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Original Article

Altered levels of sirtuin genes *(SIRT1, SIRT2, SIRT3* and *SIRT6)* and their target genes in adipose tissue from individual with obesity

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ABSTRACT

Introduction: Sirtuins regulate energy metabolism and insulin sensitivity through their ability to act as energy sensors and regulators in several metabolic tissues.
Aim: To evaluate the expression levels of sirtuin genes *SIRT1*, *SIRT2*, *SIRT3* and *SIRT6* and their target genes (*PPAR-α*, *PGC1-α*, *NRF1*, *DGAT1, PPAR-γ* and *FOXO3a*) in subcutaneous adipose tissue collected from individuals with normoweight, overweight and obesity.
Methods: Adipose tissue samples, obtained by lipoaspiration during liposuction surgery, were processed to obtain RNA, which was reverse-transcribed to cDNA. Then, we measured the expression levels of each gene by qPCR.
Results: We found differences in the mRNA expression of *SIRT1*, *SIRT2*, *SIRT3* and *SIRT6* and their target genes (*PPAR-α*, *PGC1-α*, *NRF1*, *DGAT1, PPAR-γ* and *FOXO3a*) in adipose tissue from overweight or obese subjects when compared to normoweight subjects. All genes analyzed, except *SIRT2,* showed correlation with BMI.
Conclusions: Our findings in human subcutaneous adipose tissue show that increased body mass index modifies the expression of genes encoding sirtuins and their target genes, which are metabolic regulators of adipose tissue. Therefore, these could be used as biomarkers to predict the ability of adipose tissue to gain mass of adipose tissue.

1. Introduction

The adipocytic growth characteristic of obesity is regulated by several control mechanisms at the cellular and molecular levels. Among them, the genes encoding sirtuins (*SIRT*) are important regulators of adipose tissue metabolism [1]. In mammals, sirtuins comprise seven proteins, each with different localizations and functions, which can be classified into four classes based on phylogenetic analysis: *SIRT1*, *2*, and *3* belong to class I; *SIRT4* to class II; *SIRT5* to class III; and *SIRT6* and *7* to class IV [2]. Sirtuins are characterized by NAD^+-dependent deacetylase activity [3]. In fact, while other classes of histone deacetylases (HDACs) use the Zn^{2+} active site and bind a water molecule on acetylated lysines, sirtuins transfer the acetyl group from the lysine side chain of a substrate to a NAD^+ cofactor, generating nicotinamide, 2′-*O*-acetyl-ADP-ribose and a deacetylated substrate [4].

In recent years, several experimental studies have suggested that sirtuins regulate energy metabolism and insulin sensitivity through their ability to act as energy sensors and regulators in several metabolic tissues [5]. In terms of lipid metabolism, *SIRT1* and *SIRT3* are the most studied and characterized among the members of the sirtuin family. In contrast, little is known about *SIRT2*, *SIRT4* and *SIRT6*, and *SIRT5* and *SIRT7* are thought to play a minor role in lipid metabolic homeostasis [1]. In the current study,

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we focused on analyzing the expression of *SIRT1, 2, 3* and *6* in human subcutaneous adipose tissue, some associated target genes, as well as their relationship to clinical or antropometric parameters.

Sirtuin 1 can regulate the activation of *PPAR-α* through deacetylation of *PGC-1α*, leading to increased oxidation of fatty acids [6]. Studies have shown that SIRT1 functions to protect against high-fat diet-induced metabolic damage and diabetes [7]. SIRT1 differentially affects FOXO3 function, potentiating some process and attenuating others in the presence of stress stimuli [8]. Also, FOXO3a can regulate *SIRT1* transcription and increase *SIRT1* expression through interaction with the p53 promoter [9]. Evaluation of *SIRT2* activity in adipose tissue *in vivo* has revealed a role in the inhibition of adipogenesis and accumulation of lipids in 3T3-L1 adipocytes [10] by deacetylating *FOXO1* [11].

In contrast, *SIRT3* is the main regulator of the deacetylation of mitochondrial proteins [12] and it may change under caloric restriction, suggesting that modulation of its levels may be associated with certain metabolic diseases [13]. *SIRT6* has been demonstrated to be associated with adipose metabolism, as a deficiency in this protein reduces the expression of genes related to β-oxidation and increases the expression of genes required for triglyceride synthesis, such as diacylglycerol acyltransferase 1 (DGAT1) [14]. Also, *SIRT6* expression is regulated by *NRF1,* a transcription factor that is regulated by caloric restriction [15].

Weight reduction in subjects undergoing gastric reduction surgery leads to a significant increase in *SIRT1, 3* and 6 mRNA levels in the subcutaneous adipose tissue [16]. However, the relationship between these sirtuin family members and their main target genes in subcutaneous adipose tissue has been poorly explored. Therefore, the aim of this study was to evaluate the expression levels of sirtuin genes *SIRT1*, *SIRT2*, *SIRT3* and *SIRT6* and their target genes (*PPAR-α*, *PGC1-α*, *NRF1*, *DGAT1, PPAR-γ* and *FOXO3a*) in subcutaneous adipose tissue collected from individuals with normoweight, overweight and obesity.

