Differential expression of miRNAs related to angiogenesis and adipogenesis in subcutaneous fat of obese and nonobese women

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Abstract

To disclose the mechanisms surrounding obesity, we selected microRNAs (miRNAs) that target genes involved in adipogenesis, angiogenesis, and inflammation and compared their expression levels in the subcutaneous adipose tissue of 40 obese and nonobese women. Mature miRNAs were extracted from subcutaneous adipose tissue samples that were collected during surgery and quantified by real-time polymerase chain reaction. miR-16 was overexpressed in the nonobese group (n-expression ratio = − 151.1; $P < 0.001$). Furthermore, the expression levels of two other miRNAs were significantly correlated with waist circumference in nonobese women (miR-27b, $r = 0.453$; $P = 0.027$ and miR-424-5p, $r = 0.502$, $P = 0.014$). Central and total subcutaneous adipose tissue thicknesses were correlated with miR-424-5p levels ($r = 0.506$, $P = 0.034$ and $r = 0.475$, $P = 0.046$, respectively) in the nonobese group. In the obese group, miR-424-5p expression was correlated with body mass index ($r = 0.582$, $P = 0.018$). miR-16 and miR-424 have shown correlations with body-fat-mass-related parameters. Because these miRNAs have vascular endothelial growth factor (VEGF) and its receptors as target genes, they may be involved in the alterations of angiogenesis observed in obesity. In addition, higher levels of miR-27 and miR-424 were correlated with higher fat depot measurements in nonobese women. These results highlight the importance of miRNA expression in subcutaneous adipose tissue and encourage further investigation of miRNAs as prognostic markers.

Keywords Adipose tissue · Obesity · microRNA · miR-16 · miR-424 · miR-27

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Introduction

Obesity and its related comorbidities, such as type II diabetes, cardiovascular diseases, and certain types of cancer, have been threatening the health of a growing number of people worldwide [1]. To fight this global epidemic, the study of adipose tissue biology has become increasingly important in recent years.

Evidence has accumulated indicating that obesity is associated with a state of adipose tissue remodeling characterized by adipocyte hypertrophy, increased angiogenesis and the production of proinflammatory adipokines during the progression of chronic inflammation [2, 3]. Unbalanced production of regulatory molecules by adipocytes, macrophages and endothelial cells may play a major role in this pathology.

A new component of post-transcriptional control of gene expression, performed by microRNAs (miRNAs), has been discovered, which has been related to most biological processes, including adipose tissue functionality [4, 5]. The importance of miRNAs is well recognized in regulating genetic networks and subsequent physiological processes. Altered expression levels of miRNAs in human and animal models have been associated with various disease conditions and their progression, including obesity-associated comorbidities [6–8]. miRNAs can suppress the production of target proteins by inducing the degradation of their messenger RNA (mRNA) and/or blocking their translation [9] by binding to the 3' untranslated region of mRNAs.

Although it is widely accepted that obesity is a heritable condition, even the most powerful genome-wide association study (GWAS) published [10] has so far explained only 2.7% of the variation in body mass index in the general population. Furthermore, Shungin et al. [11] conducted a genome-wide association meta-analysis of traits related to waist and hip circumferences in up to 224,459 individuals, finding that the pathway analyses implicated adipogenesis, angiogenesis, transcriptional regulation and insulin resistance as processes affecting fat distribution. Thus, the explanation of this so-called “missing heritability” in adiposity may rely on the less-explored level of gene regulation at the level of miRNA expression. Furthermore, several miRNAs and their targets have been implicated in the linkage between inflammation, angiogenesis and obesity [8, 12, 13] in the years since their discovery.

Considering this evidence, we hypothesized and aimed to determine whether specific miRNAs targeting genes involved in angiogenesis, inflammation, and adipogenesis may be differentially expressed in adipose tissue from obese and nonobese individuals. We believe this determination can improve the understanding of the molecular mechanisms underlying the accumulation of fat mass in the human body.

