# Effect of resveratrol on expression of genes involved thermogenesis in mice and humans

João Marcus Oliveira Andrade[a,c], Antônio Sérgio Barcala-Jorge[a], Gislaine Cândida Batista-Jorge[a], Alanna Fernandes Paraíso[a,c], Kátia Michele de Freitas[b], Deborah de Farias Lelis[a], André Luiz Sena Guimarães[a], Alfredo Maurício Batista de Paula[a], Sérgio Henrique Sousa Santos[a,b,*]

[a] Laboratory of Health Science, Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil
[b] Institute of Agriculture Sciences. Departments of Food Engineering, Universidade Federal de Minas Gerais, Minas Gerais, Brazil
[c] Educational Institute Santo Agostinho, Minas Gerais, Brazil

## ARTICLE INFO

## ABSTRACT

The present study aimed to evaluate the effects of resveratrol on FNDC5 and thermogenesis markers expression in the adipose tissue of mice and humans. Thirty-two male mice were randomly divided into four groups (n = 8) and fed with: Standard Diet; Standard Diet + Resveratrol (400 mg/kg); High-fat Diet; High-fat Diet + Resveratrol for eight weeks. Twenty male and female volunteers, aged 30–55 years, BMI $\geq 30$ kg/m² were divided into two groups and treated for four weeks with 500 mg *trans*-resveratrol or placebo, adipose tissue biopsies were taken. Analysis of body weight, food intake, glycemic and lipid profiles, mRNA expression from tissues and primary culture of adipocytes were performed. The main results show that resveratrol improves the glycaemic and lipid profiles along with an increase in the levels of UCP1, PRDM16, PGC1α, and SIRT1. The increase in FNDC5 expression was observed in the mouse and human subcutaneous adipose tissue. The SIRT1 antagonist in adipocyte primary culture resulted in decreased FNDC5 expression. 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, PRDM16, PGC1α and that increased FNDC5 expression in the subcutaneous adipose tissue from mice and human might be modulated by SIRT1.

## 1. Introduction

In 2012, Boström et al. identified a new hormone secreted by the skeletal muscle - irisin [1]. It was demonstrated that its secretion is dependent on the transcriptional co-activator PGC-1α [1]. The expression of FNDC5 (type I membrane protein), which is cleaved and released into the plasma, is increased by PGC-1α in muscle cells [1]. The secreted portion of FNDC5 (irisin), is recognized by yet undetermined white adipocyte surface receptors. Additionally, irisin induces the expression of uncoupling protein 1 (UCP1) and other brown adipose tissue (BAT)-associated markers of thermogenesis and browning in the adipose tissue [1–3].

Adipose tissues play essential roles in the energy homeostasis and the development of obesity and metabolic syndrome, becoming essential targets in the treatment of obesity and metabolic disorders [4].

Among the key events involved in the progression of the metabolic dysregulation in obese, insulin resistance and dysfunctional lipid storage are noteworthy. Both subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) are associated with metabolic risk factors [5].

Resveratrol (3,5,4'-trihydroxystilbene) a polyphenolic compound present in grapes and red wine induces an increase of the lifespan in many organisms by the improvement of metabolic homeostasis [6]. Resveratrol is a known activator of the sirtuin family (especially sirtuin 1 – SIRT1) [7,8]. Recently, studies have indicated an effect of resveratrol on thermogenesis and the browning process. Andrade et al. [9] reported that resveratrol reduced fat accumulation, increased oxygen consumption and the expression of thermogenesis markers such as UCP1 and BMP7. Subsequently, another study showed that resveratrol increases the level of UCP1 protein expression in two important

* Corresponding author at: Laboratory of Health Science, Universidade Estadual de Montes Claros, Av. Cula Mangabeira 562, 31401-001, Montes Claros, Minas Gerais, Brazil.
*E-mail address:* sergiosousas@ufmg.bra (S.H.S. Santos).

thermogenic tissues (brown adipose tissue and skeletal muscle), suggesting that resveratrol can contribute to increasing whole-body energy expenditure, thus reducing energetic efficiency [10].

Given the FNDC5 expression importance, it is necessary to perform additional studies so information regarding this hormone nature and its effects on energy homeostasis may be elucidated. Resveratrol, a polyphenol with important functions in obesity-related diseases, has emerged as a promising possibility for its role in the thermogenesis and browning activation in the brown and white adipose tissue, respectively.

Thus, our objective was to evaluate the effects of the resveratrol on the expression of FNDC5 in the white and brown adipose tissue of mice fed a high-fat diet and obese humans.

## 2. Materials and methods

### 2.1. Animals

The experiment was conducted with thirty-two male FVB/N mice (four weeks old), an obesity model successfully used in several other studies [9,11,12]. After an adaptation period of 7 days, they were randomly divided into four groups (n = 8) and fed with experimental diets for eight weeks: Standard Diet (ST); Standard Diet plus Resveratrol (ST + RSV); High-Fat Diet (HFD); High-Fat Diet plus Resveratrol (HFD + RSV). All experimental procedures were approved by the Animal Ethics Committee (n°023/2012). Daily dose (concentration of 400 mg/kg) [13–17].

The animals were obtained and maintained in the Universidade Estadual de Montes Claros (Montes Claros, Minas Gerais, Brazil), individually housed and placed in an air-conditioned room (22 ± 2 °C) with a 12 h light-dark cycle. They had free access to food and water during the experimental period. Resveratrol was purchased from Sigma-Aldrich Co. LLC. (Saint Louis, MO, EUA).

### 2.2. Animals diet

High-fat diet was prepared according to the protocols described previously [11,18], being composed of 24.55% carbohydrate, 14.47% protein and 60.98% fat, for a total of 5.28 kcal per 1 g of diet. Standard diet (Purina – Labina®), which was used for the regular maintenance of our mice, is composed of 50.30% carbohydrate, 31.90% protein and 17.80% fat, for a total of 2.18 kcal per 1 g of diet [11]. All of the high-fat diet components were purchased from Rhoster® LTDA (São Paulo, Brazil). The diets composition details are presented in supplementary material (Table S1).

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

The mice were housed, and the food intake was measured daily during the treatment to obtain food efficiency (food intake/body weight). Overnight fasted (12 h) mice were killed after anesthesia with Ketalar® (130 mg/kg – Pfizer Laboratório, Brazil) and Dorcipec® (0.30 mg/kg – Vallé S/A, Brazil) by decapitation. Samples of blood and white adipose tissue (epididymal, retroperitoneal, mesenteric and subcutaneous) and brown adipose tissue (interscapular) were collected, weighed and immediately frozen in dry ice and stored at −80 °C for subsequent analysis.

### 2.4. Human samples

Twenty male and female volunteers, aged 30–55 years, participated. The subjects were divided into two groups as follows: G1: treated for eight weeks with tablets containing 500 mg *trans*-resveratrol (Fluxome Inc., Stenlose, Denmark) [19] daily and G2: treated for eight weeks with placebo tablets daily (1 g/day mineral oil). To finalize the treatment under sterile conditions and using local anesthesia (Xylestesin® 2%,

Cristália, Brazil) adipose tissue biopsies (subcutaneous abdominal fat) were obtained by liposuction, cleaned, and subsequently snap-frozen in liquid nitrogen. A participant surgeon obtained all samples. This study was approved by the Human Ethics Committee from the Universidade Estadual de Montes Claros (n° CAAE 01987912.0.0000.5146). Informed consent was obtained from all included participants.

All participants had BMI ≥ 30 kg/m² considered type 1 obesity but otherwise healthy, were taking no prescriptive medicine, and had no overt endocrine disorders. All participants were practicing physical activity during the experimental period. Eligibility ultimately was based on a normal physical examination including routine clinical biochemical exams. During the trial period, the subjects were instructed to abstain from using nutritional supplements and consuming food suspected to contain resveratrol in significant amounts. Furthermore, the importance of maintaining their normal way of living was underscored.

### 2.5. Insulin sensitivity and glucose tolerance tests

D-Glucose (2 mg/g body weight) was intraperitoneally injected into overnight fasted mice for the glucose tolerance test. Glucose levels from tail blood samples were monitored at 0, 15, 30, 60, and 120 min after injection using an Accu-Check glucometer® (Roche Diagnostics, Indianapolis, USA). An insulin sensitivity test was performed on overnight-fed mice, after intraperitoneal injection of bovine insulin (0.75 units/kg body weight; Sigma-Aldrich®, St. Louis, USA). Blood samples from the tail were taken at 0, 15, 30, and 60 min blood glucose levels measurement.

