i).

#### Discussion

The main findings of this study are that mice treatment with resveratrol enhances thermogenesis and improves lipid and glycemic profile in mice fed with a standard diet. These alterations were associated with increased UCP1 expression in BAT. We suggest that high expression of SIRT1, PTEN and BMP-7 markers might be associated with increased expression of UCP1.

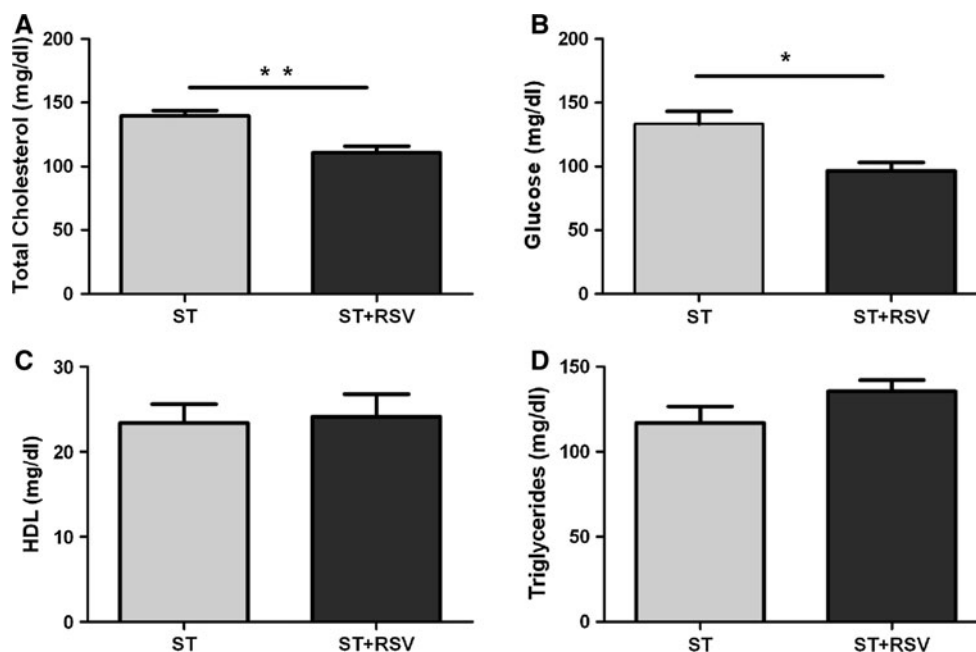
Two adipose tissue types can be distinguished in mammals, which have essential antagonistic functions: WAT stores excess of energy as triglycerides, while BAT is specialized in the dissipation of energy through the production of heat (nonshivering thermogenesis) [19]. These two tissues differ in anatomical localization, abundance, maintenance throughout the life of the animal, morphology and mainly in function [2]. Although excessive accumulation of WAT is important for the