2. Methods

2.1. Selection of patients

The group of individuals included 50 females, divided according to their body mass index (BMI) into obese (BMI ≥ 30), overweight (BMI > 25) and normal-weight (BMI < 25) groups. No individual had any major medical comorbidities. The mean age of the group was 39.5 years (range 21–63 years). Biochemical parameters, including glucose and triglycerides, were measured in the three groups (Table 1). Adipose tissue was collected from lipoaspirate of the abdomen and adjacent areas of adult individuals, performed by plastic surgeons. The study was approved by the Bioethical Committee of the Autonomous University of San Luis Potosi (CEID2013006) and all participants signed a written informed consent form.

2.2. Adipose tissue samples

Samples from human adipose tissue were washed with sterile phosphate-buffered saline (PBS) and centrifuged to eliminate any other surgical solutions. Adipose tissue samples were digested with 0.03% type I collagenase (Invitrogen) in Dulbecco's Modified Eagle's Medium (DMEM; GiBCO) and 2.0% bovine serum albumin (Sigma-Aldrich) for 60 min at 37 °C with gentle agitation. The collagenase was neutralized using an equal volume of complete cell culture medium consisting of DMEM (Invitrogen), 10% fetal bovine serum (FBS; Sigma-Aldrich) and 1% penicillin/streptomycin (ThermoFisher Scientific). Approximately 1–5 g of collagenase-digested adipose tissue was used for RNA extraction.

Table 1
Anthropometric and clinical parameters of individuals.

Parameters	Normoweight	Overweight	Obesity	p
Total of subjects	24	22	4	
Age (years)	38.04 ± 2.03	40.72 ± 1.74	41.75 ± 4.49	p = 0.5382
Weight (Kg)	60.48 ± 1.30	72.86 ± 1.80	90.28 ± 3.19	**p < 0.001
Height (m)	1.62 ± 0.01	1.64 ± 0.02	1.65 ± 0.03	p = 0.5980
BMI (Kg/m^2)	23.09 ± 0.27	27.15 ± 0.26	33.30 ± 1.34	**p < 0.001
Glucose (mg/dL)	92.25 ± 1.65	92.64 ± 2.61	89.50 ± 0.50	p = 0.8944

2.3. Total RNA extraction

Total RNA was isolated using the Trizol method (AMBION, Life Technologies, Grand Island, NY, USA). During the lysis of adipose tissue the adipocytes release oil, thus we placed tubes at room temperature for 15 min and removed the upper layer of oil and any cell debris. Next, we followed the normal RNA extraction procedure. All RNA extracted from tissue was verified in 1% agarose gels to determine the integrity of the sample through visualization of both bands of 18S and 26S rRNA, then the absorbance of all samples was measured at 260 and 280 nm giving values between 1.8 and 2.0.

2.4. Real-time q-PCR assays

We used 5–10 mg of total RNA to obtain cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, San Francisco, CA, USA). The cDNA was used for the measurement of *SIRT1, SIRT2, SIRT3 and SIRT6* mRNA expression, as well as target genes regulated by sirtuins (*PPAR-α*, *PGC1-α*, *NRF1*, *DGAT1, PPAR-γ* and *FOXO3a*), using specific primers (Table 2) by qRT-PCR and SybrGreen (BIO-RAD Laboratories, Hercules, CA, USA) in a RT thermocycler CFX96™ Real Time System (BIO-RAD). The results obtained were analyzed with the $2^{-\Delta\Delta Cq}$ method. The *GAPDH* gene was used as an internal control to normalize the gene expression of sirtuins and target genes (ΔCq).

3. Statistical analysis

Data are presented as the arithmetic mean, with each value representing the relative mRNA expression of each gene in the adipose tissue. Groups of data were statistically analyzed using GraphPad Prism v.5 software (GraphPad Software Inc., San Diego, CA, USA). For analysis of parametric data, one-way ANOVA was performed. For non-parametric data, a Mann-Whitney *U* test and Spearman correlation were used. A p-value of <0.05 was considered significant.

4. Results

4.1. Individuals characteristics

Adipose tissue samples were collected from 50 subjects who were divided according to BMI into normal weight (BMI < 25 kg/m^2), overweight (BMI > 25 kg/m^2) or obese (BMI ≥ 30 kg/m^2) groups. Among the parameters evaluated, we only observed significant differences in antropometric parameters including weight (kg) and BMI between the three groups of studied subjects (Table 1).