### 2.6. Determination of biochemical parameters

Serum was obtained after centrifugation ($600 \times g$ for 10 min at 4 °C). Total serum cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides were assayed using enzymatic kits (Wiener®, Argentina). Enzyme-linked immunosorbent assay kits were used to measure serum adiponectin (Adipo-Gen®, Seoul, Korea), and resistin (Lincoln®, St. Louis, USA) levels. Serum insulin was measured by chemiluminescence using a Rat/Mouse Insulin Kit (Millipore®, Billerica, USA) and ADVIA-Centaur equipment.

### 2.7. Reverse transcription and qRT-PCR

Total RNA from epididymal and brown adipose tissue of mice and subcutaneous adipose tissue from humans were 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. The endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (internal control), FNDC5, PGC1α, SIRT1, UCP1, PRDM16 cDNA samples were amplified using specific primers and SYBR Green reagent (Applied Biosystems®, USA) in a PlusOne platform (Applied Biosystems®).

### 2.8. Primary culture of adipocytes

Adipocytes from FVB/N mice (subcutaneous inguinal, visceral epididymal and interscapular brown adipose tissue) and human (subcutaneous white adipose tissue) samples were maintained in primary culture for 3 h in DMEM containing 5 mmol/L glucose, 10% fetal bovine serum, 20 U/mL penicillin, 20 mg/mL streptomycin, and 1% BSA. The cells were incubated under basal conditions or in the presence of 50 μM of Resveratrol (SIRT1 activator), 10 μM of Sirtinol (SIRT1 inhibitor), or both (Resveratrol + Sirtinol) for 12 h. At the end of the incubation period, samples were collected to measure SIRT1, FNDC5, UCP1, FNDC5 and PGC1α mRNA levels of expression [12].
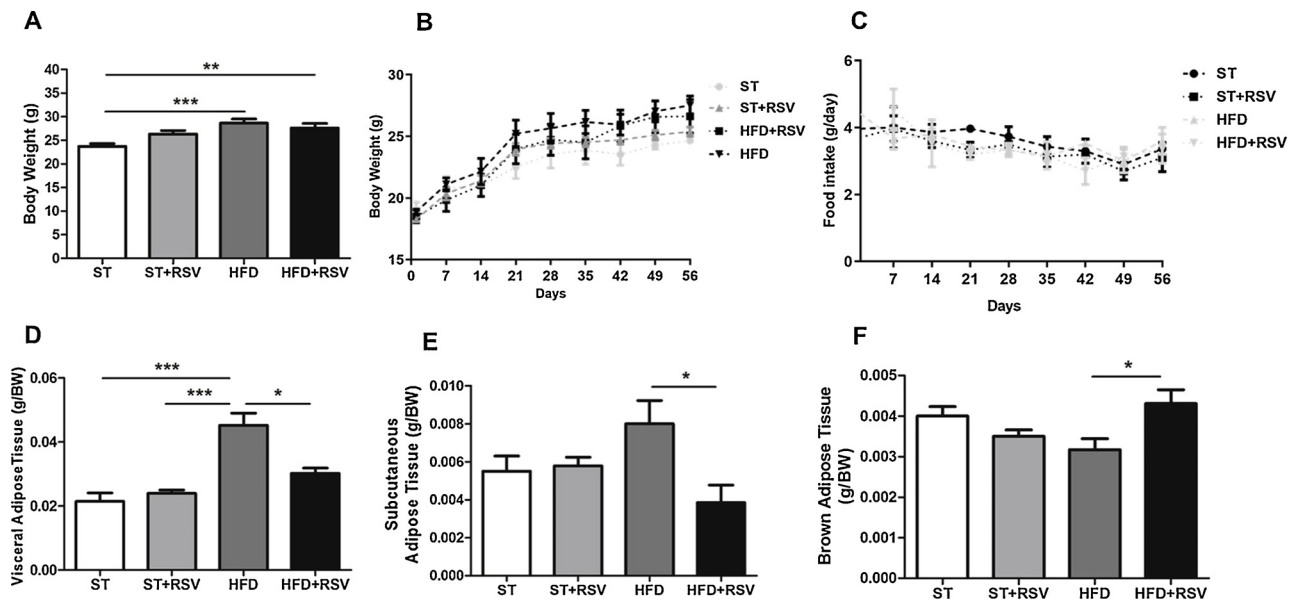
**Fig. 1.** Resveratrol regulate body adiposity. A) Body Weight (g). B) Body weight over time (g). C) Food intake over time (g/day). D) Visceral Adipose Tissue (Ratio of fat weight to body weight) (g/BW). E) Subcutaneous Adipose Tissue (Ratio of fat weight to body weight) (g/BW). F) Brown Adipose Tissue (g/BW). Data are presented as means ± SEM. *P < 0.05, **P < 0.01 and ***P < 0.001.

### 2.9. Statistical analysis

All data were transferred to GraphPad Prism software (Version 5.0®, San Diego, USA) and submitted to specific tests with statistic confidence of 95% (p < 0.05). Data were expressed as the mean ± SEM. *T*-student test, one-way ANOVA or 2-way ANOVA (glucose tolerance and insulin sensitivity tests) and the Bonferroni post-test were applied to assess the statistical significance of differences. For the anthropometric and biochemical data, the Mann–Whitney *U* test was used.

### 3. Results

The results indicated that body weight (BW) was significantly lower in ST as compared to HFD and HFD + RSV groups after eight weeks of high-fat diet feeding (P < 0.001 and P < 0.01, respectively, Fig. 1A and B). The food intake per animal (g/day) among all the groups did not show differences among groups (Fig. 1C). Regarding the body adiposity, a significant decrease in ST, ST + RSV and HFD + RSV group as compared to HFD (Fig. 1D and E) and increased brown adipose tissue mass in HFD + RSV as compared to HFD group (P < 0.05, Fig. 1F) were observed.

Serum lipid measurements from HFD group revealed higher levels of total cholesterol (ST: 102.2 ± 16.35; ST + RSV: 103.5 ± 10.51; HFD: 173.2 ± 28.25; HFD + RSV: 115.8 ± 13.50, *P* = 0.003); and triglycerides (ST: 140.3 ± 19.70; ST + RSV: 139.2 ± 18.97; HFD: 169.8 ± 13.78; HFD + RSV: 127.8 ± 17.59, *P* = 0.019) as compared to ST, ST + RSV and HFD + RSV groups (Fig. 2A and B). Difference on the levels of high-density lipoprotein cholesterol (HDL-C) were not observed among groups (*P* = 0.274) (Fig. 2C).

Serum adiponectin levels were increased in HFD + RSV as opposed to HFD (HFD: 0.575 ± 0.0721 vs. HFD + RSV: 1.201 ± 0.142; *P* = 0.046) (Fig. 2D). The resistin levels were similar among groups (Fig. 2E).

For the glycaemic profile evaluation, intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were performed (Fig. 3A and B). The results showed that HFD + RSV fed mice had decreased levels of glucose and insulin compared to HFD group on both tests. The data were confirmed by the glucose area under curve test. This state was accompanied by a remarkable increase in fasting plasma glucose levels and insulin levels in HFD group as

compared to all groups (Fig. 3C and D).

The anthropometrical and biochemical profiles of the individuals included in the study are detailed in Table 1.

As shown in Fig. 4, the results evidenced increased levels of UCP1 (*P < 0.001*), PGC1α (*P < 0.01*), PRDM16 (*P < 0.01*) and SIRT1 (*P < 0.01*) in HFD + RSV group as compared to HFD (Fig. 4B and E) in mice brown adipose tissue. On the other hand, FNDC5 expression remained unaltered among groups (*P* = 0.260) (Fig. 4A).

