Association between low SIRT1 expression in visceral and subcutaneous adipose tissues and metabolic abnormalities in women with obesity and type 2 diabetes

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A B S T R A C T
Aims: To assess the importance of adipose tissue sirtuin 1 (SIRT1) in the regulation of whole-body metabolism in humans with obesity and type 2 diabetes.

Methods: In total, 19 non-diabetic obese women, 19 type 2 diabetic women undergoing gastric bypass surgery, and 27 normal-weight women undergoing gynecological surgery (total 65 women) were enrolled. Their anthropometric variables, abdominal fat distribution and metabolic parameters, serum adiponectin concentrations, and SIRT1 mRNA and protein and adiponectin mRNA expressions in visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were measured.

Results: SIRT1 mRNA levels in VAT and SAT were similar and these levels were suppressed in obese and type 2 diabetic women compared to normal-weight subjects. These decreases in SIRT1 expression were observed in both adipocytes and non-fat cells. There was a strong association between adipose tissue SIRT1 mRNA and protein levels. Adipose SIRT1 expression correlated inversely with HOMA-IR and other insulin resistance-related parameters. Adipose SIRT1 and adiponectin mRNA expression correlated very strongly and positively. SIRT1 mRNA level in VAT correlated inversely with visceral obesity whereas its expression in SAT correlated negatively with body mass index.

Conclusions: Adipose tissue SIRT1 may play a key role in the regulation of whole body metabolic homeostasis in humans. Downregulation of SIRT1 in VAT may contribute to the metabolic abnormalities that are associated with visceral obesity.

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1. **Introduction**

The yeast protein silent information regulator 2 (SIR2) is an NAD⁺-dependent protein deacetylase that is linked to the extension of lifespan that occurs in calorie restriction [1]. SIRT1, the mammalian protein with the highest homology to SIR2, is also associated with increased longevity in rodents on restricted calorie diets [2]. Calorie restriction also produces metabolic profiles that are associated with improvements in obesity-related disorders such as insulin resistance and type 2 diabetes; it has been claimed that the beneficial cellular effects of calorie restriction are largely mediated by the induction of SIRT1 [3]. Moreover, the dependence of SIRT1 activity on NAD⁺ levels means that it may serve as a sensor of changes in the nutritional status of cells and thereby mediate the regulation of metabolic processes [4]. Thus, it appears that SIRT1 may regulate the metabolic pathways that are modulated in calorie restriction and in obesity that is mainly caused by long-term calorie overload.

Adipose tissue serves as a storage site for lipids but also as an endocrine organ that secretes biologically active molecules that participate in whole-body energy metabolism and inflammation. Thus, by dysregulating these functions, adipose tissue itself is involved in the development of obesity-related disorders [5]. In adipose tissues, SIRT1 suppresses adipocyte differentiation and lipid accumulation by repressing peroxisome proliferator-activated receptor-γ [6]. SIRT1 is also a major regulator of the transcription and secretion of adiponectin, an adipokine that appears to be an important mediator of insulin sensitivity [7,8]. In addition, SIRT1 suppresses adipose tissue inflammation by repressing nuclear factor (NF)-κB signaling [9–12]. Conversely, adipocyte SIRT1 expression is suppressed in high-fat diet fed and genetically obese rodents [8]. Thus, impaired SIRT1 expression and activity in adipose tissues has been implicated in the development of obesity-related diseases [13–15].

Although SIRT1 has been extensively studied in cultured cells and experimental animals, there are only a few studies that have examined the expression and functions of SIRT1 in human adipose tissue. One of these showed that fasting in humans increases SIRT1 mRNA expression in subcutaneous adipose tissue (SAT) [16]. Conversely, SIRT1 mRNA expression in SAT is suppressed in obese subjects [9,16]. In addition, SIRT1 mRNA levels correlate inversely with macrophage content in SAT of lean and obese human subjects [9]. Recently, SAT SIRT1 mRNA levels were found to associate significantly with energy expenditure and insulin sensitivity during hyperinsulinemic euglycemic clamps [17].