4.2. Sirtuin mRNA expression in human subcutaneous adipose tissue

We measured the relative mRNA expression of *SIRT1*, *SIRT2*, *SIRT3* and *SIRT6* in adipose tissue collected from normoweight,

Table 2
Primer sequences for real time q-PCR. List of genes and primer sequences used in the real-time PCR assay.

qPCR genes and primer sequences		
Gene name	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
SIRT1	CAGTTGGAAGATGGCGGACG	ATCACCGAACAGAAGGTTATCTCG
SIRT2	TGGGTGTTGTACGAAAGC	ACTGCTCTGTCCTGTCACCGACT
SIRT3	TGTACAGCAACCTCCAGCAG	CTCCTTGGCCAAAGTGAAAA
SIRT6	GCAGTCTTCCAGTGTGGTGTT	GCTCTCAAAGGTGGTGTC
PPAR-a	ATTCGCCATGCTGTCTTC	ATCACAGAACGGTTTCCTTAG
PGC1-a	GATGGAGACAGCTATGGTTTC	AAGTCAGTTTCGTTTGACCTG
PPAR-γ	GCTGGCCTCCTTGATGAATA	GCTGGCCTCCTTGATGAATA
FOXO3a	ATTCTGTCAGCAACATGGG	AGAGTCCGAGAGGGTTTG
NRF1	CCGAACATATGGCTACCATAG	CGTAAGAGGTGTCCTCGG
DGTA1	ACCATCCAGAACTCCATGA	GAAGAAGATGAGCCAGATGAG
GAPDH	CCCTTCATTGACCTCAACTAC	GACAAGCTTCCCGTTCTC

overweight and obese subjects. The relative mRNA expression levels of *SIRT1* in overweight and obese subjects were lower than in normoweight subjects ($p < 0.0001$; Fig. 1A). In a similar way, *SIRT3* and *SIRT6* mRNA levels were diminished in overweight subjects compared to normoweight subjects ($p < 0.0001$; Fig. 1C and D). No differences in *SIRT1* and *SIRT6* levels were observed between individual with obesity and overweight subjects (Fig. 1A and D). Interestingly, we found that the obese group had the highest expression of *SIRT2* while overweight subjects showed the lowest expression level compared to the other two groups ($p < 0.0001$; Fig. 1B).

4.3. Correlation between sirtuin expression and body mass index

We then performed a correlation analysis between the relative expression level of each sirtuin gene and subject according to BMI. Negative correlations of BMI with the expression levels of *SIRT1* (Fig. 2A), *SIRT3* (Fig. 2C) and *SIRT6* (Fig. 2D) genes were detected. We did not observe a correlation between *SIRT2* and BMI (Fig. 2B).

4.4. Expression of sirtuins and target genes

Of the target genes reported for each sirtuin gene, we chose to assess the expression of the main target genes regulated by sirtuins in adipose tissue. We evaluated the relative mRNA expression levels of *PPAR-γ*, *PGC1-α*, *PPAR-α*, *NRF1*, *DGAT1* and *FOXO3a* in collagenase-digested subcutaneous adipose tissue by real-time q-PCR. The relative mRNA expression levels of *PPAR-γ* (Fig. 3A) and *PCG1-α* (Fig. 3B) in individuals with overweight or obesity were higher than in normoweight subjects. However, *PCG1-α* showed the highest relative mRNA expression level in individuals with obesity (Fig. 3B), while the highest expression level of *PPAR-γ* was observed in the overweight group (Fig. 3A). Similar to *PCG1-α*, the expression of *PPAR-α* (Fig. 4A), *FOXO3a* (Fig. 4B) and *NRF1* (Fig. 5A) was highest in