In mice subcutaneous adipose tissue, we observed increased levels of UCP1 (*P < 0.05*), PRDM16 (*P < 0.05*) and SIRT1 (*P < 0.01*) in HFD + RSV fed mice as compared to HFD (Fig. 5B, D, and E). PGC1α expression was similar among groups (*P* = 0.538) (Fig. 5C). The FNDC5 expression was decreased in HFD group as compared to ST and HFD + RSV, pointing to an important role of resveratrol on FNDC5 expression modulation (Fig. 5A). Following the subcutaneous adipose tissue analyses, the visceral adipose tissue showed increased levels of PRDM16 (*P < 0.01*) and SIRT1 (*P < 0.05*) in HFD + RSV group as compared to HFD (Fig. 6D and E). The levels of FNDC5, UCP1, and PGC1α, on the other hand, remained similar between HFD + RSV and HFD fed mice (Fig. 6A and C).

Secondly, we analyzed the adaptive thermogenesis markers expressions in subcutaneous adipose tissue from obese volunteers treated for four weeks with tablets containing 500 mg *of trans*-resveratrol. The results displayed increased expression of FNDC5 (*P < 0.01*), UCP1 (*P < 0.01*), PRDM16 (*P < 0.05*) and SIRT1 (*P < 0.05*) were observed in the subcutaneous adipose tissue of OBESE + RSV subjects as compared to obese (Fig. 7A–C, and E). The levels of PGC1α levels remained similar between groups (Fig. 7D).

Considering the described potential effects of resveratrol to stimulate thermogenesis in the adipose tissue and its possible effect on the modulation of FNDC5 via SIRT1, we assessed the levels of thermogenesis markers in primary cell culture. The results indicated that resveratrol might be associated with the activation of FNDC5 and genes associated with the thermogenesis process (Fig. 8A and E).

### 4. Discussion

Recently, Boström et al. [1] published an auspicious mechanism for the induction of the browning process in white adipose tissue following exercise in mice, which counts with increased expression of PGC1α and
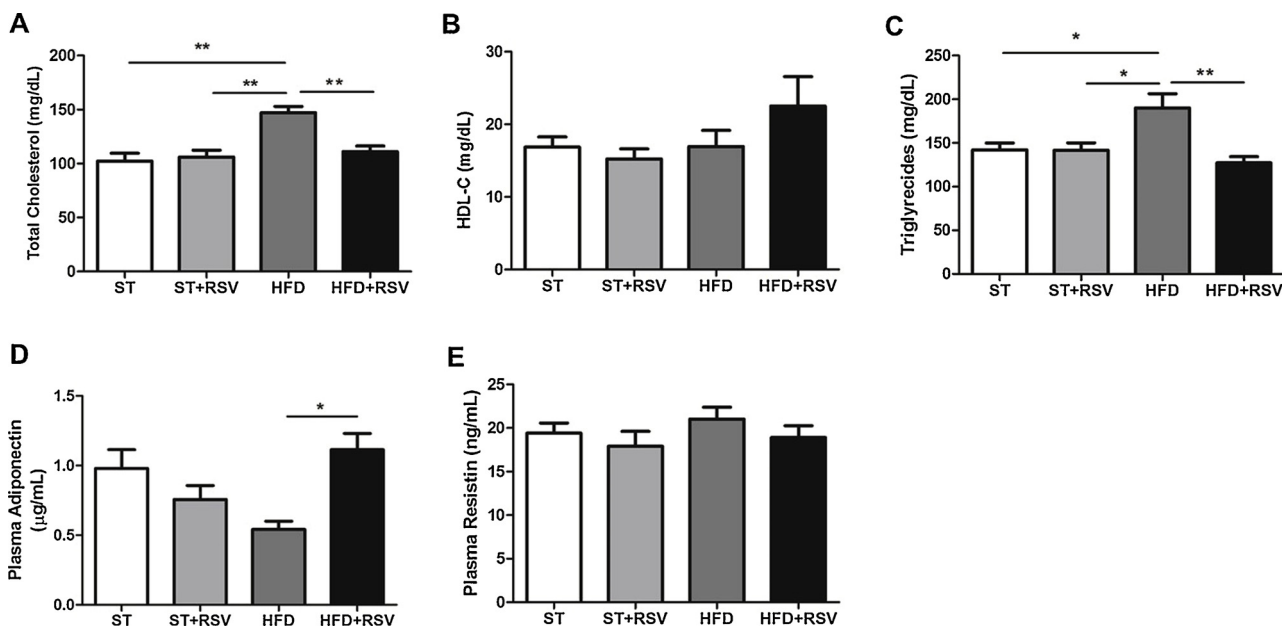
**Fig. 2.** Resveratrol ameliorated lipid plasmatic profile. Serum obtained from the animal's blood was used for the lipid plasmatic profile, assessed by ELISA, using enzymatic kits. A) Total cholesterol (mg/dL). B) HDL-c (mg/dL). C) Triglycerides (mg/dL). D) Plasma levels of adiponectin (μg/mL). E) Plasma levels of resistin (ng/mL). Data are presented as means ± SEM. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

induce the expression of the FNDC5 gene. FNDC5 actions in mice are associated with an increase in the browning of subcutaneous adipocytes, oxygen consumption stimulation, and prevention of weight gain and metabolic dysfunction development induced by diet [1,20].

The discovery of new drivers of the FNDC5 activation is encouraged as it may help in the development of relevant preventive and therapeutic alternatives to the treatment of obesity and its comorbidities. In this context, the resveratrol, which is described in the literature to be involved in the control of energy balance, by modulating food intake, body weight and energy expenditure, deserves attention

[6,10,14,21,22]. Studies have shown that resveratrol protects against metabolic stress, obesity and its comorbidities in mammals, in part, via the activation of sirtuins [23–26]. Interestingly, although the body weight was no different among groups, the visceral adipose tissue weight was decreased in HFD + RSV animals, which may indicate that the resveratrol may modulate not only adiposity but other parameters such as muscle mass, as shown in previous studies [27,28]. The mice in our study presented a small adiposity increment as compared to a study published by Kn et al. [29], which can be justified by the short-treatment period applied in our study (2 months). This finding also
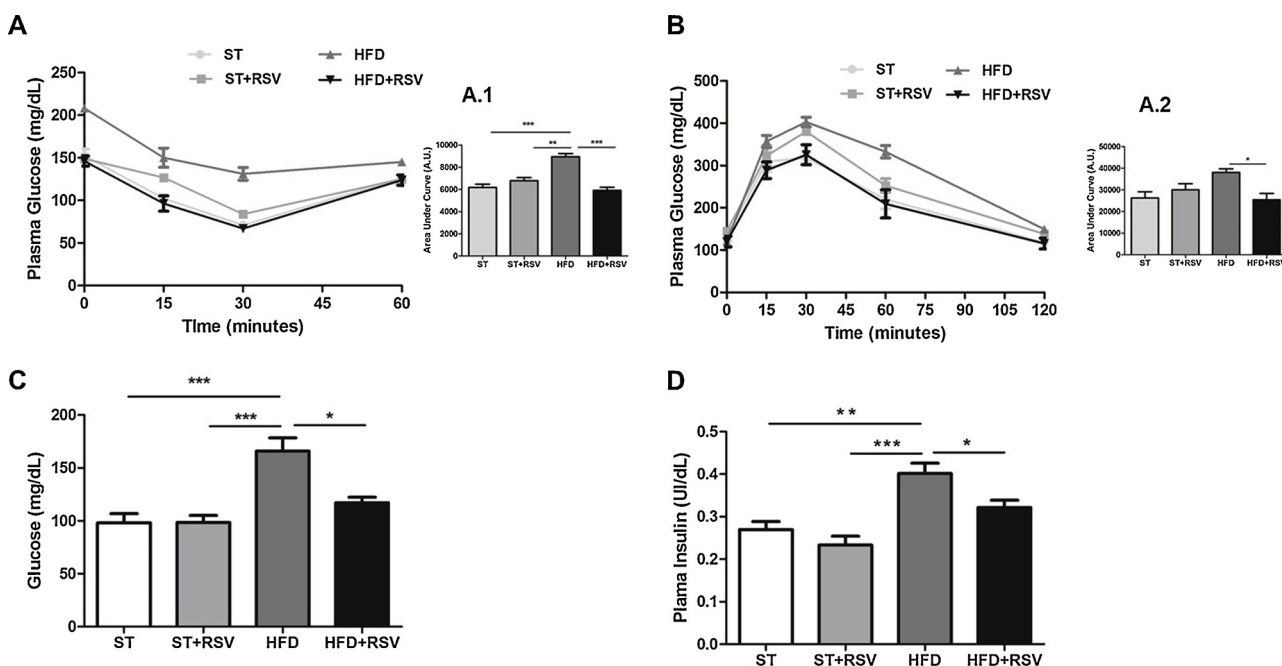