In the present study, the contribution of impaired SIRT1 expression in adipose tissues to the metabolic derangements associated with human obesity was assessed by addressing the following questions: (1) is SIRT1 expression in visceral adipose tissue (VAT) and SAT suppressed in obesity and type 2 diabetes; (2) if so, do SIRT1 mRNA levels associate with other metabolic parameters or abdominal fat distribution; and (3) do SIRT1 mRNA levels in human adipose tissues correlate with adiponectin mRNA levels in the same tissues? To answer these questions, paired VAT and SAT samples were obtained from obese, type 2 diabetic, and normal women undergoing abdominal surgery.

2. **Materials and methods**

2.1. **Study subjects**

The study cohort consisted of 19 non-diabetic obese women (BMI ≥ 30), 19 women with type 2 diabetes, and 27 normal-weight (BMI ≤ 25) women. All obese and diabetic subjects were patients who underwent laparoscopic Roux-en-Y gastric bypass at the Obesity Center of the Inha University Hospital (Incheon, Korea) between July, 2010 and March, 2011. Four days before surgery, these patients were admitted and underwent routine physical examinations, systematic fasting biological analyses, and abdominal computerized tomography (CT). In addition, normal-weight women undergoing elective abdominal surgery for benign conditions (uterine myoma, teratoma, cystadenoma, adenomyosis, or endometriosis) at the Gynecology Unit of the Asan Medical Center (Seoul, Korea) were recruited as controls. Women with secondary causes of obesity, those who were pregnant or lactating, and those with evidence of malignancy or severe hepatic or renal disease were excluded. All subjects provided written informed consent at enrollment. Some of the samples of these subjects were analyzed in previous studies. The study protocol was approved by the Institutional Review Boards of the Asan Medical Center and the Inha University Hospital. All applicable institutional regulations on the ethical use of human volunteers were followed during this research.

2.2. **Assessment of blood pressure (BP) and anthropometric measurements**

BP was measured in the morning. Each patient was seated in a quiet room for 10 min. Thereafter, three successive BP measurements using a standard mercury sphygmomanometer were obtained at 5 min intervals. The results were averaged. Anthropometric measurements were taken while the subjects were dressed in light clothing but without shoes. Height to the nearest 0.1 cm and weight to the nearest 0.1 kg were measured by using an automatic height-weight scale. Body mass index (BMI) was calculated by dividing the weight in kg by the square of the height in m.

2.3. **Estimation of abdominal fat distribution**

The distribution of abdominal fat was assessed by CT using a Siemens Somatom Scanner (Erlangen, Germany), as previously described [18,19]. The subjects were placed in a supine position, and a 10 mm thick cross-sectional scan centered on the L4–L5 vertebral disk space was obtained by using a skeletal radiograph as a reference to establish the position of the scan to the nearest millimeter. The area of total abdominal adipose tissue (TAT) was measured by delineation with a graphic pen, followed by computation of the area using an attenuation range of −190 to 30 Hounsfield units. The VAT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The SAT area was calculated by...
subtracting the VAT area from the TAT area. In addition, the ratio of the VAT area to the SAT area (VSR), which is an index of visceral obesity [20,21], was calculated.

2.4. Measurements of metabolic variables and serum adiponectin concentration

The subjects discontinued medications for diabetes and dyslipidemia 3 days before blood withdrawal. The blood samples were obtained after a 12 h fast and the plasma was separated immediately by centrifugation. Plasma glucose concentrations were measured by the glucose oxidase method, and insulin concentrations were assessed by using a human insulin radioimmunoassay kit (TFB, Tokyo, Japan). The homeostasis model assessment for insulin resistance index (HOMA-IR) was calculated by using the formula: fasting plasma insulin in μU/mL x fasting plasma glucose in mg/dL ÷ 405, as previously described [22]. Total cholesterol was determined by enzymatic procedures using an autoanalyzer (Hitachi-747; Hitachi, Tokyo, Japan). Serum adiponectin concentrations were measured by using a commercial ELISA kit (AdipoGen, Incheon, Korea) according to the manufacturer’s instructions. The assay sensitivity was 100 pg/mL, and the intra- and inter-assay coefficients of variance were 3.0–3.8 and 2.8–5.5%, respectively.