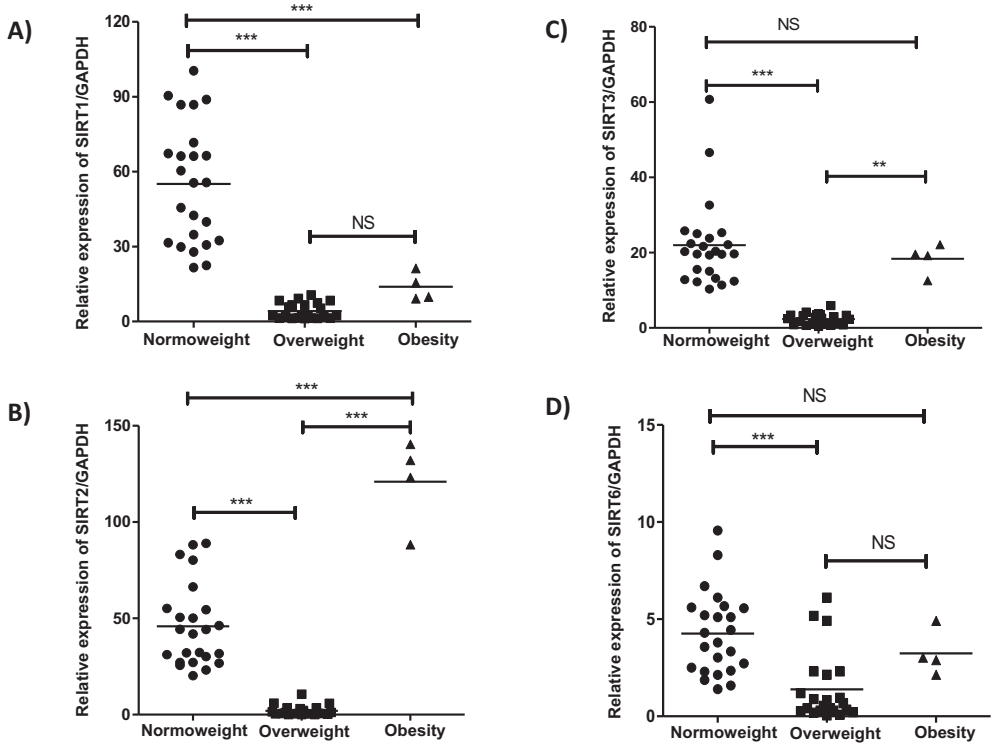

Fig. 1. Relative mRNA expression of *SIRT1, SIRT2, SIRT3* and *SIRT6* in human subcutaneous adipose tissue. Real-time q-PCR was performed on cDNA from subcutaneous adipose tissue samples collected by lipoaspiration in liposuction surgeries from normoweight, overweight and obese subjects. Relative mRNA expression levels of **(A)** *SIRT1,* **(B)** *SIRT2,* **(C)** *SIRT3* and **(D)** *SIRT6* genes in adipose tissue samples from normoweight, overweight and obese subjects. ***$p < 0.0001$; **$p < 0.001$; NS, non-significant.

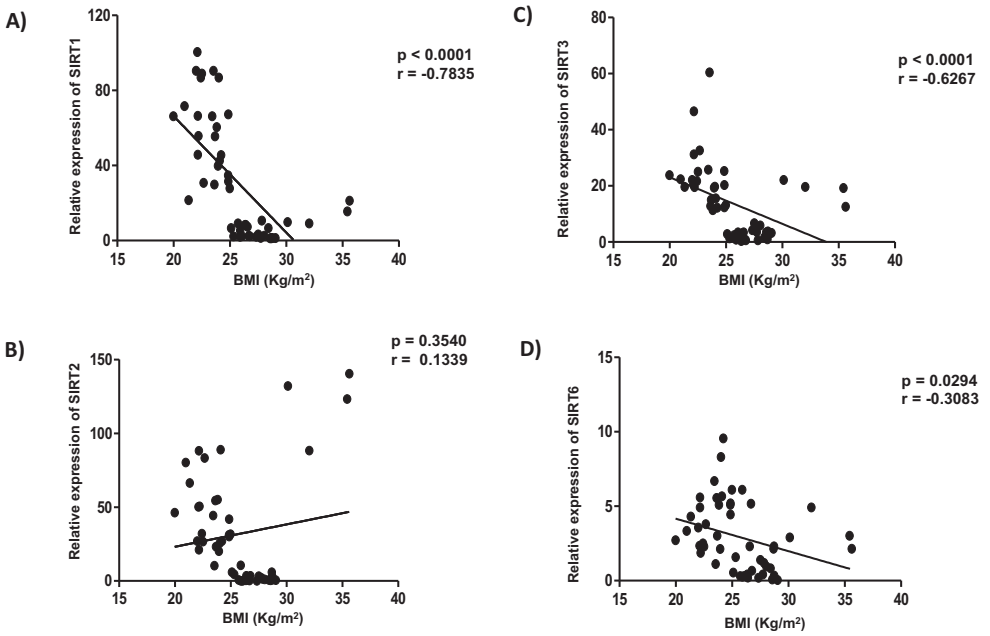

Fig. 2. Correlation between relative sirtuin mRNA expression levels and body mass index (BMI). Analysis of correlation between expression of sirtuins **(A)** *SIRT1*, **(B)** *SIRT2*, **(C)** *SIRT3* and **(D)** *SIRT6* with BMI.

