

ORIGINAL

Association of subcutaneous and visceral fat mass with serum concentrations of adipokines in subjects with type 2 diabetes mellitus

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Abstract. The goal of the study was to examine the association of subcutaneous and visceral fat mass with serum concentrations of adipokines in 130 subjects with type 2 diabetes mellitus. The levels of serum high sensitivity C-reactive protein (HS-CRP), adiponectin, high-molecular-weight (HMW) adiponectin, interleukin-18, and retinol-binding protein 4 were measured. Percentage body fat was determined by dual energy X-ray absorptiometry, and subcutaneous and visceral fat areas were measured by abdominal CT. HS-CRP had significant positive correlations with percentage body fat and subcutaneous fat area, and a particularly significant positive correlation with visceral fat area. Serum adiponectin had a negative correlation with the subcutaneous and visceral fat areas, with the strongest correlation with the visceral fat area. Similar results were obtained for HMW adiponectin. Serum adiponectin had a negative correlation with visceral fat area in subjects with a visceral fat area $< 100 \text{ cm}^2$, but not in those with a visceral fat area $\geq 100 \text{ cm}^2$. In contrast, serum HS-CRP showed a positive correlation with visceral fat area in subjects with visceral fat area $\geq 100 \text{ cm}^2$, but not in those with a visceral fat area $< 100 \text{ cm}^2$. These findings indicate that an increased visceral fat area is associated with inflammatory changes, and that inflammatory reactions may alter the functional properties of visceral fat in type 2 diabetes mellitus.

Key words: Visceral fat, C-reactive protein, Adiponectin, Type 2 diabetes mellitus, Adipokines

VISCERAL fat-type obesity is an essential element of metabolic syndrome, along with impaired glucose tolerance, hypertension, and dyslipidemia. In obesity, adipocytes are enlarged and excess body fat accumulates. Adipocytes synthesize and secrete many adipokines, and the secretory properties of enlarged adipocytes are altered in obesity [1]. Thus, serum levels of inflammatory cytokines including tumor necrosis factor α (TNF- α) and interleukin-6 (IL-6) are increased, while the serum level of adiponectin, which has an anti-inflammatory function, is reduced in obesity [2]. These changes in secretion of adipokines may induce metabolic derangements, including impairment of glucose metabolism [3].

Clinical studies have shown a relationship of vis-

ceral fat-type obesity with accompanying systemic mild inflammatory reactions in metabolic syndrome [4]. High sensitivity C-reactive protein (HS-CRP) is positively correlated with body mass index (BMI) in healthy European and American subjects [5, 6] and in healthy Japanese subjects [7]. HS-CRP also has positive correlations with visceral fat accumulation in healthy male European and American subjects [5], with percentage body fat in healthy Japanese subjects [7], and with abdominal circumference in healthy female European and American subjects [6]. Systemic inflammatory reactions occur in patients with obesity and type 2 diabetes-associated insulin resistance [8], and an increased CRP level is also a predictive factor for development of diabetes [9, 10]. Several other adipokines are related to insulin resistance, with elevation of serum retinol-binding protein 4 (RBP4) and interleukin 18 (IL-18) and a decrease in serum adiponectin found in patients with type 2 diabetes. Obesity per se could alter the synthesis and release of these adipokines, and this may affect insulin sensitivity in type 2 diabetes.

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In the present study, we examined the relationships of fat accumulation with the levels of inflammatory and anti-inflammatory cytokines to clarify the correlations between fat accumulation and inflammation-related adipokines in patients with type 2 diabetes mellitus, based on the idea that inflammation may be induced by fat accumulation. HS-CRP and IL-18 were measured as inflammatory cytokines, and adiponectin and high-molecular-weight (HMW) adiponectin were evaluated as anti-inflammatory cytokines. RBP4 was measured to examine its correlation with insulin resistance accompanied by visceral fat accumulation. We also determined the percentage body fat, subcutaneous fat area, and visceral fat area, and examined the relationship between obesity profiles and serum levels of cytokines.

