

Research Article



Expression Patterns of SIRT1, SIRT3, and TFAM in Adipose Tissue: Associations with Adiposity Indices and Insulin Resistance in Women

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ABSTRACT

Objectives: Obesity is linked to metabolic dysfunction, with mitochondrial regulators such as SIRT1, SIRT3, and TFAM playing key roles in adipose tissue health. This study examined the expression of these genes in subcutaneous and visceral adipose tissues of obese and normal-weight women, and their associations with adiposity indices and insulin resistance.

Methods: Forty-six women (22 obese, 24 normal-weight) were enrolled. Anthropometric, metabolic, and biochemical parameters were measured. mRNA levels of SIRT1, SIRT3, and TFAM were assessed in adipose tissue samples using quantitative real-time PCR.

Results: Obese women had significantly higher adiposity indices and insulin resistance markers. SIRT1 expression in subcutaneous adipose tissue and SIRT3 expression in visceral adipose tissue were lower in obese women compared to controls. SIRT1 and SIRT3 transcript levels showed significant inverse correlations with several adiposity indices and insulin resistance measures. TFAM expression did not differ significantly between groups but was inversely associated with metabolic risk factors in visceral fat.

Conclusion: Reduced SIRT1 and SIRT3 expression in adipose tissue is associated with greater adiposity and insulin resistance in obese women, suggesting a potential role for these genes in obesity-related metabolic disturbances.

Keywords: SIRT1, SIRT3, And TFAM, Subcutaneous, Visceral, Adipose Tissue, Obesity

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Introduction

Obesity is a growing global health challenge and a major risk factor for a range of metabolic disorders. The epidemic of obesity has stimulated considerable research interest into the cellular and molecular mechanisms underlying its complications (1, 2). Adipose tissue, an essential organ in energy storage and metabolic regulation, plays a critical role in maintaining energy homeostasis. It is anatomically distributed across two primary compartments: subcutaneous adipose tissue (SAT), accounting for more than 80% of total body fat, and visceral adipose tissue (VAT), predominantly located around internal organs (3). Dysfunction in adipose tissue—characterized by excessive lipid accumulation and alterations in the secretion of adipokines—has been implicated as a key contributor to obesity-associated metabolic dysfunction (2).

Among the various mechanisms implicated in adipose tissue dysfunction, mitochondrial dysfunction has received increasing attention. Emerging evidence suggests that impaired mitochondrial function in mature adipocytes disrupts energy balance and contributes to abnormal adipokine secretion, insulin resistance, and chronic inflammation (4). Mitochondrial biogenesis and function are tightly regulated by several nuclear-encoded factors, including sirtuins (SIRT), a family of NAD⁺-dependent deacetylases that modulate mitochondrial activity and cellular metabolism (5).

Sirtuins are classified into four groups, with SIRT1–3 falling under class I, SIRT4 under class II, SIRT5 under class III, and SIRT6–7 under class IV. All seven sirtuin isoforms are expressed in human white adipose tissue (WAT), though they differ significantly in subcellular localization, enzymatic activity, and tissue-specific functions. In addition to their roles in epigenetic regulation, sirtuins influence adipogenesis, lipid metabolism, and glucose homeostasis. Altered expression levels of sirtuins have been observed in both animal models and human subjects with obesity, suggesting their involvement in the development of metabolic complications (6).

Among these, SIRT1 (localized mainly in the nucleus) and SIRT3 (primarily in the mitochondrial matrix) are the most studied. Both enzymes contribute to the regulation of oxidative stress responses, energy metabolism, inflammation, and mitochondrial function. SIRT1 has been shown to directly interact with transcription factor A, mitochondrial (TFAM), an essential factor for the transcription and replication of mitochondrial DNA (mtDNA). This interaction contributes to mitochondrial genome stability and may influence biogenesis through transcriptional and post-translational regulation mechanisms (7, 8). Similarly, SIRT3 plays a central role in mitochondrial homeostasis by deacetylating and activating

numerous enzymes involved in oxidative phosphorylation and energy metabolism. Deficiency of SIRT3 has been associated with impaired ATP synthesis, increased reactive oxygen species (ROS) production, and defective mitochondrial structure and function (9). These findings underscore the significance of SIRT1 and SIRT3 in maintaining mitochondrial integrity and their potential role in adipose tissue dysfunction during obesity.