2.5. Sampling of adipose tissue

During surgery, 2–5 g VAT and SAT samples were removed. VAT was obtained from the distal portion of the greater omentum (i.e., the epiploon), and SAT was taken from the site of surgical incision (the lower abdomen). The samples were taken immediately to the laboratory in ice-cold 0.9% (w/v) saline, frozen in liquid nitrogen, and stored at −80°C for subsequent analyses.

2.6. Fractionation of adipocytes and stroma/vascular cells (SV)

SIRT1 and adiponectin mRNA expression in adipocytes and SV fractions of adipose tissue were measured. VAT was used for this purpose because there was enough of it to divide it into the two fractions. Each fresh adipose tissue sample was washed with Krebs-Ringer-Henseleit (KRH) buffer and digested with 1 mg/mL collagenase (Worthington, Freehold, NJ) in KRH buffer containing 1% (w/v) BSA for 40–60 min at 37°C. The collagenase-digest was separated from undigested tissue by filtration through 100 μm nylon mesh (Falcon, Franklin Lakes, NJ). The floating adipocyte fraction was collected and washed three times with KRH buffer. Non-floating cells isolated from the collagenase-digest were centrifuged for 15 min at 1000 rpm and the pellet, which was the SV fraction, was collected. The adipocyte and SV fractions were used for RNA extraction.

2.7. RNA isolation and real-time quantitative RT-PCR

Total RNA was extracted from adipose tissue samples by using TRIzol (Invitrogen, Carlsbad, CA; 1 mL/100 mg tissue) according to the manufacturer’s instructions. RNA purity was assessed by using a NanoDrop spectrophotometer. RNA was reverse-transcribed into cDNA by using Superscript III reverse transcriptase (Invitrogen) and oligo-dT primers. Amplification reactions using 25 μL aliquots and the SYBR Green QPCR master mix kit (Bio-Rad, Hercules, CA) were performed by using a Roche Light Cycler system (Roche Molecular Biochemicals, Mannheim, Germany). The following primers were used: human SIRT1 5’-caggtctatgctctttgtgc-3’ (forward) and 5’-cttaggacatcagagaactac-3’ (reverse); adiponectin 5’-ggaagaaagagaagcaggtgt-3’ (forward) and 5’-agaaagactggcatgtgg-3’ (reverse); monocyte chemotactic protein-1 (MCP-1) 5’-ccaaagacgtgtcatcaccaggc-3’ (forward) and 5’-gattttgctgcctgagttg-3’ (reverse); inducible NO synthase (iNOS) 5’-aacaagctatccctcttagatc-3’ (forward) and 5’-tccgagttgtagtc-3’ (reverse); TNFa 5’-gagctcataatcgaggttc-3’ (forward) and 5’-agagttctcaagtaatgtagct-3’ (reverse); CD68 5’-ctactagctgcgtagtaac-3’ (forward) and 5’-atagatggagcagaca-3’ (reverse); IL-1β 5’-gacgtaggtagtagatc-3’ (forward) and 5’-ctgtaggtagctgtagct-3’ (reverse); β-actin 5’-gagcagggctcaccaca-3’ (forward) and 5’-ggtgctcgaatgccc-3’ (reverse); and importin 8 (IP08) 5’-agagaggaagatgatgc-3’ (forward) and 5’-cgactcagctgtatagc-3’ (reverse). Expression of mRNA was quantified by the second derivative maximum method, which determines the crossing points of individual samples by using an algorithm that identifies the first turning point of the fluorescence curve. SIRT1, adiponectin, MCP-1, iNOS, TNFa, CD68, and IL-1β mRNA expression was normalized relative to the expression of β-actin or IPO8 mRNA.