Materials and methods

Study population

This cross-sectional study included 40 women who were consecutively recruited from reference centers in two hospitals in Porto Alegre in southern Brazil. The obese group (body mass index, BMI ≥ 30 kg/m²) consisted of 20 patients who underwent bariatric surgery for weight management and one who underwent cosmetic surgery (a total of 21 individuals). On the other hand, the nonobese group (BMI < 30 kg/m²) consisted of 19 patients who underwent abdominoplasty for cosmetic purposes.

The inclusion criteria for the study were patients older than 18 years of age and who had a confirmed BMI according to the group to which they belonged at the moment of enrollment. The exclusion criteria were pregnancy and chronic inflammatory diseases.

Procedures

An interview was performed at enrollment to obtain demographic and lifestyle information. The patients' ethnicities were self-defined and classified as white or nonwhite (black and brown). Subcutaneous adipose tissue and plasma samples were obtained from all patients during surgery.

Anthropometric analysis

At the moment of inclusion in the study, for nonobese patients, a complete physical examination, including measurements of weight (kilograms), height (meters), waist circumference (centimeters) and seven skinfold thicknesses (millimeters), was performed by the same researcher. The skinfold thickness measurements were performed in triplicate on the right side of the body with a Lange skinfold caliper (Beta Technology, California, USA). Biceps, triceps and calf folds were grouped into a single measurement referred to as limb subcutaneous fat (LSF). Subscapular, axillary, suprailiac and abdominal folds were grouped into a single measurement referred to as central subcutaneous fat (CSF). Total subcutaneous fat (TSF) was calculated as the total sum of LSF and CSF. BMI was calculated using the following formula: BMI = weight in kilograms/(height in meters)². All data were stored in a database using the Microsoft Excel program (Microsoft Corporation, Redmond, Washington, USA). For obese patients, only BMI was assessed because the data in the literature suggest that waist circumference and skinfold

thickness in extremely obese patients are imprecise, and no additional clinically distinguishing or relevant information would likely be obtained by performing anthropometric assessments other than BMI in these individuals [14, 15].

microRNA selection

A total of 11 miRNAs were chosen based on their complementarity to the 3'UTR regions of target genes involved in metabolic, angiogenic and inflammatory adipose pathways (Table 1). miRNAs that target our genes of interest were selected through the TargetScanHuman [16] and confirmed by miRBase [17] and miRò online databases [18]. From all miRNAs listed by the database for each gene of interest, we selected only those that showed an exact match of the mature miRNA to positions 2–8.

microRNA collection and analysis

Subcutaneous adipose tissue samples were collected from the region near the stomach by the surgeon during the procedure and were immediately stored in 1 mL of Allprotect tissue reagent® (Qiagen, Hilden, Germany) at -20°C . Approximately 100 μg of tissue was ground in 1 mL of the homogenization denaturing lysis buffer from mirVana miRNA Isolation Kit® (Life Technologies, California, USA) using a Turrax tissue homogenizer (Marconi, São Paulo, Brazil). Then, the samples were subjected to acid-phenol:chloroform extraction. Homogenate was then subjected to the mirVana miRNA Isolation Kit® protocol for

total RNA isolation. Ethanol was added to the samples, and they were passed through a filter cartridge containing a glass-fiber filter. Then, the filter was washed a few times, and total RNA was eluted with a low ionic-strength solution. After sequential filtrations with different ethanol concentrations, an RNA fraction highly enriched in RNA species ≤ 200 nucleotides was obtained and stored at -80°C . RNA integrity and quantification were measured with BioSpec-nano (Shimadzu®, Kyoto, Japan). microRNAs were enriched to a final concentration of 1.2 ng/ μL of total RNA eluted through a multiplex approach by using a pool of individual stem-loop primers of selected microRNA assays (Life Technologies®, California, USA). Subsequently, the TaqMan® microRNA Reverse Transcription Kit (Life Technologies®, California, USA) was used with a preamplification step. All cDNAs were stored at freezer temperature for no longer than 5 days. Afterwards, preamplified mature microRNAs from each sample were used in duplicate for relative quantification in a StepOnePlus™ real-time PCR System (Life Technologies®, California, USA) using hydrolysis probes for detection and following the MIQE guidelines [35]. Samples from both groups were included in each batch to avoid any measurement bias.

