Physical Activity and Exercise Promote Peroxisome Proliferator-Activated Receptor Gamma Expression in Adipose Tissues of Obese Adults

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Abstract

Background: Peroxisome proliferator-activated receptor gamma (PPARγ) has recently been studied for its potential influence on the functional response of the human body to exercise. We aimed to investigate the association of habitual physical activity (PA) with PPARγ mRNA level in the visceral and subcutaneous adipose tissues (VAT and SAT) in non-obese and obese non-diabetic adults.

Methods: VAT and SAT were obtained from 95 individuals, including 40 non-obese (BMI<30kg/m²) and 55 obese (BMI≥30kg/m²) who underwent elective abdominal surgery (Tehran, Iran, 2012-2015). The assessment of habitual PA was performed by a valid and reliable International PA Questionnaire-long form, and the metabolic equivalent of task (MET) was evaluated. Real-time quantitative reverse transcriptase-PCR evaluated the PPARγ expression in VAT and SAT.

Results: PPARγ expression in both VAT (1.18 vs. 0.37 fold change, *P*<0.001) and SAT (2.07 vs. 0.29 fold change, *P*=0.004) among obese subjects was higher than the non-obese group. After controlling for age, sex, and total energy intake, a positive association was found between total METs and PPARγ expression in both VAT and SAT among obese participants (β=0.22, *P*=0.007 and β=0.12, *P*<0.001, respectively). Among obese participants, there was a direct association between leisure time-related METs with VAT PPARγ expression (β=0.05, *P*=0.026). Moreover, in this group, an association was observed between occupation-related METs with PPARγ in both fat tissues (β=0.11, *P*=0.002 and β=0.17, *P*=0.013, respectively), and household work-related METs with SAT PPARγ (β=0.21, *P*=0.011).

Conclusion: High PA as an indispensable part of a healthy lifestyle may exert its beneficial effect by regulating PPARγ expression.

Keywords: Adipose tissue; Exercise; Non-diabetic

Introduction

Over the last few decades, the prevalence of obesity has risen dramatically in many countries throughout the world(1). Obesity is often associated with numerous comorbidities, such as metabolic syndrome, atherosclerosis, insulin resistance (IR), dyslipidemia, and type 2 diabetes mellitus (T2D) (2). Obesity is a multifactorial disease resulting from an imbalance between energy consumption and expenditure (3). Such imbalance can be affected by the interaction of lifestyle (nutrition and physical activity (PA)), environment, and genetic factors (4, 5).

The role of adipose tissue dysfunction is emphasized during the development of obesity(6). Obese individuals often have enlarged adipocytes with a reduced buffering capability for lipid storage, thereby exposing other tissues to an excessive influx of lipids, leading to ectopic fat deposition in situations where energy consumption exceeds energy spending. Furthermore, adipose tissue blood flow is reduced in obesity. Therefore, it seems that an understanding of the cellular and molecular factors involved in energy consumption and storage, appetite regulation, lipid metabolism, and adipogenesis are appropriate candidates for the development of effective therapeutic strategies against obesity (7, 8).

The most commonly studied candidate transcription factor for obesity is peroxisome proliferator-activated receptor gamma (PPARγ), which is located on chromosome 3 at band 3p25 (9, 10). PPARγ is a member of the nuclear hormone receptor superfamily that acts as a ligand-dependent transcription factor. It has a central role in fat cell differentiation, adipogenesis, inflammation, lipid metabolism, lipid storage, and insulin sensitivity (11). PPARγ has been considered in the context of its potential influence on the functional response of the human body to exercise (12). Exercise induces PPARγ signaling cascades (Fig. 1) and causes upregulation of genes related to improved lipid metabolism and insulin sensitivity (13).
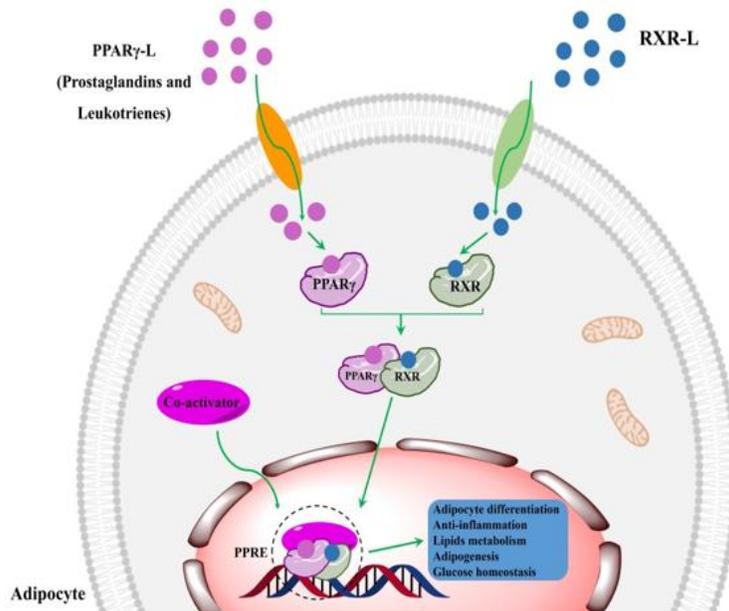