While caloric restriction has been shown to upregulate SIRT1 and SIRT3 expression and activity, obesity is associated with their downregulation in WAT. Transgenic overexpression of SIRT1 in animal models results in reduced adiposity, improved insulin sensitivity, and decreased systemic inflammation (10, 11). However, studies in human adipose tissue have reported conflicting results regarding the expression profiles of SIRT1, SIRT3, and TFAM in obesity (12, 13), indicating a need for more comprehensive investigations. Hence, the present study aimed to simultaneously assess the mRNA expression levels of SIRT1, SIRT3, and TFAM in SAT and VAT samples obtained from obese and normal-weight women. Furthermore, the relationships between these gene expressions and anthropometric, metabolic, and biochemical parameters were explored, with the goal of better understanding the molecular pathways linking adipose tissue mitochondrial function to obesity-related metabolic phenotypes.

Methods

Obese and Normal-Weight groups

This study was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1397.702). Written informed consent was obtained from all participants prior to any surgical procedure. The results pertaining to the anthropometric, clinical, and metabolic characterizations of the study population presented herein correspond with findings reported in previous studies (2, 14).

In brief, a total of 46 women aged 20 to 53 years were enrolled in the study, comprising 22 obese women (BMI ≥ 30 kg/m²) undergoing bariatric surgery and 24 normal-weight women (BMI ≤ 25 kg/m²) who were scheduled for elective surgeries such as inguinal hernia repair or cholecystectomy. Obese participants were recruited from the Bariatric Surgery Center at Erfan Hospital, while normal-weight individuals were selected from the Center for Advanced Laparoscopic Surgery at Sina and Loqman Hakim Hospitals. All participants were of Iranian ethnicity. Exclusion criteria for both obese and non-obese participants included: (1) a history of cardiovascular disease, diabetes mellitus, malignancy, acute or chronic inflammatory or infectious conditions, or known renal or hepatic dysfunction; (2) use of weight-loss medications within the past six months; (3) current pregnancy or lactation; (4) postmenopausal status; (5) a history of surgery within the previous six months; and (6) current smoking (14).

Assessment of adiposity indices

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Waist circumference (WC) was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest at the end of a normal expiration. Hip circumference was recorded at the level of the greatest protrusion of the buttocks using a non-elastic tape measure. Waist-to-hip ratio (WHR) was calculated as WC (cm) divided by hip circumference (cm), and waist-to-height ratio (WHtR) was calculated as WC (cm) divided by height (cm). All measurements were taken with participants in a standing position and by trained personnel to ensure accuracy and reproducibility.

Body adiposity index (BAI), as another index of obesity, was calculated using the following formula:

$$\text{Body Adiposity Index (BAI)} = \frac{\text{hip circumference (cm)}}{\text{height (m)}^{1.5}} - 18$$

The Abdominal Volume Index (AVI), an anthropometric tool used to estimate general abdominal volume, was calculated using the following equation based on waist circumference (WC) and hip circumference.

$$\text{Abdominal Volume Index (AVI)} = \frac{2 \text{ cm} \times (\text{WC (cm)})^2 + 0.7 \text{ cm} \times (\text{WC (cm)} - \text{hip (cm)})^2}{1000}$$

Given the sexual differences in visceral adipose tissue (VAT) estimation, the Visceral Adiposity Index (VAI) is considered an optimal method for indirectly assessing VAT function. This index is derived from a mathematical model that incorporates sex-specific differences and is calculated using the following formula, which includes values for waist circumference (WC), body mass index (BMI), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C). Weight-adjusted waist index (WWI) is used to assess adiposity by standardizing waist circumference (WC) for weight and was calculated as WC in cm divided by the square root of weight in kg ($\text{cm}/\sqrt{\text{kg}}$). Conicity index (CI) was calculated based on the values of WC, weight, and height, by using the following formula

$$\text{Conicity index (CI)} = \frac{\text{WC (m)}}{0.109 \sqrt{\text{weight (kg)} / \text{height (m)}}} - 18$$

Resting blood pressure was measured three times

on the right arm of seated participants using a manual sphygmomanometer.