An automatic baseline combined with a global threshold of 0.09 was used throughout the analysis. miRNA expression was measured using a threshold cycle (C_T). Target miRNA C_T values were normalized to miR-let-7d and miR-126 endogenous controls, which presented the lowest variation among all assays evaluated with ExpressionSuite Software version 1.0.3 (Life Technologies®,

Table 1 Selected miRNAs and target genes identified through online databases^a and literature data

miRBase ID	Mature miRNA sequence	Predicted target	References
hsa-miR-16-5p	UAGCAGCACGUAAAUUUGGCG	VEGF/VEGFR2	[19]
hsa-miR-26b-5p	UUCAAGUAAUUCAGGAUAGGU	TNFA/LEPT/RESIST/ AP2/C/EBP α /PPARG/ HSL	[20, 21]
hsa-miR-27a-3p	UUCACAGUGGCUAAGUUCGCG	PPARG/PHB	[22–24]
hsa-miR-27b-3p	UUCACAGUGGCUAAGUUCUGC	PPARG/PHB	[24, 25]
hsa-miR-125b-5p	UCCCUGAGACCCUAAUUGUGA	VEGF	[26, 27]
hsa-miR-132-3p	UAAACAGUCUACAGCCAUGGUCG	SIRT1/CREB	[28, 29]
hsa-miR-186-5p	CAAAGAAUUCUCCUUUUGGGCU	CASP10/P2RX7	[30, 31]
hsa-miR-223-3p	UGUCAGUUUGUCAAUACCCCA	PKNOX1/GLUT4	[32, 33]
hsa-miR-424-3p	CAAACGUGAGGCGCUGCUAU	VEGF/VEGFR	[19]
hsa-miR-424-5p	CAGCAGCAAUUAUGUUUUGAA	VEGF/VEGFR	[19]
hsa-miR-519d-3p	CAAAGUGCCUCCUUUAGAGUG	PPARG/PPARA	[34]

VEGF vascular endothelial growth factor, VEGFR2 vascular endothelial growth factor receptor 2, TNFA tumor necrosis factor alpha, LEPT leptin, RESIST resistin, AP2 adipocyte lipid-binding protein, C/EBP α CCAAT/enhancer binding protein α , PPARG peroxisome proliferator-activated receptor gamma, PHB prohibitin, HSL hormone-sensitive lipase, SIRT1 sirtuin-1, CREB cAMP responsive element binding protein, CASP10 caspase-10, P2RX7 P2 $\times$ 7 receptor gene, PKNOX1 PBX/knotted 1 homeobox 1, GLUT4 glucose transporter 4, PPARA peroxisome proliferator-activated receptor alpha

^aTargetScanHuman [15] miRBase [16] and miRò [17] databases

California, USA). RNU48 was also tested as an endogenous control; however, it presented a higher level of variation than the aforementioned miRNAs.

Plasma adipokines

Human leptin, resistin and adiponectin were profiled in EDTA plasma samples with ELISA kits (EMD Millipore®, Darmstadt, Germany). The assays were performed according to the manufacturer's instructions, and the absorbances were read in a Biorad® (California, USA) microplate absorbance reader.

Ethical considerations

All participants were informed about the purposes of the study and signed a free and informed consent form. This study was approved by the Research Ethics Committee of each participating center (protocol numbers 481/11, Irmandade Santa Casa de Misericórdia de Porto Alegre Hospital committee, and 11-105, Nossa Senhora da Conceição Hospital committee).

Statistical analyses

Using the Winpepi software [36] set to 95% reliability and 90% power, we estimated that a sample of at least 38 subjects was necessary to detect a difference in miRNA levels of 10% between groups.

Demographic and clinical continuous variables were compared between the two groups (obese and nonobese) using Student's *t*-test for independent samples. Categorical variables were contrasted with the chi-squared test with Yates' continuity correction or Fisher's exact test. Asymmetrically distributed data (BMI, adiponectin and resistin levels) were transformed into their natural logarithm and then used for statistical analyses.