2.8. Western blot analysis

Adipose tissue samples were homogenized in extraction buffer (20 mM HEPES, pH 7.4, 2 mM EGTA, 1 mM DTT, 10% glycerol, 1% Triton X-100, protease inhibitor cocktail), rotated end-over-end for 1 h at 4°C, sonicated, and centrifuged at 12,000 rpm for 15 min at 4°C. After delipidation, the protein concentrations in the extracts were measured with the Bradford method. The proteins in the extracts were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membranes were blocked with 5% milk in TBST buffer (10 mM Tris, 150 mM NaCl, and 0.05% Tween 20, pH 7.4) for 1 h at room temperature and then incubated overnight at 4°C with anti-SIRT1 antibody (Santa Cruz, CA; 1:1000) and anti-actin antibody (Labfrontier, Seoul, Korea; 1:4000) in blocking solution. The membranes were washed with TBST buffer and incubated for 1 h at room temperature with horseradish peroxidase-linked secondary antibodies. The proteins were detected by using ECL plus reagents (Amersham, Buckinghamshire, UK) and the resulting signals were quantified by using GelQuantNET (BiochemLabSolutions.com).

2.9. Statistical analyses

The data are presented as means ± standard errors. Non-normally distributed data (HOMA-IR, adipose tissue adiponectin mRNA levels, and circulating glucose, insulin, triglyceride, and adiponectin level data) were log-transformed prior to analysis to generate a normal distribution. Correlation coefficients between the measured variables were calculated using by
Pearson’s correlation. Partial correlation coefficients between SIRT1 mRNA levels and other metabolic parameters were estimated after adjustment for age. For pair-wise group comparisons, Student’s paired or unpaired t-tests were performed. When more than two groups were compared, ANOVA followed by Tukey test was used. For all tests, a *p*-value < 0.05 was considered to be statistically significant. All statistical analyses were performed by using SPSS (version 19.0 for IBM; SPSS, Chicago, IL).

3. Results

3.1. Characteristics of the subjects

The clinical characteristics of the obesity, diabetes, and normal-weight control groups are described in Table 1 and their metabolic characteristics are summarized in Table 2. The obesity group (32.5 ± 1.98 yr) was significantly younger than the control group (39.7 ± 2.11 yr; *p < 0.05) and the diabetes group (45.6 ± 1.90 yr; *p < 0.01). Compared to the control group, the obesity group had significantly higher systolic and diastolic BP, HOMA-IR, and fasting plasma insulin and triglyceride concentrations, and significantly lower serum adiponectin levels. Although the obesity group had significantly higher VAT and SAT areas than the control group, it had a similar ratio between the two fat depots (VSR 0.30 ± 0.04 vs 0.38 ± 0.07; *p > 0.05). This indicates a lack of association with visceral obesity in these obese subjects. The diabetes group had significantly higher BMI, systolic BP, HOMA-IR, and plasma glucose, insulin, LDL-cholesterol and triglyceride levels than the control group. Compared to the obesity group, the diabetes group was older and their BMI (29.0 ± 0.97 kg/m²) was significantly lower. However, abdominal CT revealed severe visceral obesity (VSR 0.67 ± 0.06) in these diabetic subjects.

3.2. Expression of SIRT1 in adipose tissues

For all three groups, the SIRT1 mRNA levels in the VAT and SAT were compared. In all groups, the two fat depots did not differ significantly in terms of mean SIRT1 mRNA levels (Fig. 1A) and there was a strong correlation between the SIRT1 mRNA levels in the VAT and SAT (*r = 0.467; *p = 0.002; n = 65, data not shown). This indicates that SIRT1 mRNA was expressed at similar levels in the two fat depots. However,

### Table 1 – Clinical characteristics of the obesity, diabetes, and normal weight control groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Obesity</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>27</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Menopause</td>
<td>2</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Fatty liver</td>
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<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Patients receiving treatment for Hypertension</td>
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<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Beta-receptor blocker</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin inhibitor</td>
<td>1</td>
<td></td>
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<tr>
<td>Angiotensin receptor blocker</td>
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</tr>
<tr>
<td>Calcium channel blocker</td>
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<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
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<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Metformin</td>
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<td></td>
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</tr>
<tr>
<td>Sulfonylureas</td>
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<td></td>
<td>8</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Glucosidase inhibitor</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Statins</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

The data are the numbers of subjects.