**Fig. 3.** Resveratrol ameliorated glycemic plasmatic profile. Glucose tolerance test was performed in fasted animals after glucose (2 mg/g body weight) injection. Insulin sensibility test was performed in fed animals after administration of insulin (0.75 units/kg body weight; Sigma-Aldrich®, St. Louis, USA). A) Intraperitoneal Insulin Tolerance Test (IPITT) and IPITT glucose area under the curve. B) Intraperitoneal Glucose Tolerance Test (IPGTT) and IPGTT glucose area under the curve. C) Plasma glucose (mg/dL). D) Plasma insulin (UI/L). Data are presented as means ± SEM. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

**Table 1**
Human anthropometrical and biochemical profiles.

| Variables | Placebo | | Resveratrol | | p-value |
|---|---|---|---|---|---|
| | Before | After | Before | After | |
| Body weight | 102.7 ± 10.5 | 95.9 ± 12.1 | 105.4 ± 8.8 | 98.1 ± 11.7 | 0.001 |
| BMI | 35.0 ± 3.9 | 32.8 ± 3.6 | 36.1 ± 2.8 | 33.6 ± 3.5 | 0.003 |
| Total Cholesterol | 218.4 ± 37.3 | 193.7 ± 44.1 | 221.0 ± 38.9 | 192.1 ± 43.9 | 0.031 |
| Triglycerides | 287.4 ± 45.1 | 137.1 ± 47.4 | 294.9 ± 52.8 | 189.9 ± 73.2 | 0.094 |
| Insulin | 51.7 ± 6.1 | 13.9 ± 6.1 | 46.6 ± 7.2 | 15.6 ± 4.1 | 0.154 |
| Glycaemia | 127.1 ± 33.8 | 89.4 ± 5.7 | 138.8 ± 41.0 | 117.1 ± 45.9 | 0.116 |

Body weight (kg); Body Mass Index (BMI) (kg/m$^2$), Total cholesterol (mg/dL), Triglycerides (mg/dL), Insulin (UI/mL), glycaemia (mg/dL). The p values presented on the table correspond to statistics from the comparison among the differences (before and after resveratrol treatment) in both groups (placebo and resveratrol). Statistical significance was set at < 0.05.
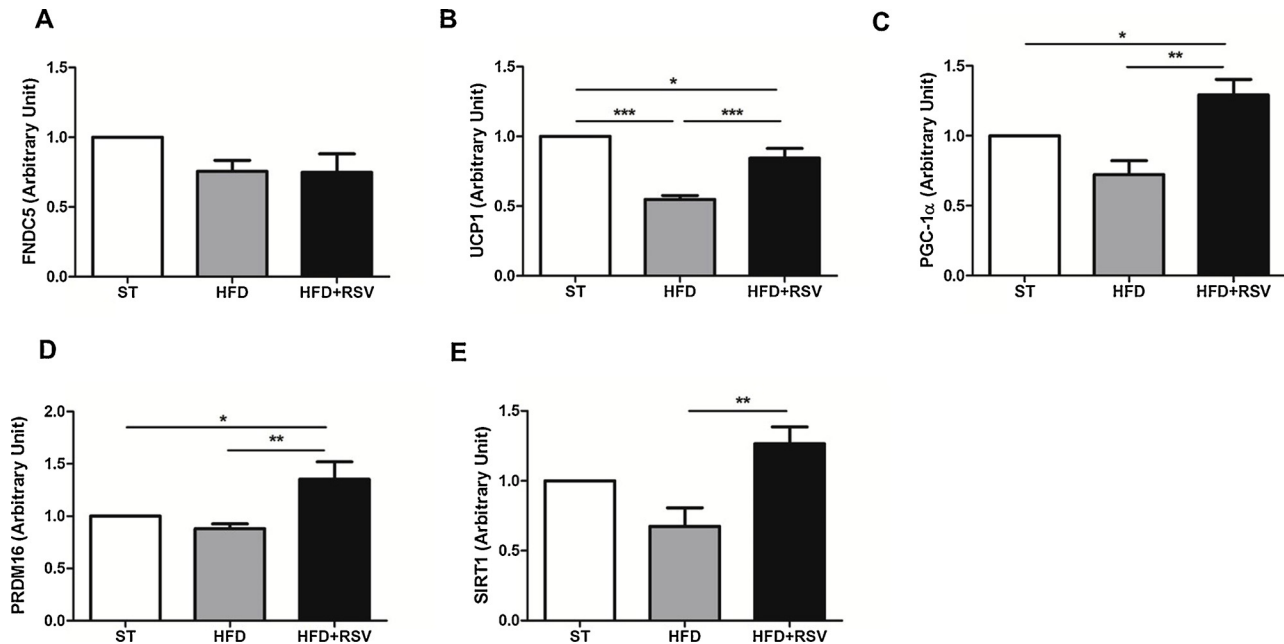


**Fig. 4.** Resveratrol modulates SIRT1 and thermogenesis markers in mouse brown adipose tissue. The mRNA expression was assessed by qRT-PCR with mRNA extracted from the mouse brown adipose tissue. A) FNDC5 mRNA expression. B) UCP1 mRNA expression. C) PGC-1α mRNA expression. D) PRDM16 mRNA expression. E) SIRT1 mRNA expression. Gene expression data were normalized to the expression of GAPDH and gene expression levels in ST group were assumed to be 1. Values are the mean ± SEM (n = 6 per group). Data are presented as means ± SEM. *P < 0.05, **P < 0.01 and ***P < 0.001.

emphasize the hypothesis that the resveratrol acts to improve the metabolism via other tissues, such as liver and skeletal muscle, synergistic to adipose tissue.

Along with the previously mentioned findings, increased adiponectin levels were observed in the HFD + RSV group as compared to HFD, although the same increase was not noticed in ST + RSV mice. A possible explanation for this finding is that several hormones and treatments exert significant effects only when the body homeostasis is brooked. In our model, the HFD-obesity is the altered situation. Adiponectin is a adipokine secreted by adipocytes that exerts several beneficial functions such as antidiabetic, cardioprotective and anti-inflammatory [30–33], thus being recognized as an important target in the treatment of obesity-associated disorders.

For the first time, we show in this study that resveratrol increases FNDC5 expression in the subcutaneous adipose tissue from mice and human, and a modulating candidate molecule is SIRT1.

The white adipose tissue has a significant role in whole-body energy homeostasis [11]. Roca-Rivada et al. [3] showed that the secretion of FNDC5 by the white adipose tissue, triggered by exercise, suggests that FNDC5 is an adipokine-like molecule and exerts critical roles in the adipose tissue [3]. FNDC5 has its mechanisms described in several different organs and tissues, and have been studied in the last few years

[2,3,20,34–37]. In our study, we showed that resveratrol is an essential activator of FNDC5, even at protein level that although no statistically significant differences were observed, a clear tendency could be seen. Furthermore, many other signaling pathways and key molecules activated by resveratrol, which includes PGC1α, MAPK, SIRT1, and UCP1, are critical in FNDC5 activation and thermogenesis activation.