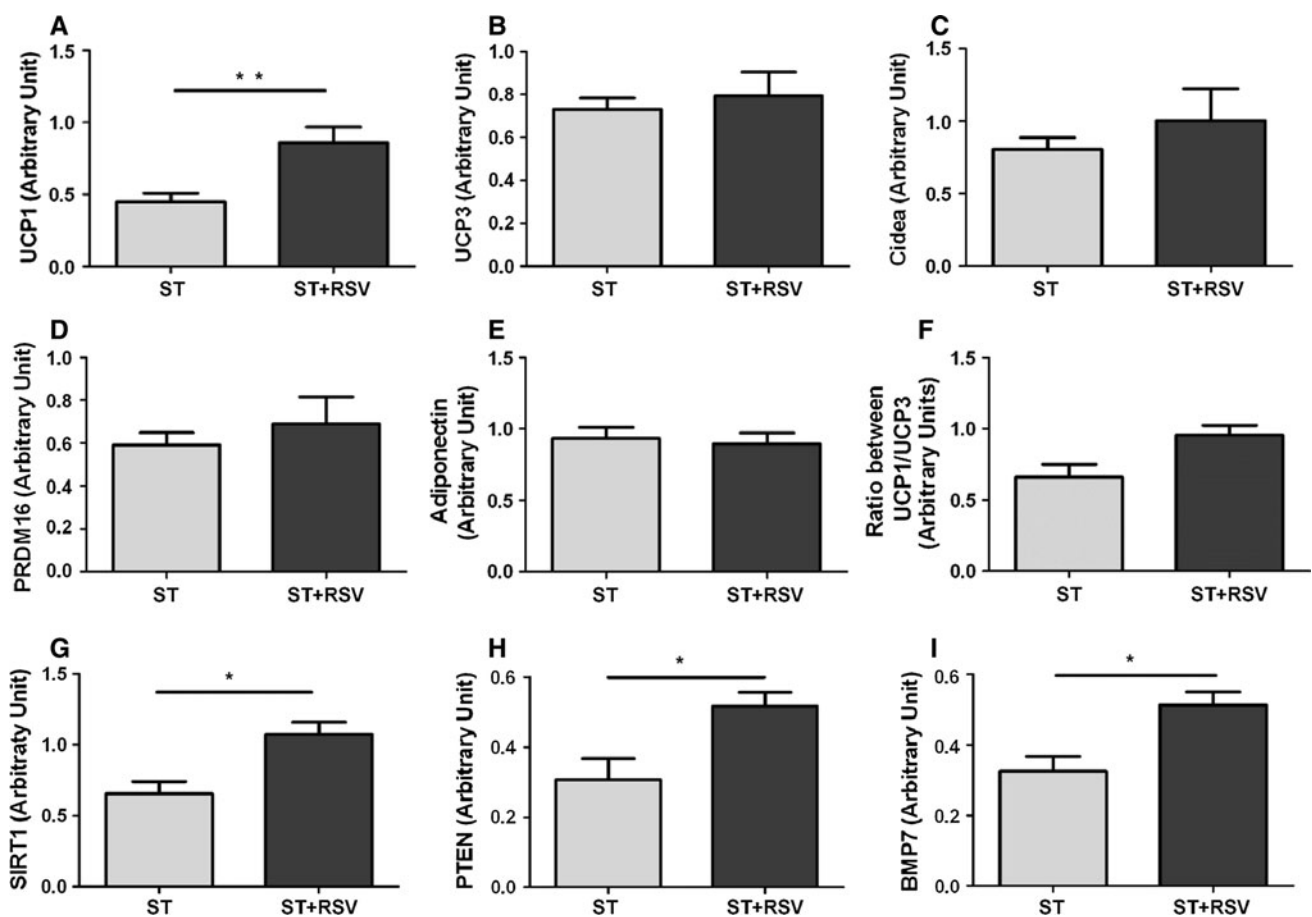
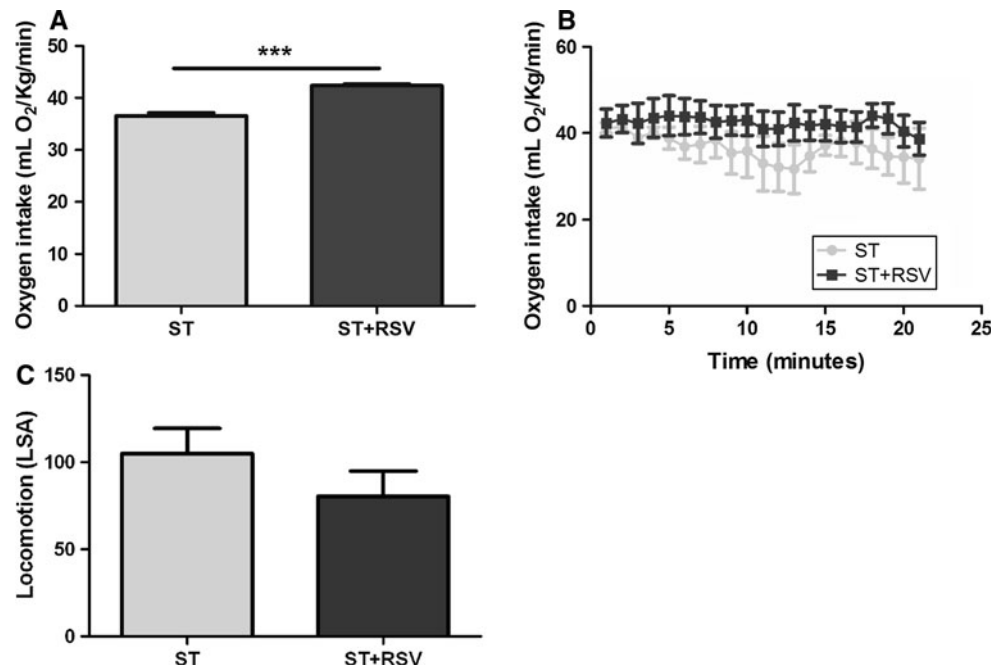
**Fig. 1** Body weight, food intake and tissue weight. **a** Body weight (g) of 4-week-old ST and ST + RSV ( $n = 8$ ) male mice. **b** Food intake (g/g bw). **c** Epididymal adipose tissue weight. **d** Retroperitoneal

