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NOTE

Visceral adiposity is associated with SIRT1 expression in peripheral blood mononuclear cells: A pilot study

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Abstract. Sirtuin1 (SIRT1) is activated during calorie restriction and appears to be related to energy balance through glucose or lipid metabolism and insulin signaling. These findings suggest that SIRT1 may play a role in the pathophysiology of visceral obesity. Therefore, we investigated the relationship between SIRT1 gene expression in circulating peripheral blood mononuclear cells (PBMCs) and abdominal visceral adiposity as measured by computed tomography. We recruited 43 men and women without history of diabetes or cardiovascular disease Biomarkers of metabolic disease and body composition by computed tomography were assessed. SIRT1 gene expression was determined using isolated PBMCs. SIRT1 expression levels negatively correlated with body mass index, waist circumference, abdominal visceral fat area, and homeostasis model of assessment of insulin resistance (HOMA-IR) and positively correlated with adiponectin levels. Results of step-wise multiple regression analysis revealed that abdominal visceral fat area and HOMA-IR were independently associated with SIRT1 expression. The significant association between abdominal visceral fat accumulation and SIRT1 gene expression in PBMCs suggests that SIRT1 may be a new therapeutic target for the prevention of disease related to obesity, especially visceral obesity.

Key words: SIRT1, Visceral fat, Obesity

SIRTUIN1 (SIRT1), a NAD+-dependent deacetylase, is activated during calorie restriction and is associated with increased lifespan [1]. SIRT1 has attracted interest because of its possible role in cellular energy balance through glucose or lipid metabolism [2, 3]. In addition, increasing evidence suggests that decreased SIRT1 expression or activity contributes to the pathogenesis of diseases related to insulin resistance, such as metabolic syndrome and diabetes mellitus [4, 5].

These findings suggest that SIRT1 may also play a role in the pathophysiology of visceral obesity. Although the biochemical mechanisms that underlie visceral obesity remain elusive, visceral obesity is associated with many metabolic diseases, and visceral fat accumulation is a common feature of aging in humans and animal models [6-8]. Moreover, a recent study showed that calorie restriction more clearly reduced visceral fat accumulation than subcutaneous fat accumulation [9]. However, the relationship between SIRT1 and visceral obesity in humans is not completely clear.

Therefore, we investigated the relationship between SIRT1 gene expression in circulating peripheral blood mononuclear cells (PBMCs) and abdominal visceral adiposity as measured by computed tomography (CT).

Materials and Methods

Subjects

We recruited 43 voluntary healthy participants who visited the Department of Family Medicine at Severance Hospital. All participants provided written informed consent, and the study protocol was approved by the institutional review board of Severance Hospital, Seoul, Korea.

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All participants were apparently healthy and had no previous diagnosis or evidence of cardiovascular disease, diabetes, moderate to severe hypertension (resting blood pressure >170/100 mm Hg), dyslipidemia, body weight fluctuation of 5 kg in the previous 6 months, acute infectious disease, or chronic inflammatory disease. In addition, we excluded patients who had taken medication that could affect metabolic function.

Study design and methods

All participants completed a lifestyle questionnaire that included items about cigarette smoking, alcohol consumption, and physical activity. Alcohol consumption was defined as consuming alcohol more often than once a week. Regular exercise was defined as physical exercise or physical work performed for more than 30 min at least three times a week. Body mass index (BMI) was calculated by dividing weight by height squared (kg/m^2). We measured waist circumference at the midpoint between the lower border of the rib cage and the iliac crest. Abdominal adipose tissue areas were quantified by CT (Tomoscan 350; Philips, Mahwah, NJ, USA). A 10-mm CT slice scan was acquired at the L4 to L5 level to measure the abdominal visceral fat area and the subcutaneous fat area. Fat attenuation was determined by measuring the mean value of all pixels within the range of -150 to -50 Hounsfield units.