The sirtuin/Sir2 is a family of NAD1-dependent deacetylase and mono-ADP-ribosyltransferase proteins. In mammals, seven sirtuin genes have been identified (SIRT1-7) [38–40]. SIRT1 regulates processes such as glucose homeostasis and insulin production, fat metabolism and cell survival (increase in lifespan). The possible mechanisms associated with the SIRT1 role in several body functions seem to be related to the allosteric role of this gene with NAD + and the acetylated substrate [26]. Interestingly, our findings revealed that the resveratrol treatment improved triglycerides and insulin levels in humans. As all individuals (placebo and intervention) performed the same physical activity program, the exercise interference is diluted in all individuals, remaining to be analyzed the resveratrol effects in the intervention group as compared to placebo. However, the physical activity is indeed only a possible reason for the lower insulin and triglycerides levels observed intra-groups. Moreover, despite the differences between before and after intervention in the placebo and resveratrol groups, statistically
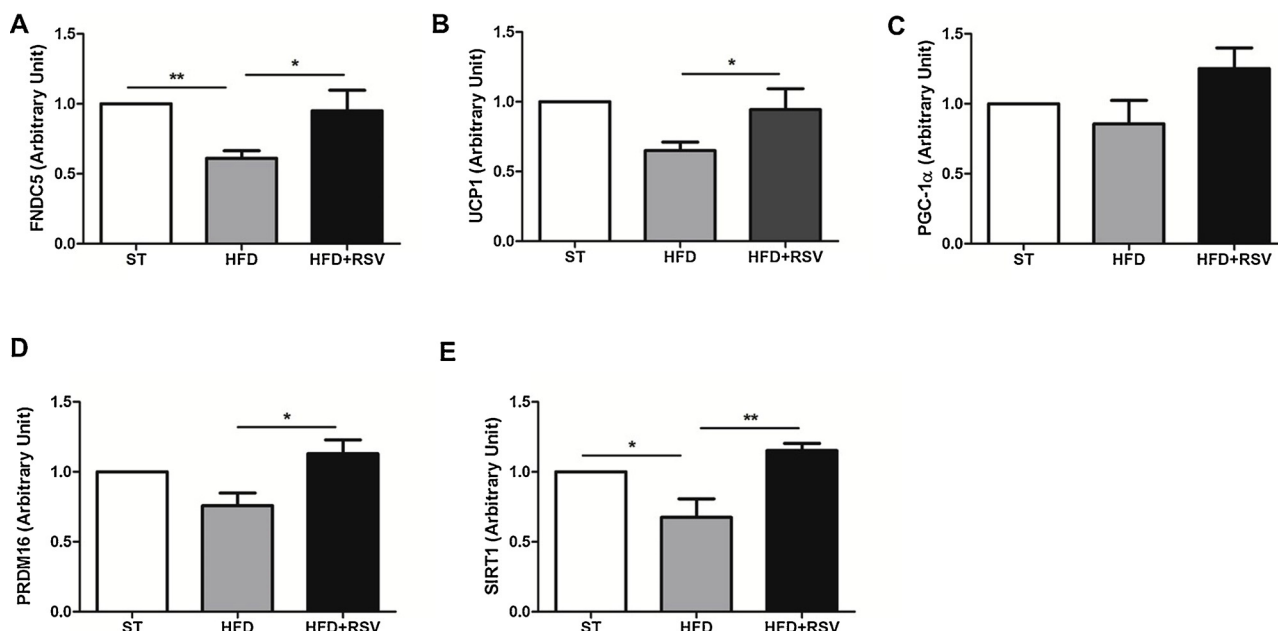
**Fig. 5.** Resveratrol modulates SIRT1 and thermogenesis markers in mouse subcutaneous adipose tissue. The mRNA expression was assessed by qRT-PCR with mRNA extracted from the mouse subcutaneous adipose tissue A) FNDC5 mRNA expression. B) UCP1 mRNA expression. C) PGC-1α mRNA expression. D) PRDM16 mRNA expression. E) SIRT1 mRNA expression. All analysis were made in subcutaneous adipose tissue. Gene expression data were normalized to the expression of GAPDH and gene expression levels in ST group were assumed to be 1. Values are the mean ± SEM (n = 6 per group). Data are presented as means ± SEM. *P < 0.05, **P < 0.01 and ***P < 0.001.

significant differences were not observed.

Qiang et al. [41] showed that the deacetylation of the PPARγ via SIRT1 promotes the browning of subcutaneous WAT by regulating the PPARγ transcriptional complex. As a consequence, increased energy expenditure and improved metabolic profile were observed [10,41] suggested that resveratrol increases UCP1 protein expression in two important thermogenic tissues [10]. Additionally, in another study,

Lagouge et al. [14] showed that mice treated with resveratrol presented an increased energy expenditure and oxygen consumption, an improved mitochondrial function and protection against metabolic disease [14].

Together, these findings corroborate to the hypothesis of this study, by showing that resveratrol induces thermoregulation and improve the metabolism via SIRT1. Andrade et al. concluded that the oral administration of resveratrol, which can improve the metabolism, may be
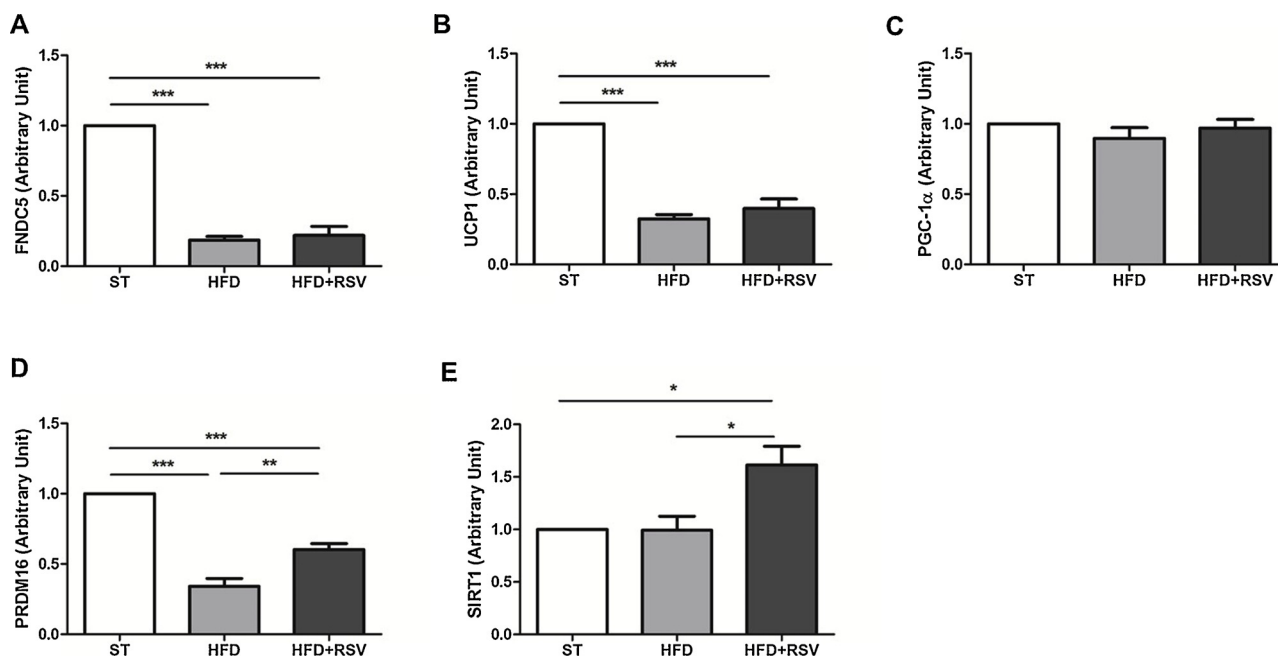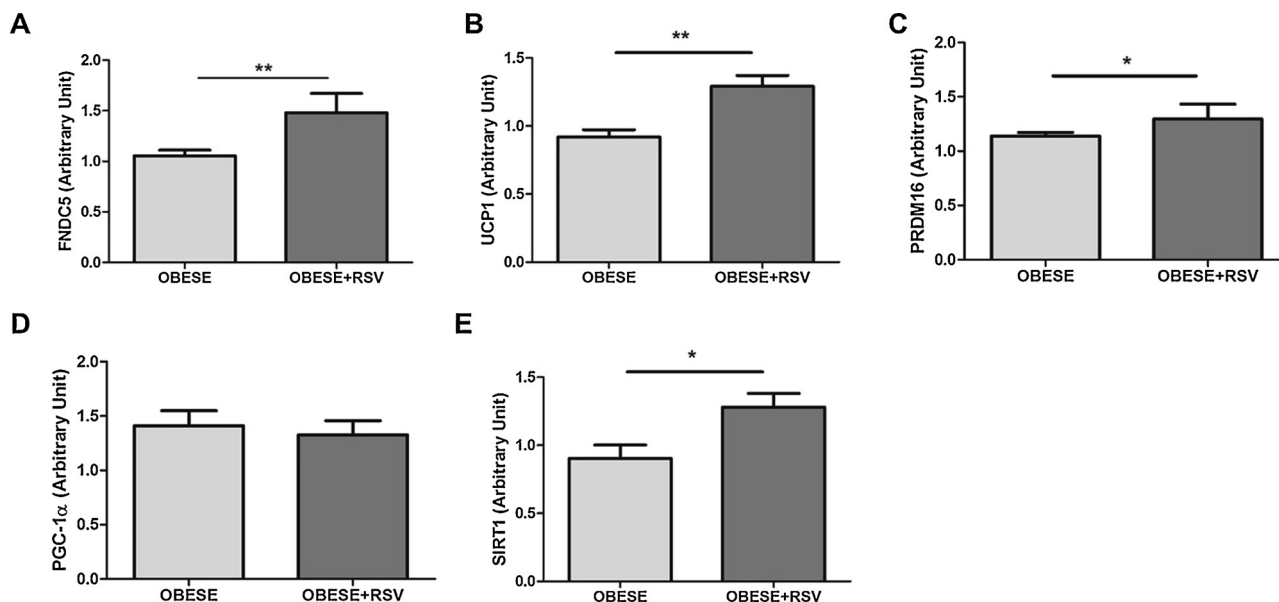