To normalize miRNA data to the internal controls and calculate the expression ratio between groups, the $2^{-\Delta\Delta C_t}$ approach [37] was used. Nonparametric groupwise Mann–Whitney *U* and Student's *t*-test for independent samples were used to compare miRNA expression between obese and nonobese patients. Additionally, plasma adipokine levels and BMI were correlated with the expression levels of individual miRNAs by using Pearson's or Spearman's statistics. All data were analyzed using the SPSS 17.0 program (SPSS Inc., Chicago, Illinois, USA). All tests were two-sided, and the level of significance was defined as a *P* value less than 0.05.

Results

All patients were female, and their mean age was 42.0 ± 11.3 years. The main characteristics of the total sample stratified by BMI (nonobese and obese) are presented in Table 2. The majority of the subjects (95%) were nonsmokers. Nonobese individuals engaged in physical activity more frequently than obese patients (Table 2, *P* = 0.011).

All three adipokine plasma levels (leptin, adiponectin, and resistin) were compared between obese and nonobese individuals and are also shown in Table 2. Plasma levels of leptin (*P* < 0.001) and adiponectin (*P* = 0.039) were higher and lower in obese subjects, respectively. In contrast, resistin plasma levels were not different between groups. In the nonobese group, leptin plasma levels were significantly correlated with waist circumference (*r* = 0.496, *P* = 0.036). However, adiponectin and resistin levels were not correlated with waist circumference (*r* = 0.240, *P* = 0.924 and *r* = 0.295, *P* = 0.235, respectively).

Table 2 Biodemographic characteristics of the studied samples

Characteristic	Group		P value
	Nonobese (N = 19)	Obese (N = 21)	
Demographic			
Age (years)	41.1 ± 12.9	42.8 ± 9.9	0.641 ^a
Clinical			
BMI (kg/m ²) ^e	23.7 ± 1.7 [21.2–27.4]	49.3 ± 10.2 [33.0–73.0]	< 0.001 ^a
Weight (kg)	60.5 ± 5.4	123.9 ± 24.0	< 0.001 ^a
Leptin (ng/dL)	4.4 ± 2.6	38.1 ± 18.6	< 0.001 ^a
Adiponectin (μg/dL) ^{de}	23.2 ± 8.7	18.0 ± 7.9	0.039 ^a
Resistin (ng/dL) ^e	0.6 ± 0.2	0.5 ± 0.2	0.272 ^a
Diabetes ^f	NA	11 (55%)	
Dyslipidemia ^f	NA	12 (60%)	
Hypertension ^f	NA	16 (80%)	
Physical activity ^g			
Yes	14 (73.68%)	6 (28.57%)	0.011 ^b
No	5 (26.32%)	15 (71.43%)	
Smoking			
Yes	0	2 (9.52%)	0.488 ^c
No	19 (100%)	19 (90.48%)	

BMI body mass index [minimum–maximum], NA not available

^aStudent's *t* test for independent samples.

^b χ^2 test with Yates' correction

^cFisher's exact test

^dTest performed without outlier individual no 143 (obese group)

^eVariable transformed into ln

^fPrevalence data available only for twenty obese individuals

^gAt least one hour/twice a week

The differential expression of each miRNA in the two groups analyzed is shown in Table 3. The most striking difference observed was in miR-16, whose expression level was approximately 150 times higher in the subcutaneous adipose tissue of nonobese than in that of the obese group (n-expression ratio = -151.121 ; $P < 0.001$), which is depicted in Fig. 1. A nonobese outlier individual was excluded from this analysis for showing a $2^{-\Delta Ct}$ value for miR-16 higher than 109.00. No significant differences were observed for the other miRNAs investigated between obese and nonobese women.