Fig. 1: Schematic activity of PPARγ. The spatial configurations of PPARγ and RXR are changed after they attach to their ligands. Then PPARγ and RXR form a heterodimer. The active PPARγ/RXR heterodimer is translocated into nucleus and bind to DNA with the help of co-activator and regulates target genes transcription. PPARγ: Peroxisome proliferator-activated receptor gamma, RXR: retinoid X receptor, PPARγ-L: PPARγ-Ligand, RXR-L: RXR-Ligand (Original)

Studies that differ in training intensity and duration report variable findings on exercise-induced gene expression of PPARγ in adipose tissues (14, 15). However, people who did not have usual PA had a chronic impact on adipose tissue gene regulation (16). It remains unknown whether PPARγ expression in adipose tissues changes with habitual PA in humans with a wide range of obesity.

Habitual PA induces energy expenditure, which may lead to amelioration of obesity-related metabolic diseases (17). Improving insight into the molecular mechanisms related to PA will be helpful in life habits. Thus, we aimed to evaluate the association of habitual PA with PPARγ gene expression in the visceral and subcutaneous adipose tissues (VAT and SAT) of obese and non-obese adults without T2D.

Materials and Methods

Participants Selection and Data Collection
In this cross-sectional study, 95 adults (age≥18year) were randomly enrolled as minor abdominal surgery patients from two Hospitals (Mostafa Khomeini and Khatamolanbia) in Tehran, Iran, between 2012-2015, and were divided into obese (BMI≥30kg/m^2, n=55) and non-obese (BMI<30kg/m^2, n=40).

Ethics approval was obtained from the Research Institute for Endocrine Sciences (RIES) of the Shahid Beheshti University of Medical Sciences (NO: IR.SBMU.ENDOCRINE.REC.1395.169) and conducted following the Declaration of Helsinki as well as RIES guidelines. Written informed consent was obtained from all participants.

Before surgery, data such as anthropometrics, demographics, dietary intake, and PA were obtained. All anthropometric characteristics, including height, weight, and waist circumference, were measured as described previously (18). BMI was calculated as weight (kg) divided by height squared (m^2).

An experienced interviewer assessed participant habitual PA using the long-form of the valid and reliable International Physical Activity Questionnaire (IPAQ)(19, 20). Data were collected and transformed to metabolic equivalent scores (MET-min-week−1). Each dominant activity was calculated by multiplying the time spent (in minutes) to subclasses of each domain. Habitual PA was calculated from sum of leisure time, occupation, household work, and transportation-related activity in MET-minutes per week. Participants were categorized as either sedentary(PA<600MET-minutes per week) or active(≥600MET-min per week)(16, 20). Dietary intake was collected using a valid and reliable semi-quantitative food frequency questionnaire (FFQ) (21).

Before surgery and after a 10-12 h overnight fast, blood samples were collected in potassium EDTA-containing tubes and plasma was separated. All biochemical components, including fasting plasma glucose (FPG), triglycerides (TG), total cholesterol (TC), and insulin levels, were measured as described previously (22). People with diabetes, cancer, on lipid-lowering or anti-obesity medication, pregnant, lactating, or dieting were excluded.