**Fig. 6.** Resveratrol modulates SIRT1 and thermogenesis markers in mouse visceral adipose tissue. The mRNA expression was assessed by qRT-PCR with mRNA extracted from the mouse visceral adipose tissue. A) FNDC5 mRNA expression. B) UCP1 mRNA expression. C) PGC-1α mRNA expression. D) PRDM16 mRNA expression. E) SIRT1 mRNA expression. All analysis were made in visceral adipose tissue. Gene expression data were normalized to the expression of GAPDH and gene expression levels in ST group were assumed to be 1. Values are the mean ± SEM (n = 6 per group). Data are presented as means ± SEM. *P < 0.05, **P < 0.01 and ***P < 0.001.

**Fig. 7.** Resveratrol modulates SIRT1 and thermogenesis markers in human subcutaneous adipose tissue. The mRNA expression was assessed by qRT-PCR with mRNA extracted from the human subcutaneous adipose tissue. A) FNDC5 mRNA expression. B) UCP1 mRNA expression. C) PGC-1α mRNA expression. D) PRDM16 mRNA expression. E) SIRT1 mRNA expression. All analysis were made in visceral adipose tissue. Gene expression data were normalized to the expression of GAPDH and gene expression levels in ST group were assumed to be 1. Values are the mean ± SEM (n = 10 per group). Data are presented as means ± SEM. *P < 0.05, **P < 0.01 and ***P < 0.001.

explained, at least in part, by an increase in the expression of UCP1 and SIRT1, and consequently increased energy expenditure and thermogenesis, thus decreasing fat accumulation in the adipose tissue. Corroborating with this hypothesis, other studies showed that obese mice with SIRT1 deficiency have brown adipose tissue dysfunction [42]. Additionally, it was shown that inflammation downregulates UCP1 in brown adipose tissue and can be restored via activation of SIRT1 and resveratrol [43]. Um et al., on the other hand, attributed the
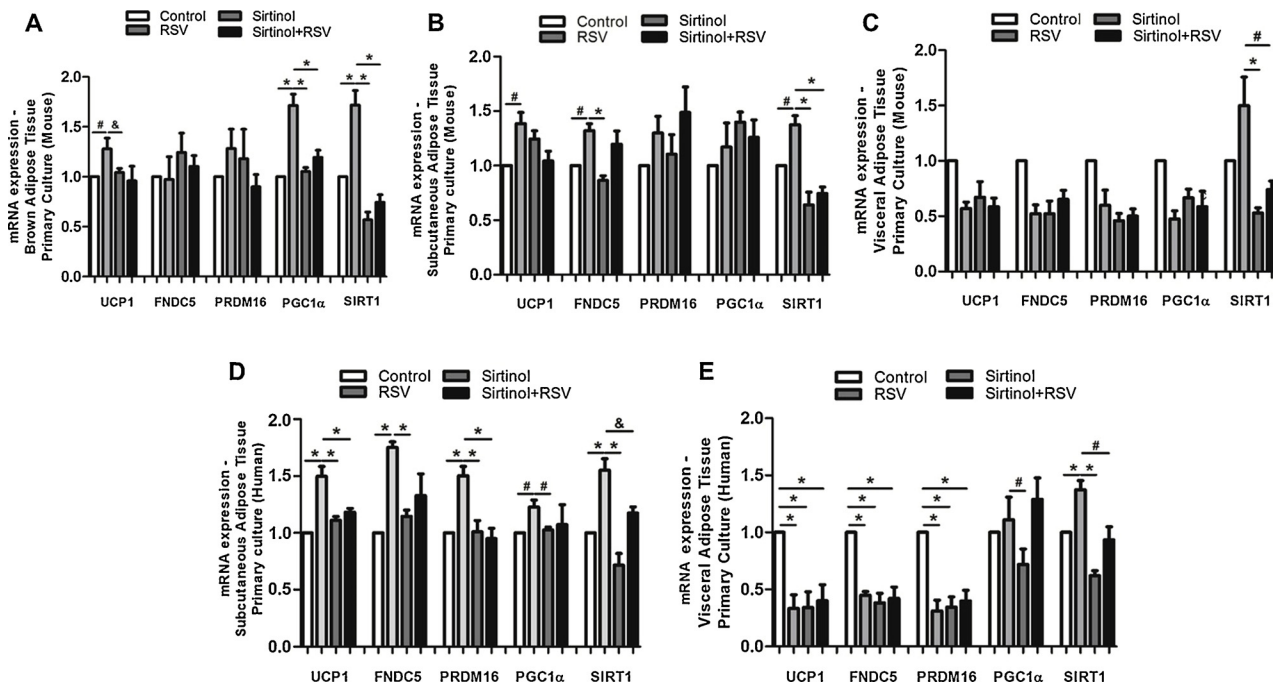


**Fig. 8.** Mouse and human primary cell culture adipocytes treated with agonist and antagonist from SIRT1. The mRNA expression was assessed by qRT-PCR with mRNA extracted from mouse and human primary cell culture adipocytes. A) UCP1, FNDC5, PRDM16, PGC1a and SIRT1 mRNA expression in mouse primary culture of brown adipose tissue. B) UCP1, FNDC5, PRDM16, PGC1a and SIRT1 mRNA expression in mouse primary culture of subcutaneous adipose tissue. C) UCP1, FNDC5, PRDM16, PGC1a and SIRT1 mRNA expression in mouse primary culture of visceral adipose tissue. D) UCP1, FNDC5, PRDM16, PGC1a and SIRT1 mRNA expression in the human primary culture of subcutaneous adipose tissue. Gene expression data were normalized to the expression of GAPDH and gene expression levels in control group were assumed to be 1. Cell culture conditions: media (DMEM containing 5 mmol/L glucose, 10% fetal bovine serum, 20 U/mL penicillin, 20 mg/mL streptomycin, and 1% BSA), followed by incubation under basal conditions or in the presence of 50 μM of Resveratrol (SIRT1 activator), 10 μM of Sirtinol (SIRT1 inhibitor), or both (Resveratrol + Sirtinol) for 12 h. Values are the mean ± SEM (n = 4 per group). Data are presented as means ± SEM. *P < 0.05, **P < 0.01 and ***P < 0.001.

resveratrol effects on the metabolism to the activation of AMPK pathways. The authors argued that the enzyme AMPK affects SIRT1 expression, in multiple ways, and can modulate PGC1a expression, thus acting similarly to the role given to SIRT1 in our study [13]. Since AMPK expression and activity was not measured in our study, it is not possible to discuss its role in the FNDC5 activation by resveratrol, but further studies are encouraged to investigate this other possible pathway by which resveratrol may act on metabolism.

PRDM16 is a gene, found in both mouse and human, and is highly expressed in the brown adipose tissue [44,45]. Several studies suggest that PRDM16 may act mainly through the modulation of transcription factors, such as PGC1α [46,47]. Seale et al. demonstrated that decreased expression of PRDM16 diminishes the thermogenic characteristics usually present in brown adipocytes [44,48].