We next evaluated the correlation of the studied miRNA expression with the measurements of central and subcutaneous adiposity of the nonobese women, i.e., waist circumference, total subcutaneous fat and central subcutaneous fat. Significant results are presented in Fig. 2. The expression of miR-27b was correlated with waist circumference ($r = 0.576$, $P = 0.016$). After adjusting for BMI, this correlation remained significant ($r = 0.453$; $P = 0.027$). miR-424-5p was also significantly correlated with waist circumference ($r = 0.562$, $P = 0.019$), even after adjusting for BMI (0.502 , $P = 0.014$). In addition, miR-424-5p levels were correlated with both TSF and CSF ($r = 0.512$, $P = 0.036$ and $r = 0.538$, $P = 0.026$, respectively), even after adjusting for BMI ($r = 0.475$, $P = 0.046$ and $r = 0.506$, $P = 0.034$, respectively). Furthermore, miR-424-3p was correlated with BMI values in the obese group ($r = 0.582$, $P = 0.018$). We did not find any significant association regarding the limb subcutaneous fat (LSF) measurement.

Fig. 1 Comparison of hsa-miR-16-5p expression between obese and nonobese women. Created by GraphPad Prism 5. ****Difference between groups, n-expression ratio = -151.121 ; $P < 0.001$

Discussion

The metabolic activity of adipose tissue has become a major focus of research due to the worldwide pandemic of obesity. In the present study, we compared the expression of selected miRNAs in subcutaneous fat between a group of obese women and a group of nonobese women to identify differences in the levels of miRNAs that target genes involved in angiogenesis, adipogenesis and inflammation between these two groups.

Table 3 Expression of analyzed miRNAs in obese and nonobese women

microRNA	Nonobese		Obese		Fold change obese/non-obese	P value
	Mean $\pm$ SD	Median [quartiles]	Mean $\pm$ SD	Median [quartiles]		
hsa-miR-16-5p	12.21 $\pm$ 23.86	6.02 [3.23–10.21]	0.08 $\pm$ 0.04	0.08 [0.06–0.08]	-151.121	$<0.001^a$
hsa-miR-26b-5p	0.28 $\pm$ 0.18 (18)	0.36 [0.11–0.43] (18)	0.35 $\pm$ 0.36	0.32 [0.17–0.38]	1.257	0.933 ^a
hsa-miR-27a-3p	0.07 $\pm$ 0.05	0.08 [0.01–0.11]	0.15 $\pm$ 0.33	0.08 [0.03–0.12]	2.074	0.616 ^a
hsa-miR-27b-3p	0.07 $\pm$ 0.05 (18)	*	0.06 $\pm$ 0.04	*	-1.148	0.578 ^b
hsa-miR-125b-5p	0.43 $\pm$ 0.37	0.43 [0.16–0.51]	0.44 $\pm$ 0.39	0.45 [0.20–0.49]	1.025	0.860 ^a
hsa-miR-132-3p	0.08 $\pm$ 0.06	*	0.09 $\pm$ 0.05	*	1.164	0.929 ^b
hsa-miR-186-5p	0.24 $\pm$ 0.14 (17)	0.22 [0.15–0.33] (17)	0.27 $\pm$ 0.17 (19)	0.23 [0.18–0.34] (19)	1.139	0.516 ^a
hsa-miR-223-3p	1.46 $\pm$ 1.22	1.27 [0.34–2.24]	2.06 $\pm$ 2.68	1.35 [0.67–2.38]	1.41	0.636 ^a
hsa-miR-424-3p	0.001 $\pm$ 0.0008 (15)	0.001 [0.0007–0.015] (15)	0.003 $\pm$ 0.007 (18)	0.001 [0.0005–0.018] (18)	2.584	0.971 ^a
hsa-miR-424-5p	0.0009 $\pm$ 0.0008 (17)	0.0008 [0.0003–0.0015] (17)	0.001 $\pm$ 0.0009 (18)	0.0009 [0.0005–0.0013] (18)	1.228	0.531 ^a
hsa-miR-519d-3p	0.0008 $\pm$ 0.0009 (16)	0.0005 [0.0001–0.001] (16)	0.001 $\pm$ 0.002 (20)	0.0007 [0.0003–0.002] (20)	1.851	0.161 ^a