Subjects and Methods

Subjects

Between March 2005 and January 2007, 130 subjects with type 2 diabetes mellitus were enrolled in the study. The subjects were hospitalized at the Jichi Medical University Saitama Medical Center for 2 weeks to learn how and why to control blood glucose practically. The subjects included 67 men and 63 women with a mean age (\pm standard deviation) of 63.7 ± 11.9 years old (range: 18–88 years old). The mean duration of diabetes was 11.8 ± 8.4 years (range: 1–39 years) and the mean HbA1c level was $8.9 \pm 1.7\%$ (range: 5.5–14.1%). Subjects with renal impairment with an estimated glomerular filtration rate (eGFR) of <30 mL/min/1.73m² and those with apparent inflammatory reactions with a serum CRP level ≥ 1.00 mg/dL were excluded. Subjects taking thiazolidinediones were also excluded from the study. The medications taken for glycemic control included a sulfonylurea ($n=82$ patients), biguanide ($n=54$), α -glucosidase inhibitor ($n=43$), glinide ($n=4$), and an insulin injection ($n=39$). Blood was collected in the morning in a supine position after an overnight fast during hospitalization, and serum HS-CRP, adiponectin, HMW adiponectin, IL-18, and RBP4 were measured. BMI, body fat rate, and subcutaneous and visceral fat areas were also measured. The study was approved by the ethical committee of Jichi Medical University for human studies. Informed consent was obtained from all the subjects.

Measurement methods

Blood was collected in tubes and centrifuged at

3000 rpm for 15 minutes at 4°C. The supernatants were decanted and frozen at -80°C until measurement. HS-CRP, adiponectin, HMW adiponectin, IL-18 and RBP4 were measured by ELISA using a High Sensitivity C-Reactive Protein (CRP) EIA Kit (American Research Products, Belmont, MA, USA); a Human Adiponectin ELISA Kit (Otsuka Pharmaceutical, Tokyo, Japan); a High-Molecular-Weight Adiponectin Kit (Fuji Rebio, Tokyo, Japan); a Human IL-18 ELISA Kit (MBL, Nagoya, Japan); and a Human RBP4 Competitive ELISA Kit (AdipoGen, Seoul, Korea), respectively.

Renal function was evaluated based on eGFR using the Modified Diet in Renal Disease equation (MDRD) (corrected for Japanese subjects) of the Japanese Society of Nephrology: $\text{eGFR (mL/min/1.73 m}^2\text{)} = 0.741 \times 175 \times \text{age}^{-0.203} \times (\text{serum creatinine (mg/dL)})^{-1.154} (\times 0.742 \text{ for females})$. Percentage body fat was measured by dual energy X-ray absorptiometry (DEXA) [11]. The subcutaneous and visceral fat areas were measured by abdominal CT and determined at the navel level in the horizontal view [12]. The visceral/subcutaneous fat area ratio was calculated by dividing the visceral fat area by the subcutaneous fat area.

Statistical analysis

All values are expressed as the mean \pm standard deviation. Categorical variables were analyzed by chi-square test. Single regression analysis was performed to evaluate correlations between parameters. StatView version 5.0 was used for all analyses, with $P < 0.05$ considered to be significant.

Results

The obesity profiles and adipokines at admission of the 130 subjects with type 2 diabetes are shown in Table 1. Age did not differ significantly between the genders. Height and body weight were significantly greater in men, as expected, but there was no significant difference in BMI. The BMI of all subjects was 24.6 ± 4.1 , with no extreme obesity in any subject. The percentage body fat in women was significantly greater than that in men. The subcutaneous fat areas were significantly greater in women than in men, but the visceral fat area did not differ significantly between the genders. The visceral/subcutaneous fat area ratios were significantly greater in men than in women ($p < 0.001$). Both serum adiponectin and HMW adiponectin were significantly higher in women, whereas serum IL-18 was signifi-

Table 1 Obesity profile and adipokines at admission in subjects with type 2 diabetes mellitus