Blood samples were obtained from each participant after a 12-hour overnight fast. We measured fasting glucose, aspartate aminotransferase, alanine aminotransferase, total cholesterol, triglycerides, and high-density lipoprotein cholesterol levels using an ADVIA 1650 chemistry system (Bayer, Terrytown, NY, USA). Low-density lipoprotein cholesterol levels were calculated using the Friedewald equation. Fasting insulin was measured by a chemiluminescence immunoassay (Roche, Indianapolis, IN, USA). Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) index (fasting insulin [units per milliliter] × fasting glucose [millimolar]/22.5). High-sensitivity C-reactive protein was measured by a latex-enhanced immunoturbidimetric assay using an ADVIA 1650 Chemistry system (Bayer). Serum adiponectin levels were determined with an enzyme immunoassay kit (Mesdia, Seoul, Korea); the inter-assay and intra-assay variability were 4.6±1.4% and 4.5±0.6%, respectively.

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was used to determine SIRT1 expression. PBMCs were collected from 5 mL of anticoagulated blood from each participant and isolated by Ficoll-Hypaque (GE Healthcare Life Science, Pittsburgh, PA. USA) gradient centrifugation (300 rpm, 30 min). Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany). First-strand cDNA was synthesized with QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR amplification was performed in a Takara Thermal Cycler Dice real-time system (Takara, Shiga, Japan) for 40 cycles of denaturing at 95°C for 10 s, annealing at 58°C for 10 s, and extension at 72°C for 10 s. The following primers were used: SIRT1: forward 5′-GCCTCACATGCAAGCTCTAGTGAC, reverse 5′-TTCGAGGATCTGTGCCAATCATAA; glyceraldehyde 3-phosphate dehydrogenase (GAPDH): 5′-GCACCGTCAAGGCTGAGAAC, reverse 5′-TGGTGAAGACGCCAGTGGA (Takara Korea, Seoul, Korea). All reactions were performed in triplicate, and target gene expression was normalized using GAPDH as the internal control.

Statistical analysis

We analyzed all data using the statistical program SAS 9.1 (SAS Institute, Cary, NC, USA). Pearson's and Spearman's correlation coefficients were calculated to evaluate the relationship between SIRT1 levels and metabolic risk factors. A step-wise multiple linear regression analysis was used to clarify which factors were significantly associated with SIRT1 levels. Significance was defined as $P<0.05$.

Results

The clinical characteristics of participants and correlation between SIRT1 expression levels and metabolic factors are summarized in Table 1. SIRT1 expression levels negatively correlated with BMI, waist circumference, and HOMA-IR levels and positively correlated with adiponectin values. In addition, the SIRT1 expression levels negatively correlated with visceral fat areas but not with subcutaneous fat areas, as determined by CT (Table 1; Fig. 1).

In stepwise multiple regression analysis including age, gender, smoking status, alcohol consumption, exercise, BMI, visceral fat area, subcutaneous fat area, mean blood pressure, fasting glucose, HOMA-IR, total cholesterol, triglyceride, high-density lipoprotein cholesterol, aspartate aminotransferase, alanine aminotransferase, high-sensitive C-reactive protein, and adiponectin, only visceral fat area and HOMA-IR were sig-

Table 1 Clinical characteristics of study participants and relationships between SIRT1 expression and metabolic factors