Biochemical and Laboratory Measurements

A peripheral blood sample was collected from the antecubital vein via sterile venipuncture in the morning after an overnight fast, prior to the start of surgery. The fasting blood samples were then centrifuged at $1200 \times g$ for 10 minutes at 4°C , and the serum was separated for analysis. The following parameters were measured: fasting blood glucose (FBG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), total cholesterol (TC), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, urea, high-sensitivity C-reactive protein (hs-CRP), and insulin, as previously described (2, 14). The insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) formula: fasting blood glucose (mg/dL) $\times$ fasting blood insulin ($\mu\text{U}/\text{mL}$) / 405.

Adipose tissue samples, RNA extraction, and Real-Time Quantitative Polymerase Chain Reaction (PCR)

Paired samples of visceral and subcutaneous adipose tissue were obtained during bariatric surgery in the obese group or during inguinal hernia or cholecystectomy in the normal-weight subjects, as described previously. After washing the samples with sterile and cold phosphate-buffered saline (PBS), the biopsy specimens were immediately frozen in liquid nitrogen and stored at -80°C for further experiments.

Total RNA was isolated from 100 mg of frozen adipose tissue using the RNeasy Lipid Tissue Mini Kit (Qiagen, Germany). The frozen adipose tissue was transferred into 1 ml of QIAzol Lysis Reagent (Qiagen, Hilden, Germany) and homogenized using a pestle and mortar. RNA isolation was then performed using the RNeasy Lipid Tissue Mini Kit as per the manufacturer's protocol. Before proceeding with reverse transcription, the quality and quantity of the isolated RNA were assessed using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific), and RNA integrity was evaluated by running the samples on a 1% agarose gel. Complementary DNA (cDNA) was synthesized from 1 μg of RNA using the PrimeScript 1st Strand cDNA Synthesis Kit (Takara, Japan). A standard curve for each primer set was generated using serial dilutions of cDNA synthesized from a pool of RNA extracted from both visceral (VAT) and subcutaneous adipose tissue (SAT). The amplification efficiency for each primer set, calculated from the slope of the standard curve, ranged between 90–100%.

Relative mRNA expression was assessed by real-time PCR using BioFACT™ 2X Real-Time PCR Master Mix (For SYBR Green I) on a Step-One-Plus™

real-time PCR system (ABI Applied Biosystems). The gene-specific primers for target genes, including SIRT1, SIRT3, and TFAM, along with the reference gene β -actin, are provided in Table 1. The PCR program involved an initial denaturation step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 95°C for 20 seconds, and annealing at 60°C for 15 seconds. All expressions were quantified in duplicate, and a melting curve analysis was performed to confirm the specificity of the amplified products. For each sample, the difference in cycle threshold (Ct) values (Δ Ct) between the target gene and the reference gene was calculated. Since the amplification efficiency (E) calculated from the standard curve ranged from 90% to 100%, relative quantification was performed using the $2^{-\Delta$ Ct method.

Results

Participant Characteristics

As mentioned earlier (14), the comparison between the obese and normal-weight groups revealed no statistically significant differences in age, systolic blood pressure (SBP), diastolic blood pressure (DBP), and circulating levels of FBG, urea, HDL-C, TG, AST, and ALT. In contrast, the obese group exhibited significantly elevated circulating levels of LDL-C, total cholesterol, uric acid, creatinine, albumin, total protein, hs-CRP, insulin, HbA1c, and HOMA-IR compared to the normal-weight group. Regarding adiposity indices, all anthropometric measurements-including BMI, WC, hip circumference, BAI, AVI, CI, and WHtR-

were significantly higher in the obese group relative to the non-obese group. Notably, other obesity indices such as WHR and WWI demonstrated an increasing trend in women with obesity compared to those with normal weight; however, these differences did not reach conventional statistical significance thresholds ($p = 0.088$ and $p = 0.073$, respectively).