Additionally, it is well described in the literature, that different mechanisms may induce the thermogenic process. The primary mechanism that triggers this process seems to be the increase in the expression of UCP1. Also, the UCP1 is believed to be responsible for the increase of thermogenesis and browning in the adipose tissue.

In our study, the treatment with resveratrol-induced an increase in UCP1 expression in BAT and subcutaneous WAT. Alberdi et al. [10] found similar data, showing that resveratrol induces a significant increase in SIRT1 expression in BAT and in PGC1α, which is a potent inducer of mitochondrial biogenesis, an important part of the thermogenic program. Increased PGC1α expression activates the UCP1 gene.

In conclusion, the main findings of the present study show that resveratrol induced significant ameliorations in the metabolic profile and increased thermogenesis markers and FNDC5 in the subcutaneous adipose tissue of the mice and human. Moreover, SIRT1 might be an important marker involved in the association between FNDC5 and Thermogenesis.

## Declarations of interest

None.

## Funding

## Appendix A. Supplementary data

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## References

[1] P. Bostrom, J. Wu, M.P. Jedrychowski, A. Korde, L. Ye, J.C. Lo, K.A. Rasbach, E.A. Bostrom, J.H. Choi, J.Z. Long, S. Kajimura, M.C. Zingaretti, B.F. Vind, H. Tu, S. Cinti, K. Hojlund, S.P. Gygi, B.M. Spiegelman, A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis, Nature 481 (7382) (2012) 463–468.

[2] J.I. Castillo-Quan, From white to brown fat through the PGC-1alpha-dependent myokine irisin: implications for diabetes and obesity, Dis. Model. Mech. 5 (3) (2012) 293–295.

[3] A. Roca-Rivada, C. Castelao, L.L. Senin, M.O. Landrove, J. Baltar, A. Belen Crujeiras, L.M. Seoane, F.F. Casanueva, M. Pardo, FNDC5/irisin is not only a myokine but also an adipokine, PLoS One 8 (4) (2013) e60563.

[4] S.H. Santos, J.M. Andrade, Angiotensin 1-7: a peptide for preventing and treating metabolic syndrome, Peptides 59 (2014) 34–41.

[5] S. Baglioni, G. Cantini, G. Poli, M. Francalanci, R. Squecco, A. Di Franco, E. Borgogni, S. Frontera, G. Nesi, F. Liotta, M. Lucchese, G. Perigli, F. Francini, G. Forti, M. Serio, M. Luconi, Functional differences in visceral and subcutaneous fat pads originate from differences in the adipose stem cell, PLoS One 7 (5) (2012) e36569.

[6] J.A. Baur, K.J. Pearson, N.L. Price, H.A. Jamieson, C. Lerin, A. Kalra, V.V. Prabhu, J.S. Allard, G. Lopez-Lluch, K. Lewis, P.J. Pistell, S. Poosala, K.G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K.W. Fishbein, R.G. Spencer, E.G. Lakatta, D. Le Couteur, R.J. Shaw, P. Navas, P. Puigserver, D.K. Ingram, R. de Cabo, D.A. Sinclair, Resveratrol improves health and survival of mice on a high-calorie diet, Nature 444 (7117) (2006) 337–342.

[7] R.H. Houtkooper, E. Pirinen, J. Auwerx, Sirtuins as regulators of metabolism and healthspan, Nat. Rev. Mol. Cell Biol. 13 (4) (2012) 225–238.

[8] A. Satoh, L. Stein, S. Imai, The role of mammalian sirtuins in the regulation of metabolism, aging, and longevity, Handb. Exp. Pharmacol. 206 (2011) 125–162.

[9] J.M. Andrade, A.C. Frade, J.B. Guimaraes, K.M. Freitas, M.T. Lopes, A.L. Guimaraes, A.M. de Paula, C.C. Coimbra, S.H. Santos, 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, Eur. J. Nutr. 53 (7) (2014) 1503–1510.

[10] G. Alberdi, V.M. Rodriguez, J. Miranda, M.T. Macarulla, I. Churruca, M.P. Portillo, Thermogenesis is involved in the body-fat lowering effects of resveratrol in rats, Food Chem. 141 (2) (2013) 1530–1535.

[11] L. de Pinho, J.M. Andrade, A. Paraiso, A.B. Filho, J.D. Feltenberger, A.L. Guimaraes, A.M. de Paula, A.P. Caldeira, A.C. de Carvalho Botelho, M.J. Campagnole-Santos, S.H. Sousa Santos, Diet composition modulates expression of sirtuins and renin-angiotensin system components in adipose tissue, Obesity 21 (9) (2013) 1830–1835.

[12] J.M. Oliveira Andrade, A.F. Paraiso, Z.M. Garcia, A.V. Ferreira, R.D. Sinisterra, F.B. Sousa, A.L. Guimaraes, A.M. de Paula, M.J. Campagnole-Santos, R.A. dos Santos, S.H. Santos, Cross talk between angiotensin-(1-7)/Mas axis and sirtuins in adipose tissue and metabolism of high-fat feed mice, Peptides 55 (2014) 158–165.

[13] J.H. Um, S.J. Park, H. Kang, S. Yang, M. Foretz, M.W. McBurney, M.K. Kim, B. Viollet, J.H. Chung, AMP-activated protein kinase-deficient mice are resistant to the metabolic effects of resveratrol, Diabetes 59 (3) (2010) 554–563.

[14] M. Lagouge, C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeq, J. Milne, P. Lambert, P. Elliott, B. Geny, M. Laakso, P. Puigserver, J. Auwerx, Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha, Cell 127 (6) (2006) 1109–1122.

[15] C. Moussa, M. Hebron, X. Huang, J. Ahn, R.A. Rissman, P.S. Aisen, R.S. Turner, Resveratrol regulates neuro-inflammation and induces adaptive immunity in Alzheimer's disease, J. Neuroinflammation 14 (1) (2017) 1.

[16] S. Bo, G. Ciccone, A. Castiglione, R. Gambino, F. De Michieli, P. Villois, M. Durazzo, P. Cavallo-Perin, M. Cassader, Anti-inflammatory and antioxidant effects of resveratrol in healthy smokers a randomized, double-blind, placebo-controlled, crossover trial, Curr. Med. Chem. 20 (10) (2013) 1323–1331.

[17] R.S. Turner, R.G. Thomas, S. Craft, C.H. van Dyck, J. Mintzer, B.A. Reynolds, J.B. Brewer, R.A. Rissman, R. Raman, P.S. Aisen, Alzheimer's Disease Cooperative Study, A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease, Neurology 85 (16) (2015) 1383–1391.

[18] J.D. Feltenberger, J.M. Andrade, A. Paraiso, L.O. Barros, A.B. Filho, R.D. Sinisterra, F.B. Sousa, A.L. Guimaraes, A.M. de Paula, M.J. Campagnole-Santos, M. Qureshi, R.A. dos Santos, S.H. Santos, Oral formulation of angiotensin-(1-7) improves lipid metabolism and prevents high-fat diet-induced hepatic steatosis and inflammation in mice, Hypertension 62 (2) (2013) 324–330.

[19] M.M. Poulsen, P.F. Vestergaard, B.F. Clasen, Y. Radko, L.P. Christensen, H. Stodkilde-Jorgensen, N. Moller, N. Jessen, S.B. Pedersen, J.O. Jorgensen, High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition, Diabetes 62 (4) (2013) 1186–1195.

[20] S. Raschke, M. Elsen, H. Gassenhuber, M. Sommerfeld, U. Schwahn, B. Brockmann, R. Jung, U. Wisloff, A.E. Tjonna, T. Raastad, J. Hallen, F. Norheim, C.A. Drevon, T. Romacho, K. Eckardt, J. Eckel, Evidence against a beneficial effect of irisin in humans, PLoS One 8 (9) (2013) e73680.

[21] Y. Jimenez-Gomez, J.A. Mattison, K.J. Pearson, A. Martin-Montalvo, H.H. Palacios, A.M. Sossong, T.M. Ward, C.M. Younts, K. Lewis, J.S. Allard, D.L. Longo, J.P. Belman, M.M. Malagon, P. Navas, M. Sanghvi, R. Moaddel, E.M. Tilmont, R.L. Herbert, C.H. Morrell, J.M. Egan, J.A. Baur, L. Ferrucci, J.S. Bogan, M. Bernier, R. de Cabo, Resveratrol improves adipose insulin signaling and reduces the inflammatory response in adipose tissue of rhesus monkeys on high-fat, high-sugar diet, Cell Metab. 18 (4) (2013) 533–545.