	n=43	*r*	*P*
Age (y)	30.35±5.70	-0.20	0.20
Men (%)	28 (65.12)		
Adiposity index			
BMI (kg/m^2)	25.38±4.60	-0.33	0.04*
Waist (cm)	86.52±12.19	-0.38	0.01*
Visceral fat area (cm^2)	85.48±45.53	-0.50	<0.01*
Subcutaneous fat area (cm^2)	189.23±97.58	-0.16	0.32
Metabolic variables			
Systolic BP (mmHg)	122.00±15.13	-0.23	0.15
Diastolic BP (mmHg)	73.00± 9.99	-0.21	0.18
Fasting glucose (mg/dL)	78.07±8.69	0.12	0.45
Fasting insulin (μIU/mL)	5.51 (3.90–7.56)	-0.17	0.29
HOMA-IR	1.05 (0.73–1.59)	-0.31	0.04*
Total cholesterol (mg/dL)	175.44±28.89	-0.06	0.70
Triglyceride (mg/dL)	85.74±33.22	-0.20	0.21
HDL-cholesterol (mg/dL)	55.36±14.06	0.26	0.09
LDL-cholesterol (mg/dL)	102.94±28.47	-0.14	0.36
AST (IU/L)	20 (17–22)	-0.24	0.12
ALT (IU/L)	18 (13–29)	-0.30	0.05
hsCRP (mg/L)	0.05 (0.03–0.09)	-0.17	0.26
Adiponectin (μg/mL)	5.42±2.49	0.33	0.03*
SIRT1 expression	1.82±1.40		
Smoking status (%)			
Nonsmokers	30 (69.77)		
Former smokers	4 (9.30)		
Current smokers	9 (20.93)		
Alcohol consumption (%)	13 (30.23)		
Exercise (%)	8 (18.6)		

BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model of assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; hsCRP, high-sensitivity C-reactive protein.
Data are expressed as mean±standard deviation or median with interquartile range (25th–75th percentile). The Shapiro-Wilcoxon test was used to test the Gaussian distribution of parameters. Coefficients (*r*) and *P*-values were calculated by the Pearson correlation model (normally distributed variables; age, BMI, waist, visceral fat area, subcutaneous fat area, systolic BP, diastolic BP, fasting glucose, total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, and adiponectin) or Spearman's correlation model (non-normally distributed variables; fasting insulin, HOMA-IR, AST, ALT, and hsCRP). *, $P<0.05$

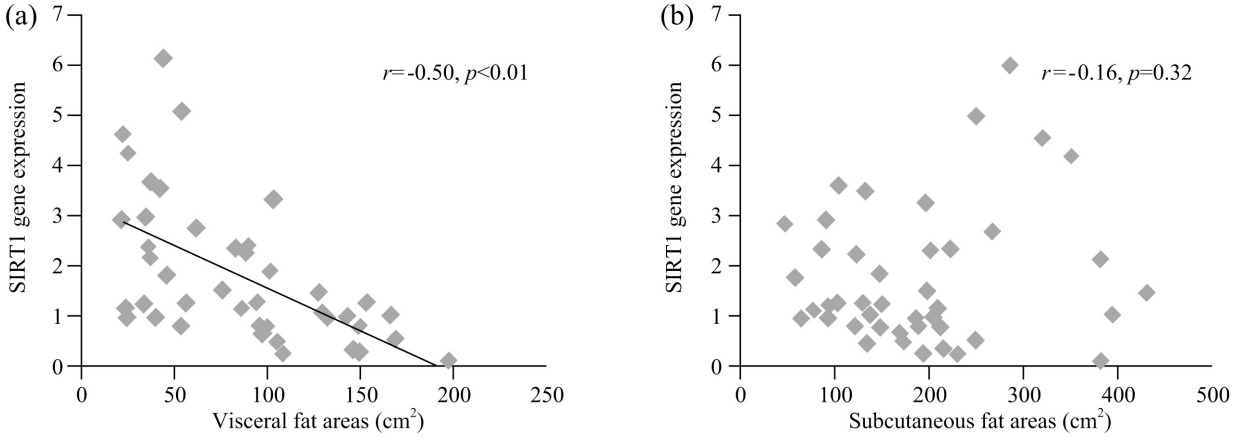

Fig. 1 Relationships between SIRT1 expression and abdominal visceral fat areas (a) and subcutaneous fat areas (b).

Table 2 Step-wise multiple linear regression analysis to identify clinical variables associated with SIRT1 expression as a dependant variable.