The mRNA Expression of SIRT1, SIRT3, and TFAM in SAT and VAT from the Obese and Normal-Weight Groups

Table 2 presents the mRNA expression levels of SIRT1, SIRT3, and TFAM in SAT and VAT of both study groups. The obese group showed lower SIRT1 mRNA expression in SAT ($p = 0.055$) compared to the normal-weight group. For SIRT3, there was no significant difference in expression in SAT between the groups, but VIS samples from the obese group had significantly reduced SIRT3 mRNA levels ($p = 0.013$). Additionally, TFAM mRNA expression showed no change in both VAT and SAT of the obese group compared to non-obese individuals.

Univariate Correlations of mRNA Expression of All Studied Genes with Adiposity Indices, Insulin Resistance, and Metabolic Parameters

We assessed the Spearman correlation coefficient of SIRT1, SIRT3, and TFAM mRNA levels with adiposity indices and metabolic parameters in SAT (Table 3) and VAT (Table 4) across the entire population. Our

Table 1. Forward and reverse primers used for real-time PCR

Primer	Forward sequence	Reverse sequence
SIRT1	5'-TGC GGGAATCCAAAGGATAA-3'	5'-CAGGCAAGATGCTGTTGCA-3'
SIRT3	5'-CATTCCAGACTTC AGATCGC -3'	5'-AGCAGCCGGAGAAAAGTAGT -3'
TFAM	5'-CAAGTTGTCCAAAGAAACCTGTAAG -3'	5'-GCCACTCCGCCCTATAAGC-3'
β -actin	5'-TCCTTCCTGGGCATGGAGT-3'	5'-ACTGTGTTGGCGTACAGGTC-3'

Table 2. Comparison of SIRT1, SIRT3, and TFAM mRNA Expression Levels in Subcutaneous and Visceral Adipose Tissue Between Obese and Normal-Weight Women

	Non-obese group					Obese group					p-value
	Mean	SD	Variance	Max	Min	Mean	SD	Variance	Max	Min	
Transcript level of SIRT1 in SAT	0.0364	0.1234	0.0152	0.5743	0.0012	0.0065	0.0078	0.0001	0.0377	0.0007	0.055
Transcript level of SIRT3 in SAT	0.0487	0.1899	0.0360	0.8766	0.0000	0.0066	0.0086	0.0001	0.0436	0.0012	0.76
Transcript level of TFAM in SAT	0.01377	0.01508	0.00023	0.05219	0.00031	0.01247	0.01720	0.00030	0.06887	0.00111	0.77
Transcript level of SIRT1 in VAT	0.01565	0.04146	0.00172	0.18946	0.00044	0.00529	0.00295	0.00001	0.01160	0.00087	0.75
Transcript level of SIRT3 in VAT	0.03441	0.12102	0.01465	0.54337	0.00003	0.01092	0.00743	0.00006	0.02739	0.00113	0.013
Transcript level of TFAM in VAT	0.0207	0.0370	0.0014	0.1627	0.0003	0.0075	0.0081	0.0001	0.0382	0.0006	0.23

Transcript levels of SIRT1, SIRT3, and TFAM in subcutaneous (SAT) and visceral (VAT) adipose tissue samples from obese (n=22) and normal-weight (n=24) women. Data are presented as mean $\pm$ standard deviation (SD), Maximum and Minimum. Statistical comparisons between groups were performed using Mann-Whitney U tests. Significant differences ($p < 0.05$) are indicated in bold.

Table 3. Correlation of SIRT1, SIRT3, and TFAM Expression in Subcutaneous Adipose Tissue (SAT)