[22] J.L. Fiori, Y.K. Shin, W. Kim, S.M. Krzysik-Walker, I. Gonzalez-Mariscal, O.D. Carlson, M. Sanghvi, R. Moaddel, K. Farhang, S.K. Gadkaree, M.E. Doyle, K.J. Pearson, J.A. Mattison, R. de Cabo, J.M. Egan, Resveratrol prevents beta-cell dedifferentiation in nonhuman primates given a high-fat/high-sugar diet, Diabetes 62 (10) (2013) 3500–3513.

[23] M. Kaeberlein, T. McDonagh, B. Heltweg, J. Hixon, E.A. Westman, S.D. Caldwell, A. Napper, R. Curtis, P.S. DiStefano, S. Fields, A. Bedalov, B.K. Kennedy, Substrate-specific activation of sirtuins by resveratrol, J. Biol. Chem. 280 (17) (2005) 17038–17045.

[24] K.T. Howitz, K.J. Bitterman, H.Y. Cohen, D.W. Lamming, S. Lavu, J.G. Wood, R.E. Zipkin, P. Chung, A. Kisielewski, L.L. Zhang, B. Scherer, D.A. Sinclair, Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan, Nature 425 (6954) (2003) 191–196.

[25] H. Dai, L. Kustigian, D. Carney, A. Case, T. Considine, B.P. Hubbard, R.B. Perni, T.V. Riera, B. Szczepankiewicz, G.P. Vlasuk, R.L. Stein, SIRT1 activation by small molecules: kinetic and biophysical evidence for direct interaction of enzyme and activator, J. Biol. Chem. 285 (43) (2010) 32695–32703.

[26] M.T. Borra, B.C. Smith, J.M. Denu, Mechanism of human SIRT1 activation by resveratrol, J. Biol. Chem. 280 (17) (2005) 17187–17195.

[27] B.T. Bennett, J.S. Mohamed, S.E. Alway, Effects of resveratrol on the recovery of muscle mass following disuse in the plantaris muscle of aged rats, PLoS One 8 (12)

(2013) e83518.

[28] J. Sun, C. Zhang, M. Kim, Y. Su, L. Qin, J. Dong, Y. Zhou, S. Ding, Early potential effects of resveratrol supplementation on skeletal muscle adaptation involved in exercise-induced weight loss in obese mice, BMB Rep. 51 (4) (2018) 200–205.

[29] B.P. Kn, V. Gopalan, S.S. Lee, S.S. Velan, Quantification of abdominal fat depots in rats and mice during obesity and weight loss interventions, PLoS One 9 (10) (2014) e108979.

[30] E. Nigro, O. Scudiero, M.L. Monaco, A. Palmieri, G. Mazzarella, C. Costagliola, A. Bianco, A. Daniele, New insight into adiponectin role in obesity and obesity-related diseases, Biomed Res. Int. 2014 (2014) 658913.

[31] P.A. Kern, G.B. Di Gregorio, T. Lu, N. Rassouli, G. Ranganathan, Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression, Diabetes 52 (7) (2003) 1779–1785.

[32] B. Lee, J. Shao, Adiponectin and energy homeostasis, Rev. Endocr. Metab. Disord. 15 (2) (2014) 149–156.

[33] K. Ohashi, N. Ouchi, Y. Matsuzawa, Anti-inflammatory and anti-atherogenic properties of adiponectin, Biochimie 94 (10) (2012) 2137–2142.

[34] T. Kurdiova, M. Balaz, M. Vician, D. Maderova, M. Vlcek, L. Valkovic, M. Srbecky, R. Imrich, O. Kyselovicova, V. Belan, I. Jelok, C. Wolfrum, I. Klimes, M. Krssak, E. Zemkova, D. Gasperikova, J. Ukropec, B. Ukropcova, Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies, J. Physiol. 592 (5) (2014) 1091–1107.

[35] F. Norheim, T.M. Langleite, M. Hjorth, T. Holen, A. Kielland, H.K. Stadheim, H.L. Gulseth, K.I. Birkeland, J. Jensen, C.A. Drevon, The effects of acute and chronic exercise on PGC-1alpha, irisin and browning of subcutaneous adipose tissue in humans, FEBS J. 281 (3) (2014) 739–749.

[36] I. Gouni-Berthold, H.K. Berthold, J.Y. Huh, R. Berman, N. Spenrath, W. Krone, C.S. Mantzoros, Effects of lipid-lowering drugs on irisin in human subjects in vivo and in human skeletal muscle cells ex vivo, PLoS One 8 (9) (2013) e72858.

[37] M.T. Vamvini, K.N. Aronis, G. Panagiotou, J.Y. Huh, J.P. Chamberland, M.T. Brinkoetter, M. Petrou, C.A. Christophi, S.N. Kales, D.C. Christiani, C.S. Mantzoros, Irisin mRNA and circulating levels in relation to other myokines in healthy and morbidly obese humans, Eur. J. Endocrinol. 169 (6) (2013) 829–834.

[38] S. Kyrylenko, A. Baniahmad, Sirtuin family: a link to metabolic signaling and senescence, Curr. Med. Chem. 17 (26) (2010) 2921–2932.

[39] T. Finkel, C.X. Deng, R. Mostoslavsky, Recent progress in the biology and physiology of sirtuins, Nature 460 (7255) (2009) 587–591.

[40] V.D. Longo, B.K. Kennedy, Sirtuins in aging and age-related disease, Cell 126 (2) (2006) 257–268.

[41] L. Qiang, L. Wang, N. Kon, W. Zhao, S. Lee, Y. Zhang, M. Rosenbaum, Y. Zhao, W. Gu, S.R. Farmer, D. Accili, Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of Ppargamma, Cell 150 (3) (2012) 620–632.

[42] X.B. Zheng, H.Y. Ai, S.H. Yuan, H.Y. Cao, H. Liang, J.P. Weng, F. Xu, Effect of SIRT1 deficiency on function of brown adipose tissue in obese mice, Zhonghua yi xue za zhi 96 (23) (2016) 1859–1862.

[43] M.K. Nohr, N. Bobba, B. Richelsen, S. Lund, S.B. Pedersen, Inflammation downregulates UCP1 expression in brown adipocytes potentially via SIRT1 and DBC1 interaction, Int. J. Mol. Sci. 18 (5) (2017).

[44] P. Seale, B. Bjork, W. Yang, S. Kajimura, S. Chin, S. Kuang, A. Scime, S. Devarakonda, H.M. Conroe, H. Erdjument-Bromage, P. Tempst, M.A. Rudnicki, D.R. Beier, B.M. Spiegelman, PRDM16 controls a brown fat/skeletal muscle switch, Nature 454 (7207) (2008) 961–967.

[45] P. Lee, J.T. Zhao, M.M. Swarbrick, G. Gracie, R. Bova, J.R. Greenfield, J. Freund, K.K. Ho, High prevalence of brown adipose tissue in adult humans, J. Clin. Endocrinol. Metab. 96 (8) (2011) 2450–2455.

[46] S. Kajimura, P. Seale, K. Kubota, E. Lunsford, J.V. Frangioni, S.P. Gygi, B.M. Spiegelman, Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex, Nature 460 (7259) (2009) 1154–1158.

[47] E. Hondares, M. Rosell, J. Diaz-Delfin, Y. Olmos, M. Monsalve, R. Iglesias, F. Villarroya, M. Giralt, Peroxisome proliferator-activated receptor alpha (PPARalpha) induces PPARgamma coactivator 1alpha (PGC-1alpha) gene expression and contributes to thermogenic activation of brown fat: involvement of PRDM16, J. Biol. Chem. 286 (50) (2011) 43112–43122.

[48] P. Seale, S. Kajimura, W. Yang, S. Chin, L.M. Rohas, M. Uldry, G. Tavernier, D. Langin, B.M. Spiegelman, Transcriptional control of brown fat determination by PRDM16, Cell Metab. 6 (1) (2007) 38–54.