Variables	Model r^2	SE	F	*P*
Visceral fat areas(cm^2)	0.29	0.01	12.36	<0.01*
Fasting glucose (mg/dL)	0.08	0.02	3.67	0.07
HOMA-IR	0.08	0.29	4.43	0.04*
Subcutaneous fat area (cm^2)	0.05	0.01	3.15	0.09

All variables left in the model are significant at the 0.15 level. No other variable met the 0.15 significance level for entry into the model. Variables included in the model for SIRT1 expression: age, gender, smoking status, alcohol consumption, exercise, BMI, visceral fat area, subcutaneous fat area, mean blood pressure, fasting glucose, HOMA-IR, total cholesterol, triglyceride, high-density lipoprotein cholesterol, aspartate aminotransferase, alanine aminotransferase, high-sensitive C-reactive protein, and adiponectin. *, $P<0.05$

nificantly associated with SIRT1 expression, explaining 37% of the variance in SIRT1 levels (Table 2).

Discussion

Visceral obesity is strongly associated with age-related phenomenon such as insulin resistance, atherosclerosis, inflammation, oxidative stress and mitochondrial dysfunction, but the biochemical mechanisms underlying visceral obesity remain elusive [10]. SIRT1 has been the focus of research because of its role in regulating longevity, although clinical studies have not yet provided direct evidence for its ability to prevent age-related medical complications [1, 11]. However, SIRT1 transcription is decreased in the visceral adipose tissue of morbidly obese patients with severe hepatic steatosis, and genetic variation in SIRT1 was associated with visceral obesity in a Belgian case-control association study [12, 13].

In view of these findings, we hypothesized that the metabolic-sensor SIRT1 may be associated with visceral adiposity. Our results showed that abdominal visceral fat, as measured by CT, negatively correlated with SIRT1 gene expression in 43 healthy participants.

Although it is unclear whether visceral adiposity affects SIRT1 expression or SIRT1 regulates fat distribution, there are some possible explanations for this relationship. Visceral fat increases free fatty acid flux and inhibits insulin action, whereas SIRT1 has been shown to decrease lipogenesis and increase fatty acid oxidation [14]. Furthermore, SIRT1 has a positive effect on insulin signaling [4]. In line with previous studies, we found an independent relationship between SIRT1 levels and insulin resistance indices. In addition, the protective role of SIRT1 in oxidative stress, which is increased with visceral fat accumulation and metabolic syndrome, was recently evaluated [15-17]. Mitochondrial function is another possible mechanism to explain the relationship between SIRT1 and visceral adiposity. Visceral adiposity is associated with altered mitochondrial activity and reduced glucose tolerance in the elderly, whereas SIRT1 promotes mitochondrial activity and biogenesis in multiple tissues [18, 19]. Lastly, adipokine or proinflammatory cytokine levels may predict this relationship. Visceral fat accumulation leads to hypoadiponectinemia and an increase in proinflammatory cytokines, which promote insulin resistance [20]. In contrast, resveratrol stimulation of SIRT1 expression increased serum adiponectin concentrations, improved hyperinsulinemia, and produced anti-inflammatory effects in the visceral adipose tissue of obese Zucker rats [21]. Similarly, our results show that SIRT1 correlates positively with serum adiponectin levels. Further studies are needed to elucidate the precise mechanism between SIRT1 and visceral adiposity.

The present study has several limitations. The cross-sectional design of the study does not allow clarification of any causal mechanisms. The small sample size is another limitation; a much larger population in a prospective clinical trial setting is required to better understand the metabolic role of SIRT1 in visceral adiposity. Moreover, we evaluated SIRT1 mRNA levels, which may not correspond precisely to protein levels. Thus, the relationship between SIRT1 and visceral

adiposity requires further investigation.

In summary, we found that visceral adiposity is associated with SIRT1 expression in peripheral blood mononuclear cells in apparently healthy Korean adults. Our findings suggest that SIRT1 may be a new therapeutic target for the prevention of diseases related to obesity, especially visceral obesity. The pathophysiological and clinical significance of this finding requires further investigation.

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Conflict of Interest

Nothing to declare.

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