### Table 2 – Metabolic parameters and abdominal fat distribution of the obesity, diabetes, and normal-weight control groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Obesity</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>27</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>39.7 ± 2.11</td>
<td>32.5 ± 1.98</td>
<td>45.6 ± 1.90*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2 ± 0.36</td>
<td>39.7 ± 1.32*</td>
<td>29.0 ± 0.97*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>115.0 ± 3.23</td>
<td>142.2 ± 3.79</td>
<td>128.6 ± 3.78*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>73.5 ± 2.43</td>
<td>84.1 ± 2.11</td>
<td>80.7 ± 2.48</td>
</tr>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>98.9 ± 3.26</td>
<td>98.1 ± 3.50</td>
<td>216.9 ± 11.24*</td>
</tr>
<tr>
<td>Plasma insulin (µU/mL)</td>
<td>6.5 ± 0.94</td>
<td>24.1 ± 2.57</td>
<td>14.6 ± 2.82*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.6 ± 0.24</td>
<td>5.9 ± 0.69</td>
<td>8.0 ± 1.61</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>179.7 ± 7.32</td>
<td>178.1 ± 7.42</td>
<td>195.2 ± 11.95</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>42.0 ± 2.43</td>
<td>48.4 ± 2.12</td>
<td>40.9 ± 2.86</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>93.9 ± 5.67</td>
<td>113.8 ± 6.12</td>
<td>117.9 ± 7.94</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>72.1 ± 5.81</td>
<td>112.5 ± 10.89*</td>
<td>199.4 ± 23.63*</td>
</tr>
<tr>
<td>Serum adiponectin (µg/mL)</td>
<td>7.05 ± 1.07</td>
<td>2.87 ± 0.53*</td>
<td>5.26 ± 1.74</td>
</tr>
<tr>
<td>Abdominal CT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAT area (cm²)</td>
<td>365.7 ± 83.4</td>
<td>679.7 ± 35.5*</td>
<td>404.7 ± 31.3*</td>
</tr>
<tr>
<td>SAT area (cm²)</td>
<td>86.9 ± 17.1</td>
<td>152.6 ± 14.2*</td>
<td>158.8 ± 17.7</td>
</tr>
<tr>
<td>VSR</td>
<td>0.38 ± 0.07</td>
<td>0.30 ± 0.04</td>
<td>0.67 ± 0.06*</td>
</tr>
</tbody>
</table>

The data are shown as means ± SE. *p < 0.05 vs the control group; †p < 0.05 vs the obesity group.

Abbreviations: BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein; CT, computerized tomography; VAT, total adipose tissue; VAS, visceral adipose tissue; SAT, subcutaneous adipose tissue; VSR, the ratio of visceral adipose tissue area to subcutaneous adipose tissue area.
3.3. Correlations between SIRT1 mRNA expression in adipose tissues and metabolic parameters and abdominal fat distribution

In the whole study cohort, SIRT1 mRNA levels in both VAT and SAT correlated strongly and negatively with fasting plasma glucose and triglyceride concentrations, and VSR (left column in Table 3). In addition, VAT SIRT1 mRNA correlated significantly and inversely with HOMA-IR and SAT SIRT1 mRNA correlated negatively with VAT area. After adjustment for age (right column in Table 3), the inverse correlations between VAT SIRT1 mRNA level and fasting plasma glucose, HOMA-IR, and VSR remained significant and there were significant negative correlations between SAT SIRT1 mRNA level and BMI, fasting insulin, HOMA-IR, and VAT area.

3.4. Relationship between SIRT1 and adiponectin mRNA expression in adipose tissues

Analysis of the relationship between SIRT1 mRNA and adiponectin mRNA expression in VAT revealed a strong positive correlation ($r = 0.451; p < 0.001$, Fig. 2A). This correlation was even stronger in SAT ($r = 0.598; p < 0.001$, Fig. 2B). Moreover, when adipocytes in VAT were separated from SV cells, a remarkably close relationship between isolated adipocyte SIRT1 and adiponectin mRNA levels was observed ($r = 0.678; p = 0.001$, Fig. 2C). While adiponectin mRNA expression in both VAT ($r = 0.284; p < 0.05$) and SAT ($r = 0.451; p < 0.001$) associated significantly with serum adiponectin
concentrations, the SIRT1 mRNA levels in either fat depot did not correlate significantly with serum adiponectin levels (data not shown).