RNA Extraction and Real-time PCR
During the surgery, approximately 100mg of VAT and SAT specimens were collected from each participant and immediately frozen. The samples were incised, weighed, and homogenized using a homogenizer (RETSCH MM 400, Germany). Based on previous studies (22, 23), total RNA was extracted from collected fatty tissues using TRIzol reagent (Invitrogen U.S. Cat. No. 15596-026) as per manufacturer's instruction. The quality of the extracted RNA was evaluated by Nanodrop spectrophotometer (ND-1000, USA) and by measuring the ratio of absorption (260/280 and 260/230nm). To remove the genomic DNA, DNase I was added to the extracted total RNA. Fermentas kit (Thermo Scientific, USA) was utilized to synthesis complementary DNA (cDNA). Primers were designed using the National Center for Biotechnology Information (NCBI) Database and then checked via Gene-Runner software (version 4.0). GAPDH was used

as the endogenous control for normalization. The sequences of the primers were: PPARγ(Forward): 5'-CCTCATGAAGAGCCTTCCAAC-3', PPARγ(Reverse): 5'-ACCCTAGCATCCTTCACAAGC-3'; GAPDH(Forward): 5'-CTGCTCCTCCTGTTCGACAGT-3', and GAPDH(Reverse): 5'-CCGTTGACTCCGACCTTCAC-3'.

The real-time quantitative reverse transcriptase-PCR (RT-qPCR) amplification was performed in a 25-μl reaction volume by the SYBR Green Master Mix (Biofact, Korea). The amplification thermal cycling was performed by Rotor-Gene 6000 (Corbett Research, Sydney, Australia), and conditions were as follows: 95 °C for 5 min, followed by 40 cycles at 95 °C for 5s, 62 °C for 20s, and 72 °C for 30s. The relative gene expression was calculated by the $2^{-\Delta Ct}$ method (24). Melting curve analysis was performed, and all experiments were performed in duplicate and repeated at least 2 times.

Statistical Analysis

Histogram and the Kolmogorov–Smirnov tests were used to evaluate the normal distribution of variables. Based on the distribution of variables, continuous variables were described as mean±standard deviation (SD), or median (interquartile 25, 75%). Skewed variables such as TG and insulin levels were log-transformed before being used in parametric analyses. To compare continuous and categorical parameters between obese and non-obese participants, *t*-test and Chi-square tests were used, respectively. Linear regression was used to evaluate habitual PA and its components with PPARγ expression in VAT and SAT based on participant obesity status, and the β coefficient (95% confidence interval (CI)) was reported. The initial model was crude, and model 2 was adjusted for age, sex, and total energy in-take (kcal). Data were analyzed using SPSS statistical analysis software (version 15.0; SPSS Inc, Chicago, IL, USA), and *P*-value <0.05 was considered statistically significant.

Results

Participants' demographic information and biochemical data are presented in Table1. The mean BMI±SD(range) of non-obese and obese participants was 24.6±2.82 (18.73-29.69) and 43.1±6.11(30.49-58.68)kg/m², respectively. The mean age±SD(range) of non-obese and obese participants was 48.3±14.9 [21-84] and 37.05±10.7 [18-66] years, respectively. Participants with obesity had significantly higher total energy intake, cholesterol, HOMO-IR, and insulin levels than their non-obese counterparts. There was also no significant difference between obese and non-obese groups concerning total PA and its sub-types except transportation and leisure time-related activities, which in non-obese participants was higher than that among obese ones (Table 1).

Define sedentary. Define METs. Define non-obese and obese

PPARγ mRNA level was significantly upregulated in both VAT (1.18 vs. 0.37, *P*<0.001) and SAT (2.07 vs. 0.29, *P*=0.004) in obese participants compared to non-obese group.

According to the RT-qPCR results, there was not a statistically significant difference in PPARγ expression in VAT and SAT between active and sedentary MET groups in the non-obese subjects, while among obese subjects, a significant downregulation of PPARγ was observed in VAT and SAT of the active participants compared to the sedentary MET group (Fig. 2).