	Transcript level of SIRT1 in SAT		Transcript level of SIRT3 in SAT		Transcript level of TFAM in SAT	
	Correlation Coefficient	p-value	Correlation Coefficient	p-value	Correlation Coefficient	p-value
Age, years	0.104	0.495	.048	0.754	0.118	0.440
BMI, Kg/m ²	-0.323	0.031	-0.191	0.208	0.012	0.937
VAI	-0.073	0.633	-0.291	0.052	-.0167	0.273
LAP	-0.322	0.031	-0.290	0.054	-0.182	0.232
WHR	-0.162	0.286	0.256	0.089	-0.282	0.061
BAI	-0.285	0.058	-0.277	0.066	0.046	0.767
AVI	-0.329	0.027	-0.177	0.244	-0.074	0.630
Waist, cm	-0.337	0.024	-0.169	0.266	-0.082	0.594
Hip, cm	-0.382	0.010	-0.270	0.073	-0.014	0.927
WWI	-0.069	0.652	-0.043	0.779	0.028	0.853
CI	-0.282	0.061	-0.162	0.289	-0.033	0.831
FBS, mg/dl	-0.304	0.043	-0.551	0.000	-0.125	0.412
Waist to Height Ratio	-0.306	0.041	-0.208	0.170	-0.051	0.740
TG, mg/dl	0.128	0.401	-0.292	0.051	-0.057	0.712
hs.CRP, mg/dl	-0.380	0.010	-0.291	0.052	-0.061	0.689
HOMA-IR	-0.501	0.000	-0.348	0.019	-0.023	0.880
Insulin, μ U/mL	-0.446	0.002	-0.264	0.080	0.014	0.925

Correlation coefficients were calculated using Spearman's rank correlation test.

SIRT1, Sirtuin 1; SIRT3, Sirtuin 3; TFAM, Mitochondrial Transcription Factor ABMI, body mass index; VAI, visceral adiposity index; BAI, body adiposity index; WWI, weight-adjusted waist index; CI, conicity index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, Waist to Height Ratio; FBG, fasting blood glucose; TG, triglycerides-CRP, high-sensitivity C-reactive Protein; *Note: Significant correlations ($p < 0.05$) are indicated in bold.*

Table 4. Correlation of SIRT1, SIRT3, and TFAM Expression in Visceral Adipose Tissue (VAT) with Obesity Indices and Biochemical Parameters

	Transcript level of SIRT1 in VAT		Transcript level of SIRT3 in VAT		Transcript level of TFAM in VAT	
	Correlation Coefficient	p-value	Correlation Coefficient	p-value	Correlation Coefficient	p-value
Age, years	0.021	0.895	-.066	0.668	-0.190	0.216
BMI, Kg/m ²	-.0194	0.207	0.457	0.002	-0.375	0.012
VAI	0.200	0.193	-0.036	0.816	0.137	0.377
LAP	-0.105	0.500	0.346	.021	-0.187	0.225
WHR	-0.172	0.265	-0.058	0.709	-0.064	0.679
BAI	-0.299	0.049	0.382	0.011	-0.274	0.072
AVI	-0.199	0.196	0.329	0.029	-0.215	0.160
Waist, cm	-0.202	0.188	0.322	0.033	-0.217	0.158
Hip, cm	-0.175	0.255	0.414	0.005	-0.274	0.072
WWI	-0.206	0.179	0.059	0.704	0.130	0.400
CI	-0.237	0.122	0.231	0.131	-0.112	0.471
FBG, mg/dl	-0.051	0.742	0.194	0.208	-0.064	0.679
Waist to Height Ratio	-0.258	0.091	0.331	0.028	-0.249	0.104
TG, mg/dl	0.267	0.080	-0.052	0.738	0.195	0.204
hs.CRP, mg/dl	-0.386	0.010	0.377	0.012	-.0353	0.019
HOMA-IR	-0.231	0.131	0.385	0.010	-0.355	0.018
Insulin, μ U/mL	-0.224	0.145	0.366	0.015	-0.356	0.018

Correlation coefficients were calculated using Spearman's rank correlation test.

SIRT1, Sirtuin 1; SIRT3, Sirtuin 3; TFAM, Mitochondrial Transcription Factor ABMI, body mass index; VAI, visceral adiposity index; BAI, body adiposity index; WWI, weight-adjusted waist index; CI, conicity index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; WHtR, Waist to Height Ratio; FBG, fasting blood glucose; TG, triglycerides-CRP, high-sensitivity C-reactive Protein; HOMA-IR, homeostasis model assessment of insulin resistance. *Note: Significant correlations ($p < 0.05$) are indicated in bold.*

results revealed that the mRNA expression of SIRT1 in SAT was inversely correlated with BMI, LAP, AVI, WC, hip circumference, waist-to-height ratio, FBG, hs-CRP concentration, HOMA-IR, and insulin levels. Additionally, SAT mRNA expression of SIRT3 showed an inverse correlation with VAI, FBG, and HOMA-IR.