Associations between SIRT1 mRNA and expression levels of inflammatory genes such as CD68, MCP-1, TNFα, IL-1β, iNOS were also examined in VAT and SAT. Among them, only IL-1β mRNA showed a significant negative correlation (r = –0.270; p < 0.05; n = 59) with SIRT1 mRNA in VAT. The relationship between SIRT1 mRNA and expression levels of the other genes did not reach a statistical significance in neither of the two fat depots (data not shown).

4. Discussion

There is increasing evidence that SIRT1 plays an important role in the regulation of energy metabolism and glucose homeostasis [3]. This, together with the observation that adipose tissue itself participates in the pathogenesis of obesity-related disorders [5], has focused attention on the role that impaired SIRT1 expression in adipose tissues may play in the development of obesity-related diseases. While there are increasing studies on SIRT1 in cultured cell lines and experimental animals, only a few studies on SIRT1 in human adipose tissues have been performed. Moreover, most of the latter studies only examined SAT. By contrast, in the present study, SIRT1 expression in both the VAT and SAT of women with obesity, type 2 diabetes, and normal weight was assessed.

![Fig. 2 – Close association between SIRT1 and adiponectin mRNA expression in human adipose tissue. Visceral adipose tissue (VAT, A) and subcutaneous adipose tissues (SAT, B) were removed from normal-weight, obese non-diabetic, and diabetic women (total n = 65) during surgery. Adipocyte fractions were isolated from visceral adipose tissues as described in the legend of Fig. 1C (n = 22) (C). The lines indicate the correlations between SIRT1 and adiponectin mRNAs in the adipose tissue preparations. SIRT1 and adiponectin mRNA levels are presented in arbitrary units (A. U.).](image)

The present study showed that the SIRT1 mRNA levels in VAT correlated highly with those in SAT and did not differ significantly from those in SAT. Moreover, as shown by previous reports on SAT of obese humans [9,16], SIRT1 mRNA expression in both VAT and SAT was markedly suppressed in
subjects with obesity and type 2 diabetes (Fig. 1A). Furthermore, there was a strong correlation between SIRT1 mRNA and protein levels (Fig. 1C). This indicates that the reduction in SIRT1 mRNA levels in the adipose tissues of obese and type 2 diabetic subjects is likely to reflect alterations in SIRT1 protein expression faithfully. The present study also showed clearly that in adipose tissues of obese and type 2 diabetic subjects, SIRT1 expression was suppressed in both adipocytes and non-adipocyte cells; moreover, the degree of suppression in both cell compartments was similar (Fig. 1B).

Next, to explore the association between adipose SIRT1 suppression and metabolic derangements, correlations between SIRT1 mRNA levels in adipose tissues and metabolic parameters were examined. SIRT1 mRNA levels in adipose tissues correlated inversely with stigmata of insulin resistance (Table 3). After adjustment for age, significant negative correlations between SIRT1 mRNA levels in both fat depots and the insulin resistance index HOMA-IR were detected. This is consistent with the observation that SIRT1 mRNA level in SAT of non-diabetic offspring of type 2 diabetic patients correlated significantly with energy expenditure and insulin sensitivity during hyperinsulinemic euglycemic clamp [17]. In addition, in the present study, SAT SIRT1 mRNA associated inversely with BMI, as has been reported previously [9], and VAT area. Even more interestingly, suppression of SIRT1 in VAT associated much more closely with visceral obesity than with adiposity per se. Depot-specific differences in metabolic function have been noticed before: subcutaneous fat accumulation represents the normal physiological buffer for excessive energy intake with limited energy expenditure whereas VAT associates more strongly with an adverse metabolic risk profile [23]. Considering these observations, the present results suggest that SIRT1 may play key roles in regulation of metabolism in both fat depots.