Table1: General characteristics of study subjects according to obesity status

Variables	Non-obese (n= 45)	Obese (n= 55)	P value
Age(yr)	48.3±14.9	37.05±10.7	<0.001
Body mass index(kg/m²)	24.6±2.82	43.1±6.11	<0.001
Female (%)	70	85	0.083
Fasting plasma glucose(mg/dl)	84.5±17.8	91.9±17.7	0.020
Total energy intake(kcal/d)	2424.5±747.5	3203±1031.7	<0.001
Cholesterol(mg/dl)	167.9±44.4	184.4±38.3	0.012
Waist circumference(cm)	87.7±10.5	123.6±15.3	<0.001
Triglycerides(mg/dl)	77.5(64.2, 132.2)	106.0(71.0, 155.0)	0.006
Insulin(μU/mL)	4.61(2.73, 8.56)	12.53(6.0, 22.99)	<0.001
Sedentary (%)	37	63	0.211
Total physical activity(MET-min/week)	999(231, 2928)	540(181.5, 1531.8)	0.113
Occupation(MET-min/week)	0.00(0.00, 297.0)	0.00(0.00, 198.0)	0.712
Transportation(MET-min/week)	66.0(0.00, 264.0)	0.00(0.00, 99.0)	0.003
Household work(MET-min/week)	180.0(0.00, 1080.0)	147.5 (0.00, 840.0)	0.555
Leisure time(MET-min/week)	0.00(0.00, 264.0)	0.00(0.00, 94.2)	0.014

Data are represented as mean±SD or median (IQ 25, 75%), and one sample *t*-test or Chi-square test was used.

Table2: The association of physical activity and its components with PPARγ expression from subcutaneous adipose tissue in non-obese and obese participants

Variable		Non-obese β (95% CI)	P value	obese β (95% CI)	P value
Total physical activity	Model 1	0.49(0.12-2.06)	0.334	0.14(0.05-0.40)	<0.001
	Model 2	0.15(0.01-1.59)	0.116	0.12(0.04-0.37)	<0.001
Leisure time	Model 1	0.87(0.19-4.01)	0.855	0.57(0.09-3.61)	0.546
	Model 2	0.69(0.13-3.68)	0.667	0.55(0.08-3.80)	0.548
Occupation	Model 1	0.29(0.05-1.69)	0.167	0.17(0.05-0.65)	0.010
	Model 2	0.26(0.04-1.78)	0.169	0.17(0.04-0.69)	0.013
Household work	Model 1	0.33(0.08-1.44)	0.140	0.28(0.10-0.79)	0.016
	Model 2	0.24(0.04-1.30)	0.098	0.21(0.07-0.70)	0.011
Transportation	Model 1	0.46(0.09-2.27)	0.341	0.18(0.02-2.11)	0.173
	Model 2	0.43(0.08-2.33)	0.328	0.23(0.02-2.89)	0.255

CI: confidence interval
Model 1: crude
Model 2: adjusted for age, sex, and total energy intake

The association of total MET and its components (leisure time, occupation, household work and transportation) in SAT and VAT with PPARγ expression in non-obese and obese participants are presented in Tables 2 and 3, respectively. After controlling for age, sex, and total energy intake, significant associations were found between total MET, occupation and household

work with SAT PPARγ level among obese participants (*P*<0.05). Moreover, significant associations were found between total MET, leisure time and occupation with VAT PPARγ expression in obese subjects (*P*<0.05).
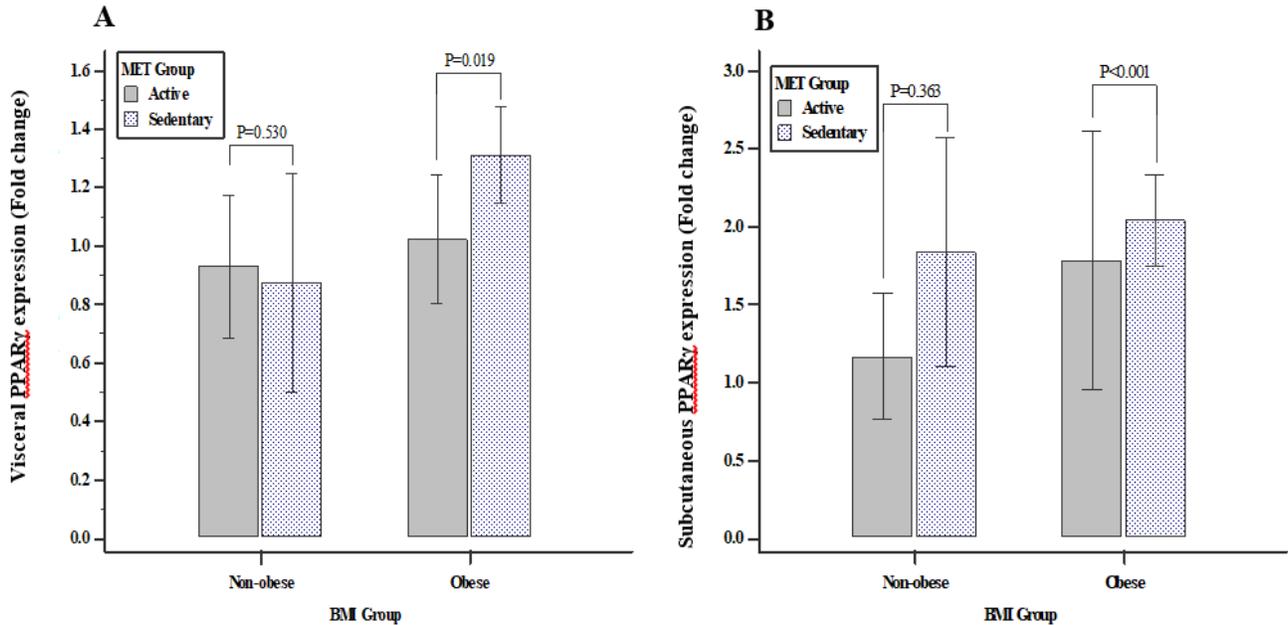