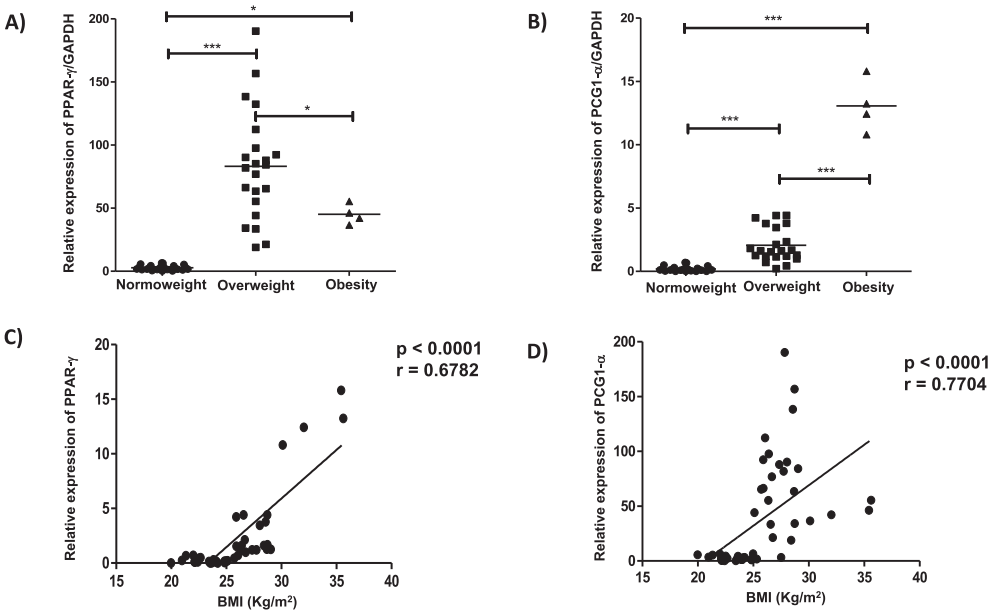

Fig. 3. Relative mRNA expression levels of sirtuin target genes regulated by *SIRT1* in subcutaneous adipose tissue. Relative mRNA expression levels of **(A)** *PPAR-γ* and **(B)** *PGC1-α*, both target genes regulated by *SIRT1*, in subcutaneous adipose tissue samples obtained by lipoaspiration from normoweight, overweight and obese subjects. *p < 0.01; ***p < 0.0001; Correlation analysis between body mass index (BMI) and relative mRNA expression of **(C)** *PPAR-γ* and **(D)** *PGC1-α*.

the obese group. In contrast, *DGAT1*, a target gene of *SIRT6*, showed a different expression profile in the studied subjects, with overweight or obese subjects presenting lower relative mRNA expression compared to normoweight subjects (Fig. 5B).

Next, we explored whether the relative expression of these genes was related to the changes in sirtuin expression. To achieve this, correlation tests between sirtuins and the evaluated targets genes were performed (Table 3). We found a negative correlation between *SIRT1* and the target genes *PPARγ* ($r = -0.74$, $p \leq 0.0001$), *PGC1-α* ($r = -0.69$, $p \leq 0.0001$) and *FOXO3a* ($r = -0.56$, $p \leq 0.0001$). In contrast, *NRF1* was positively associated with *SIRT3* expression ($r = 0.67$, $p \leq 0.0001$). Meanwhile, *SIRT6* showed a positive correlation with *DGAT1* ($r = 0.51$, $p \leq 0.0001$) and a negative correlation with *PPARα* ($r = -0.47$, $p = 0.001$). Finally, it is worth mentioning that we found a strong correlation between the mRNA expression levels of *NRF1* and *SIRT2* ($r = 0.8$, $p \leq 0.0001$), despite there being no reported association between these genes.

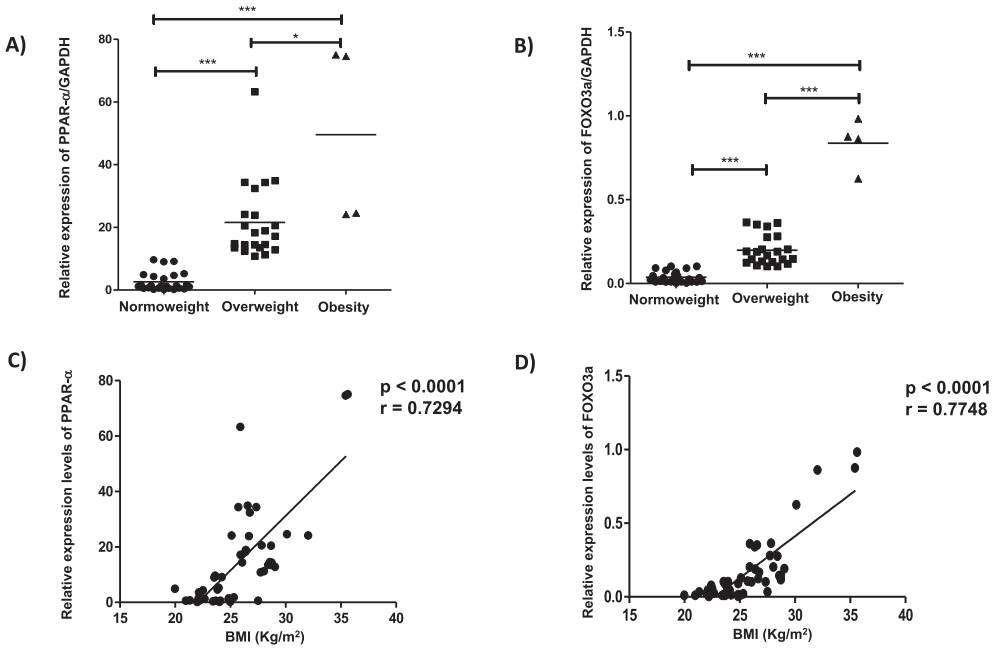

Fig. 4. Relative mRNA expression levels of sirtuin target genes regulated by *SIRT1* and *SIRT6* in subcutaneous adipose tissue. Relative mRNA expression levels of **(A)** *PPAR-α* and **(B)** *FOXO3a*, both target genes regulated by *SIRT1* and *SIRT6*, on collagenase-digested subcutaneous adipose tissue samples obtained by lipoaspiration from normoweight, overweight and obese subjects. *p < 0.01; ***p < 0.0001; Correlation analysis between body mass index (BMI) and relative mRNA expression of **(C)** *PPAR-α* and **(D)** *FOXO3a*.