Data presented as $2^{-\Delta Ct}$ mean $\pm$ SD and median [quartiles], when the data deviated significantly from the normal distribution; (number of included individuals presented when different from the total); fold change values were calculated with mean; fold change lower than one have been inverted; ^aMann-Whitney U test; ^bStudent's t-test for independent samples; *Median value not shown due to the symmetric distribution of the variable

Fig. 2 Significant correlations between miRNAs and anthropometric measures among nonobese women. Created by SPSS program. Nonobese group. **a** Correlation between expression levels of hsa-miR-27b-3p and waist circumference (Pearson's coefficient=0.576; $P=0.016$); **b** correlation between expression levels of hsa-miR-424-5p and waist circumference (Pearson's coefficient=0.562;

$P=0.019$); **c** correlation between expression levels of hsa-miR-424-5p and total subcutaneous fat (Pearson's coefficient=0.512; $P=0.036$); **d** correlation between expression levels of hsa-miR-424-5p and central subcutaneous fat (Pearson's coefficient=0.538; $P=0.026$)

In our sample, miR-16 was significantly overexpressed in the subcutaneous adipose tissue of nonobese compared to the expression in obese women. Elevated levels of a miRNA reduce the translation of its target mRNAs [9]. A previous study [38] computationally and experimentally predicted the vascular endothelial growth factor (*VEGF*) gene as one of the miR-16 targets, which was also shown in our online databases research (Table 1). Chamorro-Jorganes et al. [39] demonstrated that miR-16 and miR-424 participate in the regulation of VEGF receptor-2 (*VEGFR2*), fibroblast growth factor-1 (*FGFR1*) and *VEGF* genes in vascular endothelial cells in mice. Furthermore, they showed that miR-16 reduces blood vessel formation *in vivo*. In a review by Holmes et al. [40], *VEGF* and *VEGFR2* genes have been related

to the proliferation, migration, survival and permeability of endothelial cells. Additionally, it has been demonstrated that, without correct neovascularization, adipocyte hypertrophy generates a pseudohypoxic state in adipose tissue [41, 42]. Saad et al. [43] demonstrated that some miRNAs, including anti-angiogenic and proapoptotic miRNAs (such as miR-16) in adipose-derived mesenchymal stem/stromal cells, exhibited reduced expression under hypoxic conditions. Saad et al. also claimed that this group of miRNAs may negatively modulate angiogenesis by repressing *VEGF* expression under normoxic conditions. Accordingly, overexpression of miR-16 in the subcutaneous fat of nonobese individuals would maintain low expression of the abovementioned proangiogenic target genes, creating an unfavorable

environment for cell proliferation. As a result, adipogenesis would be impaired in this scenario due to the lack of new vessel formation. Moreover, miR-16 has been described as a suppressor of cell cycle progression by targeting innumerable G1 cyclins [44]. The importance of the *VEGF* gene has also been highlighted in a recent GWAS [11], which considered it to be one of the components of a gene set with a biological effect on body fat regulation. In a recent review by Elias et al. [45] about the actions of *VEGF-A* in the control of obesity and insulin resistance in adipose tissue, the authors noted conflicting reports regarding the local and systemic levels of *VEGF* in obesity. There is evidence showing that *VEGF-A* concentration in serum correlates significantly with BMI, with higher *VEGF-A* levels observed in overweight and obese subjects and animal models. However, other authors have failed to reproduce these results, and some studies have reported decreased *VEGF-A* expression in adipose tissue of obese mice and obese humans.

In contrast with our results, Ortega et al. [46] found elevated levels of miR-16 during adipocyte differentiation in white adipose tissue, suggesting its role in the biogenesis of this tissue. However, many miRNAs have been shown to be upregulated in obesity and downregulated in adipogenesis or *vice versa* [46, 47]. miR-16 has already been described as an endogenous control in previous studies [28, 48]. However, a comprehensive search for miRNAs to be used as endogenous controls in human adipose tissue performed by Neville et al. [49] did not include miR-16 among the 13 most stable miRNAs found in human adipose tissue.