In adipose tissues, SIRT1 is thought to regulate the transcription and secretion of adiponectin [7,8], which sensitizes cells to insulin, enhances fatty acid oxidation, suppresses hepatic gluconeogenesis, and protects against chronic inflammation [24]. Adiponectin is produced exclusively by mature adipocytes [25] and circulates at rather high concentrations [25,26]. Circulating adiponectin levels are decreased in obese humans, particularly those with visceral obesity, and correlate inversely with insulin resistance [27]. Adiponectin and SIRT1 expressions are both reduced in the adipose tissues of high fat diet-induced obese and db/db diabetic mice [8]. In 3T3-L1 adipocytes, SIRT1 increases adiponectin transcription by activating forkhead transcription factor O1 (FoxO1) and by enhancing the interaction between FoxO1 and CCAAT/enhancer-binding protein-α [8]. However, to our knowledge, a direct relationship between SIRT1 and adiponectin expressions has not been demonstrated in human adipose tissues previously. The present study revealed a remarkably strong correlation between SIRT1 mRNA and adiponectin mRNA in VAT, and this association was even stronger in SAT (Fig. 2A and B). Since adiponectin is exclusively expressed by adipocytes [25] and VAT has a greater proportion of non-fat cells than SAT [23], our observations support the notion that there may be a connection between SIRT1 and adiponectin gene transcription. In line with this, there was also a remarkably close relationship between SIRT1 and adiponectin mRNA levels in isolated adipocytes (Fig. 2C).

There are probably multiple mechanisms in adipose tissues by which SIRT1 regulates the metabolic adaptations that are linked to human obesity. For example, SIRT1 may serve as a negative regulator of inflammatory pathways that are activated by calorie overload in adipose tissues [10]. Supporting this is that there is an inverse relationship between SIRT1 mRNA levels in adipose tissue and high-sensitivity C-reactive protein concentrations in human subjects [17]. Moreover, Gillum et al. [9] demonstrated recently that SIRT1 mRNA expression in SAT correlates negatively with adipose tissue macrophage content in lean and obese adolescents. In addition, in vitro studies have shown that SIRT1 can repress inflammatory gene expression in macrophages [12] as well as in adipocytes [11] by suppressing c-Jun N-terminal kinase and NF-κB signaling. In the present study, we demonstrated that SIRT1 expression was lower in non-fat cells (which include macrophages) as well as in adipocytes of obese and type 2 diabetic patients (Fig. 1B). Furthermore, we observed a significant negative correlation between SIRT1 mRNA and a pro-inflammatory cytokine IL-1β mRNA in VAT.

The present study had some limitations. First, the cross-sectional design made it difficult to determine the causality of the observed relationships. Second, subjects in our study were limited to Korean women; therefore, our findings may not be directly applicable to Korean men or to other ethnic group. Body fat distribution has been found to differ among ethnic groups, with Asians having more VAT at a given BMI than would be expected for Caucasians [28,29]. Moreover, although the prevalence of obesity is lower in Asians than in Caucasians, obesity-related health risks are higher in Asians [30,31]. Third, insulin resistance was measured by the HOMA-IR method instead of the hyperinsulinemic glucose clamp, which is a gold standard technique for assessing insulin resistance. However, it has been demonstrated that measurements obtained by the homeostasis model correlate well with those obtained by the glucose clamp technique [22].

In summary, the present study showed that SIRT1 expression in VAT as well as in SAT was suppressed in women with obesity and type 2 diabetes compared to normal-weight women. These decreases in SIRT1 expression were observed in both adipocytes and non-fat cells. In addition, SIRT1 mRNA levels in adipose tissues correlated significantly and negatively with an insulin resistance index and other insulin resistance-related metabolic parameters. Interestingly, SIRT1 mRNA expression in VAT correlated inversely with visceral obesity whereas its expression in SAT correlated negatively with BMI. Moreover, SIRT1 expression showed a remarkable positive correlation with adiponectin mRNA levels in both fat depots, and a significant negative relationship with a proinflammatory cytokine IL-1β in VAT. Thus, the present study provides evidence showing that SIRT1 in adipose tissues may play a key role in the regulation of whole-body metabolic homeostasis and suggests that down-regulation of SIRT1 in VAT may contribute to the metabolic abnormalities that are associated with visceral obesity.
Confl icts of interest

The authors declare no conflict of interest.

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