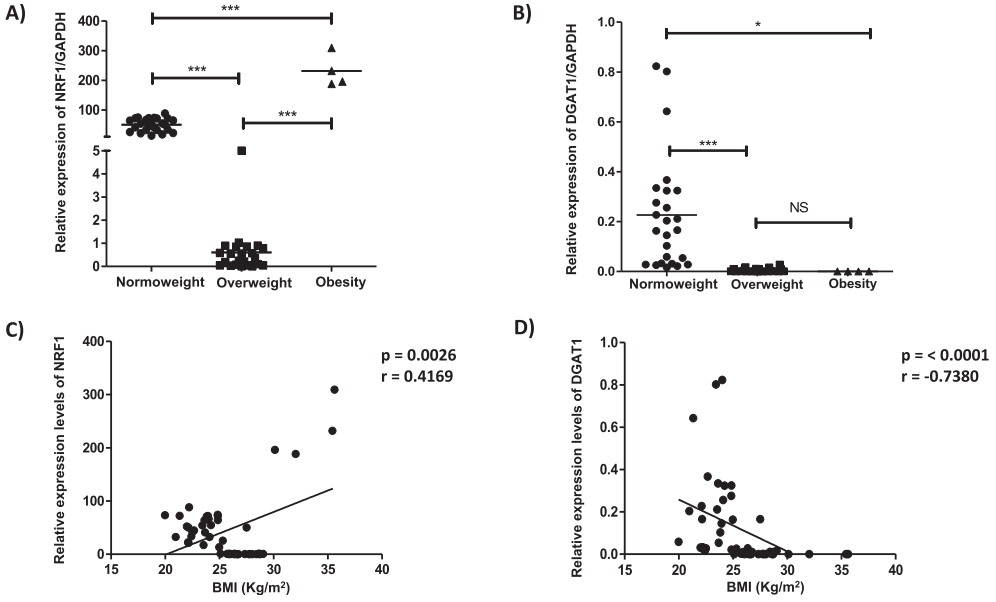

Fig. 5. Relative mRNA expression levels of sirtuin target genes regulated by *SIRT3* and *SIRT6* in subcutaneous adipose tissue. Relative mRNA expression of **(A)** *NRF1*, a target gene regulated by *SIRT3*, and relative mRNA expression of **(B)** *DGAT1*, a target gene regulated by *SIRT6*, on collagenase-digested subcutaneous adipose tissue samples obtained by lipoaspiration from normoweight, overweight and obese subjects. *p < 0.01; ***p < 0.0001; NS, non-significant. Correlation analysis between body mass index (BMI) and relative mRNA expression of **(C)** *NRF1* and **(D)** *DGAT1*.

4.5. Association between expression of sirtuin target genes and body mass index

Finally, we performed a correlation analysis between the relative expression levels of sirtuin targets and subject BMI. We found a strong positive correlation between the relative mRNA expression levels of *PPAR-γ* (Fig. 3C), *PGC1-α* (Fig. 3D), *FOXO3a* (Fig. 4D) and *PPAR-α* (Fig. 4C) with BMI. Additionally, *NRF1* (Fig. 5C) showed a positive correlation with BMI. In contrast, studied subjects showed a significant decrease in the relative mRNA expression levels of the *DGAT1* gene, with a negative correlation observed between *DGAT1* expression and BMI (Fig. 5D).

5. Discussion

Considerable research has focused on the function of sirtuins. In

Table 3
Correlations between expression of sirtuins and their target genes in subcutaneous adipose tissue.

Correlations of subcutaneous adipose tissue *SIRTs* expression		
	r	p value
SIRT1 and *PPARγ*	−0.74	$p \leq 0.0001$
SIRT1 and *PGC1-α*	−0.69	$p \leq 0.0001$
SIRT1 and *FOXO3a*	−0.56	$p \leq 0.0001$
SIRT2 and *NRF1*	0.8	$p \leq 0.0001$
SIRT3 and *NRF1*	0.67	$p \leq 0.0001$
SIRT6 and *DGTA1*	0.51	$p \leq 0.0001$

relation to metabolic health, studies have revealed that the activity of sirtuins influence cellular metabolism, and the factors that influence metabolic health, such as caloric restriction, physical activity or altered sirtuin function [5]. In this way, altered function of certain sirtuins may lead to the dysregulation of target genes underlying the metabolic deficiencies associated with certain metabolic diseases, such as obesity. In this study, we evaluated the expression levels of the sirtuin genes *SIRT1, SIRT2, SIRT3* and *SIRT6*, along with their target genes.