Despite the fact that both miR-16 and miR-424 share the same seed region, implying similar targets, we did not find statistically significant differences in miR-424 subcutaneous adipose tissue expression between obese and nonobese studied individuals. However, we observed that miR-424 levels were directly correlated with BMI in the obese group and with higher waist, TSF and CSF measurements in the nonobese group. These findings suggest that miR-424 acts in contrast to miR-16 in relation to subcutaneous fat accumulation. In a study by Zhang et al. [50], miR-424 levels were shown to be increased in cancer cells due to a hypoxic microenvironment, leading to apoptotic resistance. Similarly, the greater the adiposity (waist and sum of skinfolds), the higher is the level of hypoxia in white adipose tissue (WAT) and the miR-424 levels due to an excessive adipocyte number sharing the same vascular supply. This evidence led us to speculate that miR-16 and miR-424 have different effects according to the set of genes in each pathway they regulate. Thus, we consider that the final result of miR-424 on the outcome depends on a fine and delicate balance of its duality shown so far: on one side, being overexpressed in hypoxia and, on the other side, acting as an inhibitor of angiogenic factors. Therefore, due to these data, more studies are necessary to explain this duality mentioned.

In relation to miR-27, our results have shown that higher levels of miR-27b in nonobese individuals were correlated with higher waist measurements. Several authors have implicated miR-27b in preadipocyte differentiation [22, 23, 25, 47]. Although miR-27b has never been correlated with waist circumference or with any other anthropometric measurement of subcutaneous fat as far as we know, Kang et al. [24] described this miRNA as an inhibitor of adipogenesis. Therefore, by targeting prohibitin (*PHB*), miR-27 induces downregulation of *PPAR γ* and causes mitochondrial impairment, which leads to a reduction of adipogenesis, as observed in a mouse 3T3-L1 *in vitro* cell line culture [51]. Moreover, expression of miR-27 has already been related to hypoxia [22], which is increased in obesity. As a result, higher levels of miR-27 are expected to be correlated with higher fat depot measurements, as shown in our analysis.

We are aware that our study presents some limitations. First, our research was performed only in female individuals. However, as emphasized by a recent genome-wide association meta-analysis [11], there is great sexual dimorphism in the genetic regulation of traits related to fat distribution. Moreover, gender is known to influence miRNA expression in human WAT [34]. As a result, having only women enrolled makes our sample more homogeneous, avoiding gender differences and resulting in more robust results. Furthermore, no studies on male individuals focusing on the expression of the miRNAs that we explored were found in the literature, which hampers the ability to compare our results between genders. Another limitation was the lack of other analyses, such as *VEGF* expression level evaluation, histology and microarray analysis in our sample. However, it is worth noting that a comprehensive analysis of all possible miRNAs expressed in humans is outside the scope of the present study. Furthermore, this study has a significantly higher number of enrolled individuals when compared with other publications on the same topic [28, 34, 46, 52]. Additionally, as far as we know, there are no other studies correlating subcutaneous fat measurements and miRNA expression. As our results were generated from *in vivo* human tissue and with very sensitive methodologies, we have a more realistic scenario than studies performed with cultured cells and animal models.

In summary, our findings show that miR-16 was highly overexpressed in nonobese WAT and probably acted as an anti-angiogenic factor by binding *VEGF* and *VEGFR2* genes in the sample analyzed. In addition, we found significant correlations of miR-27b and miR-424 expression in subcutaneous adipose tissue with BMI and anthropometric measurements in obese and nonobese women, suggesting that miR-27b and miR-424 play a possible role in modulating body fat distribution but act in a direction opposite to that of miR-16. Furthermore, our data suggest that miR-16 and miR-424 may have opposing effects, and together, they

may be, at least in part, responsible for the fine-tuning of adipogenesis in adipose tissue. Considering the complex network of miRNA regulation, these results need to be further explored and validated in other studies and with other methodologies. Hence, because the prevalence of obesity poses a heavy clinical burden, innovative strategies to understand the molecular mechanisms that underlie normal and pathophysiological regulation of adipose tissue are needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study complied with the ethical principles outlined in the Declaration of Helsinki. All enrolled participants were informed and signed informed consent at the beginning of their participation in the study.

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