In terms of VAT, the mRNA expression of SIRT1 showed a significant inverse relationship with BAI and hs-CRP concentration. Furthermore, VAT TFAM transcript levels were significantly inversely correlated with BMI, hs-CRP concentration, HOMA-IR, and insulin levels.

Lastly, VAT SIRT3 mRNA expression demonstrated a significant positive correlation with BMI, LAP, BAI, AVI, WC, hip circumference, waist-to-height ratio, hs-CRP concentration, HOMA-IR, and insulin levels.

Discussion

The involvement of SIRT1, SIRT3, and TFAM in the molecular mechanisms underlying obesity and its associated metabolic disorders has been documented in numerous studies, though with some conflicting results (12, 13, 15). Despite this growing body of evidence, their precise clinical relevance in obesity remains incompletely understood. In our study, we observed significantly reduced mRNA expression of SIRT1 in SAT of obese women compared to normal-weight controls. This downregulation was paralleled by lower SIRT3 expression in VAT of the obese group. These findings are consistent with prior reports; for example, Mariani et al. demonstrated higher SIRT1 expression in normal-weight individuals relative to obese patients. Furthermore, diminished SIRT1 expression has been linked to severe liver steatosis in obese patients, suggesting a role in hepatic metabolic dysfunction (16, 17). Similarly, Boyle et al. reported reduced SIRT3 activity in skeletal muscle of women with maternal obesity and gestational diabetes mellitus (18), highlighting the systemic impact of these molecules in metabolic regulation.

The biological roles of sirtuins, particularly SIRT1 and SIRT3, are well-established in regulating key pathways involved in glucose and lipid metabolism, inflammation, and insulin signaling, all critical factors in obesity-related complications (6, 19). Experimental evidence shows that high-fat diets decrease SIRT1 levels in white adipose tissue, contributing to metabolic abnormalities characteristic of obesity. Conversely, animal models with SIRT1 overexpression are protected against high-fat diet-induced metabolic disturbances such as glucose intolerance, weight gain, inflammation, and hepatic steatosis (20). Interestingly, SIRT3 deletion in high-fat diet-fed mice has been reported to improve insulin sensitivity by enhancing mitochondrial function and glucose disposal, indicating complex tissue- and context-specific roles (10, 21). Our correlation analyses support these mechanistic insights, revealing inverse relationships between SIRT1 expression and insulin resistance, hyperinsulinemia, inflammatory marker hs-CRP, and various adiposity indices. Similarly, SIRT3 expression inversely correlated with insulin resistance and VAI, reinforcing their roles in metabolic homeostasis.

Regarding TFAM, its specific disruption in adipose tissue has been shown to increase energy expenditure and mitigate insulin resistance and hepatic steatosis in high-fat diet-induced obesity models. Enhanced mitochondrial oxidative capacity in adipose tissue confers protective metabolic effects, reducing susceptibility to obesity and insulin resistance. Therapeutic strategies promoting brown adipose tissue expansion or “browning” of white

adipose tissue capitalize on these benefits, as increased mitochondrial oxidation improves metabolic outcomes (22). Our finding of an inverse correlation between TFAM expression and insulin resistance and adiposity parameters further underscores its potential role in metabolic regulation.

Collectively, these data provide compelling evidence that alterations in SIRT1 and SIRT3 expression contribute to the pathophysiology of obesity and related metabolic dysfunction. However, several limitations of our study warrant consideration. Firstly, the cross-sectional design precludes causal inference and limits the ability to generalize findings beyond the studied population. Secondly, longitudinal studies including both sexes are necessary to validate and extend these observations. Thirdly, detailed mechanistic investigations are essential to delineate the precise roles and regulatory networks involving these genes in obesity development and progression.

Conclusion

In conclusion, while our findings align with and expand upon existing literature, further research is needed to clarify the clinical significance and therapeutic potential of targeting SIRT1, SIRT3, and TFAM in obesity and metabolic disease.

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Conflict of interests

The authors declare no conflict of interest.

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