Sirtuins, especially *SIRT1*, play a role in the regulation of several metabolic and inflammatory pathways, and this has been well studied [16]. *SIRT1* has been discovered to be a regulator of adipose tissue inflammation and macrophage infiltration [17]. Furthermore, in agreement with our results, previous studies have shown that lower levels of *SIRT1* expression are present in the adipose tissue of individuals with obesity, and that these levels are correlated with BMI [18]. These factors contribute to a state of chronic inflammation, which exacerbates the negative conditions associated with obesity [19]. It has been shown that consumption of a high-fat diet can reduce *SIRT1* levels in white adipose tissue through inflammasome mediated caspase 1, contributing to the metabolic dysfunction induced by this diet among other factors [20].

In relation to *SIRT1* targets, we found that *PPARγ, PGC1-α* and *FOXO3a* mRNA levels were increased in the overweight and obese groups compared with the normoweight group, and the expression levels of these target genes were strongly correlated with BMI. *PPARγ* is a well-characterized regulator of energy metabolism; however, the connection between *PPARγ* mRNA expression and obesity is not clear. Furthermore, previous studies have shown conflicting results. Some reports have described no difference in *PPARγ* expression in subcutaneous adipose tissue between obese and non-obese subjects [21,22], whereas others have shown differences [29–33]. In particular, Bortolotto et al. and Redonnet et al. reported that *PPARγ* expression is increased in the subcutaneous adipose tissue of individuals with obesity [23,24], while Vidal-Puig et al. and Sewter et al. reported that only *PPARγ2* expression is increased in obese versus lean subjects [25,26]. In contrast, Sewter et al., Rodríguez-Acebes and Leyvraz found a decrease in *PPARγ1* mRNA expression in subcutaneous adipocytes [26–28]. Here, we found that *PPARγ* expression is increased in the adipose tissue of overweight and obese subjects, and this was related to diminished *SIRT1* expression. The negative correlation between *SIRT1* and *PPARγ* is consistent with the capacity of *SIRT1* to repress *PPARγ* function. The observation that adipose tissue from overweight and obese humans display increased expression of *PPARγ* mRNA proportional to BMI could be explained by the expansion in adipose tissue mass of these patients.

Similar to the *PPARγ* data, studies focused on *PGC1-α* in adipose tissue from human subjects have been, thus far, conflicting [29,30]. The first report of *PGC1-α* in adipose tissue found no difference between patients with obesity and lean controls [29]. In contrast, Semple et al. reported that *PGC1-α* mRNA levels in subcutaneous fat are three-fold lower in morbidly obese than in slim subjects. However, it is important to consider the type of obesity according to BMI [29]. On the other hand, mitochondrial biogenesis is regulated by both *SIRT1* and *SIRT3* through a process involving the transcriptional coactivator *PGC1-α* [31]. While *SIRT1* activates *PGC1-α* by direct deacetylation, *SIRT3* promotes *PGC1-α* expression [32]. In this regard, compared with overweight subjects, obese group showed elevated *PGC1-α* expression coinciding with an increase in *SIRT3* expression, which could be related. However, low levels of *SIRT1* could indicate low PGC1-α deacetylation, and consequently, lower activation, as *PGC1-α* levels were found to be inversely correlated with *SIRT1* mRNA.

FOXO3a is activated during exposure to high glucose, resulting in caspase activation with subsequent apoptotic cell death [33]. To our knowledge, this is the first report of *FOXO3a* levels in the adipose tissue of individuals with obesity, although several studies have shown increased levels in different tissues. FOXO3a protein expression has been reported to be upregulated in hearts of rats with high-fat diet-induced obesity, which suggests that *FOXO3a* may be involved in high-fat diet-induced tissue injury [34]. Additionally, higher levels of *FOXO3a* phosphorylation in the colon of mice fed a high-fat diet through PI3K/Akt pathway has been reported. This induces NF-kB activation, suggesting that Akt-*FOXO3* signaling could be associated with intestinal inflammation and its related to metabolic disorders [35]. *SIRT1* can differentially affect *FOXO3a* function, as *SIRT1* and the transcription factor *FOXO3a* form a complex when cells are exposed to oxidative stress. Our results showed an increase in expression of the *FOXO3a* gene but diminished *SIRT1* levels in subcutaneous adipose tissue. In this regard, we can hypothesize that in obesity, *FOXO3a* levels are regulated independently of *SIRT1*. In support of this, *FOXO3a* mRNA levels have previously reported to be increased more than two-fold in the adipose tissue of *PPARγ*-deficient mice with no change in *SIRT1* mRNA levels [36].