Fig. 2: A) PPARγ expression in visceral fat tissue in active and sedentary non-obese and obese participants; B) PPARγ expression in subcutaneous fat tissue in active and sedentary non-obese and obese participants; Data are represented as mean±1SEM

Table 3: The association of physical activity and its components with *PPARγ* gene expression from visceral adipose tissue of non-obese and obese participants

Variable		Non-obese		obese	
		β (95% confidence interval)	*P* value	β (95% confidence interval)	*P* value
Total physical activity	Model 1	3.14(0.79-12.43)	0.103	0.24(0.09-0.64)	0.005
	Model 2	3.23(0.38-27.64)	0.284	0.22(0.07-0.66)	0.007
Leisure time	Model 1	0.28(0.06-1.25)	0.096	0.18(0.03-1.14)	0.068
	Model 2	0.25(0.04-1.41)	0.117	0.05(0.00-0.70)	0.026
Occupation	Model 1	0.63(0.16-2.46)	0.511	0.16(0.04-0.56)	0.004
	Model 2	0.96(0.19-4.93)	0.963	0.11(0.03-0.46)	0.002
Household work	Model 1	2.50(0.77-8.16)	0.129	0.46(0.17-1.20)	0.112
	Model 2	1.87(0.47-7.40)	0.371	0.60(0.22-1.67)	0.329
Transportation	Model 1	0.62(0.14-2.66)	0.518	0.00(0.00-0.00)	0.861
	Model 2	0.73(0.13-3.96)	0.711	0.00(0.00-0.00)	0.778

Model 1: crude
Model 2: adjusted for age, sex and total energy intake

Discussion

In this study, it was demonstrated that total PA in obese participants was positively associated with PPARγ expression in both VAT and SAT. Regarding the component of total PA, there was also direct association between occupation and household work with SAT PPARγ level in obese participants. Furthermore, positive association between leisure time and occupation with VAT PPARγ expression was observed in obese participants.

Frequent PA increases energy expenditure and may facilitate weight management(25). The physiological mechanisms of responses to PA are quite well clarified, but the genetic basis of these complicated processes remains mostly unclear(12). PPARγ, a key transcription factor in the activation of adipocyte metabolism, modulates gene expression in adipocytes and affects cellular and systemic lipid metabolism related to obesity, diabetes and cardiovascular diseases (26). This study showed that total MET is directly associated with PPARγ gene expression in VAT and SAT among obese subjects. Our findings support the hypothesis that regular PA can change the adipose tissue PPARγ mRNA levels. Previous studies demonstrated that exercise training robustly activated PPARγ in rodents and humans (27, 28). After intensive physical training for 4 weeks, SAT PPARγ expression increased significantly in response to physical training(27). Furthermore, these findings indicated up-regulation of PPARγ level from fat tissues of rats (28, 30). Intensiveness of physical training showed an elevation of adipose tissue PPARγ expression in low-intensity, moderate intensity and high strength group of rats compared to controls (15). These studies focused on the intervention of periodic training and assessed the PPARγ expression in response to different intensity of exercises; however, regular PA is important because this was a crucial component of lifestyle. Given this, it appears that chronic higher PA, regardless of its type(e.g., leisure- or occupational-related), in addition to the periodic exercise,

may up-regulate PPARγ expression in fat deposits (31).