adipose tissue weight. **e** Mesenteric adipose tissue weight. **f** Skeletal muscle weight. **g** Brown adipose tissue weight. \* $P < 0.05$ , in comparison with ST group

**Fig. 2** Circulating levels. **a** Plasma levels of total cholesterol. **b** Plasma levels of glucose. **c** Plasma levels of high-density protein. **d** Plasma levels of triglycerides. \* $P < 0.05$  and \*\* $P < 0.01$  in comparison with ST group



**Fig. 3** Oxygen consumption and locomotion levels. **a**, **b** Oxygen consumption (mL O<sub>2</sub>/kg/min). **b** Locomotion levels (LSA). \*\*\* $P < 0.001$  in comparison with ST group



**Fig. 4** Analyses of mRNA expression of thermogenesis-related targets by qRT-PCR in brown adipose tissue. **a** mRNA expression of UCP1. **b** mRNA expression of UCP3. **c** mRNA expression of Cidea. **d** mRNA expression of PRDM16. **e** mRNA expression of

adiponectin. **f** mRNA expression of SIRT1. **g** mRNA expression of PTEN. **h** mRNA expression of BMP-7. \* $P < 0.05$  and \*\* $P < 0.01$  in comparison with ST group

development of insulin resistance, it is believed that some fat depots are more linked to risk factors for disease than others. The main adipose depots of interest are found in the abdomen and around the testis of males (epididymal) and around the ovaries of females (periovarian) and have been identified as an important metabolic and inflammatory organ that modulates the action and metabolism of brain, liver, muscle and vascular endothelial cells [20–22].

Recently, a new process was described called “browning” of WAT. Brown adipocytes may appear after thermogenic stimuli at anatomical sites corresponding to WAT. The brown adipocytes appearing in WAT derive from precursor cells different from those in classical BAT and are closer to the white adipocyte cell lineage [23–25]. UCPs are closely involved in “browning process.” Understanding the biological processes controlling brown adipocyte activity and differentiation could help the design of BAT-focused strategies to increase energy expenditure and fight obesity.

In the present study, the decreased body fat mass is not associated with alteration in total body weight. Previous studies have found that UCP1 levels are associated with changes in the adiposity index and significantly contribute to thermogenesis and adaptations of energy expenditure. Decrease in UCP1 expression is associated with increased adiposity [26, 27]. Another study indicated that UCP1 enhances leptin action at the level of the hypothalamus, suggesting that this molecule contributes to the energy control balance not only through the regulation of energy expenditure but also through appetite control by modulating leptin action [28].

Recent studies have shown that polymorphic variations in UCP1 gene have a strong association with reduced HDL-cholesterol levels, increased LDL-cholesterol levels [29, 30], or triglycerides [31], and increased systolic and/or diastolic blood pressure [32]. On the other hand, UCP1 also appears to act in the regulation of glucose homeostasis. Several works have reported that polymorphisms -3826A/G, -1766A/G and -112A/C in the promoter region, Ala64Thr in exon 2 and Met299Leu in exon 5 of UCP1 gene are possibly associated with obesity and/or type 2 diabetes mellitus [33].