SIRT2 is the most abundantly expressed member of the sirtuin family in adipose tissue [10]. It has been reported that *SIRT2* is downregulated in visceral adipose tissue from individuals with obesity in a Hif1α-dependent way [8]. However, the effects of Hif1α on lipid catabolism are depot-specific and affect visceral but not subcutaneous fat. In our expression assays, we found that the *SIRT2* gene is downregulated in subcutaneous adipose tissue of overweight subjects when compared to normoweight subjects, which is in agreement with the results of a study by Krishnan et al. The group with obesity in our study showed higher *SIRT2* expression compared to overweight or normoweight subjects. As previously mentioned, subcutaneous and visceral adipocytes are regulated in different ways [45], and this is the first report of *SIRT2* levels in subcutaneous fat. In this regard, we hypothesize that diminished *SIRT2* levels at an early stage of overweight are the result of a high-energy state, as it has been demonstrated that *SIRT2* expression is regulated in response to changes in cellular energy status [37]. However, the implications of the increased *SIRT2* mRNA in obesity are unknown.

SIRT3 is a mitochondrial matrix protein that regulates the

acetylation status of multiple targets [38]. Here, we measured *NRF1 gen* which encoded Nrf1 protein and belongs to the transcriptional activator family, and is a target of *SIRT3*. Similar to the targets of the other sirtuins, *NRF1* has been reported to be diminished in the subcutaneous adipose tissue of morbidly obese individuals [39]. Our results showed that both *SIRT3* and *NRF1* levels behave similarly in the subcutaneous adipose tissue of normoweight and overweight groups, in agreement with previous results. However, the subcutaneous adipose tissue of individuals with obesity was found to have higher levels of *NRF1* mRNA compared to the expression levels in normoweight or overweight subjects, and *NRF1* showed a positive correlation with increasing BMI. Additionally, up-regulation of *NRF1* in the obese group is supported by an increase in *PGC1-α* expression in the same group, as *NRF1* transcription is regulated by *PGC1-α* [40]. It is necessary to further study the consequences of these results. Nevertheless, van Tienen et al. found that *NRF1* overexpression in adipocytes could induce inflammation in these cells through an adipokine expression profile, leading to insulin-resistant adipocytes [41]. In this way, elevated levels of *NRF1* in obesity may be implicated in the inflammatory processes characteristic of this condition. The strong correlation between the mRNA levels of *NRF1* and *SIRT2* should be explored in the future.

Finally, we measured *SIRT6* expression levels and the expression of two of its targets: *DGAT1* and *PPAR-α*. Recent reports have shown that fat-specific *SIRT6* knockout promoted high-fat diet-induced obesity in mice, and it is also associated with increased inflammation in the adipose tissue. The same authors found that Sirt6 protein levels are significantly diminished in the abdominal adipose tissue of obese group with a BMI greater than 27 [42] similar to our results in subcutaneous adipose tissue. Furthermore, it has been reported that overexpression of *SIRT6* resulted in an increase in *SIRT6* binding to the *DGAT1* promoter, causing a reduction in *DGAT1* transcription levels [43]. Our data showed that *DGAT1* is diminished in the subcutaneous adipose tissue of overweight and obese group. In support of these data, *DGAT1* has been reported to be decreased in the white adipose tissue of diet-induced obese mice [43]. Furthermore, there is evidence that down-regulation of *DGAT1* could affect insulin sensitivity and inflammation. In this way, a recent report demonstrated that *DGAT1* protects adipocytes from lipid-induced ER stress and inflammation during lipolysis, as adipocyte-specific *DGAT1* deficiency induces the activation of inflammatory adipose tissue macrophages [44]. In addition, increased *DGAT1* in insulin-resistant subjects treated with thiazolidinedione was reported to be positively associated with insulin sensitivity in obese group [45]. Taken together, the diminished levels of *DGAT1* in obesity may contribute to inflammatory status. More studies are necessary to elucidate the causes and consequences of diminished levels of *DGAT1* in the adipose tissue of individuals with obesity.

In regard to *PPARα*, its levels are known to be low in preadipocytes, but expression is induced during adipocyte differentiation [46]. It has been reported that *PPARα* plays a critical role in limiting white adipose tissue inflammation during chronic lipolytic activation [47], and chronic obesity is associated with excessive lipolysis [48]. We found that the adipose tissue from obese and overweight group had higher *PPARα* expression than the normoweight group, and expression was strongly correlated with BMI. This suggests that the upregulation of *PPARα* is associated with its anti-inflammatory capacity, representing a mechanism to counteract the proinflammatory processes that exist in obesity. In support of this, *PPARα* mRNA levels in the liver have been reported to be increased in a murine model of obesity [49], where it was found to protect against obesity-induced chronic inflammation [50]. Finally, as this study focused on expression at the mRNA level, future research should aim to confirm protein levels and function. Therefore, these could be used as biomarkers to predict the ability of adipose tissue to gain mass of adipose tissue.

6. Conclusion

We conclude that the expression of sirtuin genes *SIRT1*, *SIRT2*, *SIRT3* and *SIRT6*, along with their target genes (*PPAR-α*, *PGC1-α*, *NRF1*, *DGAT1,* PPAR-γ and *FOXO3a*), have a different profile in the subcutaneous adipose tissue from individuals with normoweight, overweight and obesity. Some of the genes investigated in this study could be the focus of future research aimed to elucidate their possible role in increased BMI.

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Appendix A. Supplementary data

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