In the present study, PPARγ expression was reduced in active individuals compared to sedentary individuals. However, after regression modeling, a positive association was observed between PPARγ levels and PA in obese individuals. In fact, with each unit of increase in PA, PPARγ levels increased 12 and 22% in SAT and VAT, respectively. Since the persistence of the healthy metabolic state depends on adipogenesis in SAT, as the largest white adipose tissue, PPARγ has an important role, as the key regulator to activate differentiation of preadipocytes by promoting the expression of metabolic adipocyte genes (32). Exclusive activation of PPARγ in adipocytes induced systemic insulin sensitization; the effect was comparable to treatment with anti-diabetic drugs in the thiazolidinedione class (33). In previous studies where the subjects were prediabetic, there was a decrease in PPARγ expression, a sign of an unhealthy metabolic state or lipotoxicity (34). In the same way, those studies that had no limitation for fasting blood sugar (FBS) and diabetic or prediabetic individuals among the samples reported reduction (27, 35) or elevation in PPARγ gene (36), depends on the proportion of normoglycemic individuals.

Although applying a questionnaire to evaluate the METs is a subjective manner, and it is prone to recall-bias, previous validation studies indicated that IPAQ reasonably accurate measures of the average long-term PA (37). Interestingly, there was no substantial difference between obese and non-obese participants concerning total PA components, including occupational, leisure, and housework. Leisure-time PA refers to exercise, sports, or recreation, which is characterized by high, moderate, or intensive activity that is not related to regular work, housework, or transport activities directly associated with PPARγ expression. The current recommendations mostly concentrated on the health benefit of Leisure-time PA on preventing and treating metabolic disorders; however, occupation, transportation, and housework may affect adipose tissue metabolism (16). Housework-related activity is an important

factor that could have contributed to enhancing the total activity level, which female adults primarily carry out.

The PPARγ mRNA expression in both VAT and SAT in participants with obesity was higher than that in non-obese ones. Consistent with our findings, *PPARγ* gene expression in VAT and SAT in individuals with obese(BMI>30kg/m^2) was higher compared to lean subjects(BMI<25kg/m^2)(27). Besides, the findings of a study demonstrated a significant increase in PPARγ expression in SAT in morbid obese cases (36). Furthermore, in a study which categorized their participants similar to ours, PPARγ expression in SAT in obese (BMI>30kg/m^2) participants were higher than in non-obese(BMI<30kg/m^2); however, there was no significant difference between groups regarding VAT PPARγ expression (36). Unlike our findings, Afzal et al showed a significant lower PPARγ level in obese individuals compared to participants with normal weight (37). Moreover, like our results, Montague et al found an inverse correlation between BMI and PPARγ expression in adipocytes (38). These controversial results in the different studies could be an important issue in the molecular mechanism of PPARγ in the onset and progression of obesity. It seems that classifying participants, according to their BMI, can help in the accuracy of results and needs more investigation to clear the main role of this gene in obesity. Changes may be related to the chronic inflammatory in obese adipose tissue.

In the current study, some limitations need to be described. Because of the cross-sectional nature of the study, causal inferences cannot be made. However, as it is less likely that PPARγ impresses body movement, we assumed that PA might affect PPARγ. Second, as the findings of the current study were exploratory, the sample size in our study was not enough to have a stratified analysis based on overweight/normal. Third, although our sampling was a random selection study and all subjects undergoing abdominal surgery (obesity, appendectomy and hernia repair, etc.) were included, most young people had undergo obesity surgery and older people general surgery. Therefore, the obese group had a younger mean age than the control group. Finally, the lack of measuring serum concentration of inflammatory factors were another limitation.

The strengths of the current study include this is the first study providing data on the usual PA and its association with the adipose tissues PPARγ mRNA levels. Also, the observational design of the current study reflected long-term PA on PPARγ in a population with a wide range of BMI.

Conclusion

Usual PA positively associated with *PPARγ* gene expression in VAT and SAT only in participants with obesity. Besides, PA components, including leisure, occupation, and household-related activity levels among obese participants, were associated with higher PPARγ gene expression. These findings revealed that higher PA as an indispensable part of a healthy lifestyle might affect its benefit by regulating *PPARγ* gene expression.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

Conflict of interest

The authors declare that there is no conflict of interest.

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