Evidences suggest that increased expression of UCP1 is associated with a decrease in adiposity, due to the increase in brown adipocyte number [34]. Additionally, UCP1 induces an increase in oxygen consumption and energy expenditure, leading to weight loss. In fact, our study showed that resveratrol produce a decrease in body adiposity, possibly by increasing UCP1 levels in BAT.

Previous studies have found that aside from UCP1, UCP3 levels are also associated with changes in the adiposity index and significantly contribute to thermogenesis and adaptations of energy expenditure [18, 35]. Our study revealed an increase in the ratio of UCP1/UCP3. Queiroz

et al. [18] suggest that physical training increases the ratio of UCP1/UCP3 expression, which might mediate the induction of higher energy efficiency.

Mitochondrial biogenesis in BAT and muscle is controlled, in large part, by the transcriptional coactivator PGC-1 $\alpha$  [36]. PGC-1 $\alpha$ , in turn, is positively regulated by SIRT1 [37]. In our study, we did not find any increases in PGC-1 $\alpha$  expression, however resveratrol induces increase in oxygen consumption. Moreover, Baur et al. [4] suggest that the acetylation status of PGC-1 $\alpha$  in the resveratrol-fed mice was threefold lower than diet-matched controls. These findings were accompanied by increased mitochondrial biogenesis.

A recent report indicates that PTEN overexpression in mice is associated with high levels of UCP1 [38], accompanied by reduced body size, reduced levels of IGF1, improved insulin sensitivity and increased energy expenditure. Moreover, transgenic PTEN mice have an increased energy expenditure, which is associated with lower adiposity and lower body weight despite being hyperphagic [38].

A new study showed that BMP-7 promotes the differentiation of brown preadipocytes [39]. BMP-7 activates a full program of brown adipogenesis, including induction of early regulators of brown fat rate PRDM16 [40] and PGC-1 $\alpha$  (PPAR $\gamma$  coactivator-1 $\alpha$ ) [41], increased expression of brown fat-defining marker UCP1 and adipogenic transcription factor PPAR $\gamma$ , and mitochondrial biogenesis via a p38 MAPK- and PGC-1 $\alpha$ -dependent pathway [39]. Additionally, Schulz et al. showed that genetic ablation of the type 1A BMP receptor (BMP1a) in brown adipogenic progenitor cells leads to a severe paucity of BAT, affecting regulatory mechanism of thermoregulation and energy homeostasis [42].

In addition, it was reported that a gain of function of NAD-dependent deacetylase SIRT1 promotes browning of WAT by deacetylating PPAR- $\gamma$  on Lys268 and Lys293 [10]. Sirt1-dependent deacetylation of Lys268 and Lys293 is required to recruit the BAT program coactivator PRDM16 to PPAR- $\gamma$ , leading to selective induction of BAT genes and repression of visceral WAT genes associated with insulin resistance [10].

Lagouge et al. [13] showed that mice treated with resveratrol associated with high-fat diet present increased energy expenditure and oxygen consumption, mediated by PGC-1 $\alpha$  activity through SIRT1. Recently, another study by Alberti et al. [3] suggests that resveratrol increases UCP1 protein expression in two important thermogenic tissues, BAT and skeletal muscle. Together, these findings confirm our data showing that resveratrol, via activation of SIRT1, can induce thermoregulation and improves metabolic homeostasis. In the present study, we have similar findings to those showed by Lagouge et al. [13] and Alberti



et al. [3]; however, our experiment was performed in mice fed a standard diet. The present data opens a perspective to reduce adiposity in nonobese population.

Taken together, our findings suggest that oral administration of resveratrol increases UCP1 expression by stimulation of SIRT1, which might mediate the induction of higher energy efficiency, improved metabolic parameters and decreased fat mass. The relationship between UCP1 and SIRT1 mRNAs might be responsible for the maintenance of body balance, allowing for changes in body composition, without change in body weight.

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**Conflict of interest** There is no conflict of interest to disclose for any of the authors.

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