	All subjects	Men	Women	P value
Number of patients (N)	130	67	63	
Age (years)	63.7 ± 11.9	62.2 ± 11.9	65.4 ± 11.6	0.06
Height (cm)	159.0 ± 10.1	166.4 ± 7.3	151.1 ± 5.7	< 0.001
Body weight (kg)	62.6 ± 13.8	68.4 ± 13.3	56.3 ± 11.3	< 0.001
BMI	24.6 ± 4.1	24.6 ± 4.0	24.6 ± 4.2	0.48
Percentage body fat (%)	27.2 ± 7.5	22.1 ± 5.5	32.0 ± 5.9	< 0.001
Subcutaneous fat area (cm ²)	166.7 ± 81.7	138.7 ± 73.2	196.4 ± 80.2	< 0.001
Visceral fat area (cm ²)	126.8 ± 60.1	126.9 ± 61.8	126.8 ± 58.7	0.50
Visceral /subcutaneous fat area ratio	0.85 ± 0.37	0.99 ± 0.36	0.70 ± 0.31	< 0.001
HS-CRP (mg/dL)	0.17 ± 0.21	0.15 ± 0.19	0.20 ± 0.22	0.12
Adiponectin (µg/mL)	10.3 ± 7.2	9.1 ± 6.1	11.5 ± 8.2	0.03
HMW-adiponectin (µg/mL)	6.4 ± 6.3	5.5 ± 5.5	7.4 ± 7.0	0.04
IL-18 (pg/mL)	267 ± 113	286 ± 116	247 ± 107	0.02
RBP4 (µg/mL)	70.3 ± 34.9	73.4 ± 29.6	67.0 ± 39.7	0.15

Values are shown as the mean ± standard deviation. P value for men vs. women

BMI, body mass index; HS-CRP, High sensitivity C-reactive protein; HMW-adiponectin, High molecular weight adiponectin; IL-18, Interleukin-18; RBP4, Retinol-binding protein 4

Table 2 Correlation of adipokine levels with percentage body fat, subcutaneous fat area, and visceral fat area in subjects with type 2 diabetes mellitus

	Percentage body fat (n = 118)	Subcutaneous fat area (n = 130)	Visceral fat area (n = 130)
HS-CRP	0.286**	0.319**	0.346**
Adiponectin	-0.156	-0.262**	-0.332**
HMW-adiponectin	-0.183*	-0.275**	-0.339**
IL-18	0.136	0.135	0.232**
RBP4	-0.139	-0.132	0.054

Values are correlation coefficients (a negative sign indicates a negative correlation).

*, $P < 0.05$, **, $P < 0.01$ HS-CRP, High sensitivity C-reactive protein; HMW-adiponectin, High molecular weight adiponectin; IL-18, Interleukin-18; RBP4, Retinol-binding protein 4

Table 3 Correlation of adipokine levels with percentage body fat, subcutaneous fat area, and visceral fat area in subjects with type 2 diabetes mellitus

	Men			Women		
	Percentage body fat (n = 57)	Subcutaneous fat (n = 67)	Visceral fat (n = 67)	Percentage body fat (n = 61)	Subcutaneous fat (n = 63)	Visceral fat (n = 63)
HS-CRP	0.260	0.412**	0.363**	0.235	0.209	0.341**
Adiponectin	-0.279*	-0.293*	-0.360**	-0.312*	-0.390**	-0.327**
HMW-adiponectin	-0.305*	-0.288*	-0.354**	-0.331**	-0.411**	-0.341**
IL-18	0.225	0.246*	0.272*	0.303*	0.195	0.188
RBP4	-0.096	-0.162	-0.052	-0.064	-0.067	0.145

Values are correlation coefficients (a negative sign indicates a negative correlation). *, $P < 0.05$, **, $P < 0.01$

HS-CRP, High sensitivity C-reactive protein; HMW-adiponectin, High molecular weight adiponectin; IL-18, Interleukin-18; RBP4, Retinol-binding protein 4

cantly higher in men.

Correlations of various adipokines with the percentage body fat and subcutaneous and visceral fat areas are shown in Table 2. HS-CRP was significantly correlated with all three parameters, with a highly significant positive correlation between HS-CRP and visceral fat area. Serum adiponectin had a negative correlation with the subcutaneous and visceral fat areas, with the

strongest correlation with the visceral fat area. Similar results were obtained for HMW adiponectin. Serum IL-18 was significantly correlated only with the visceral fat area.

Gender differences in the correlations of adipokines with obesity profiles are shown in Table 3. The positive correlation of HS-CRP with the subcutaneous fat area disappeared in women. The negative correlations

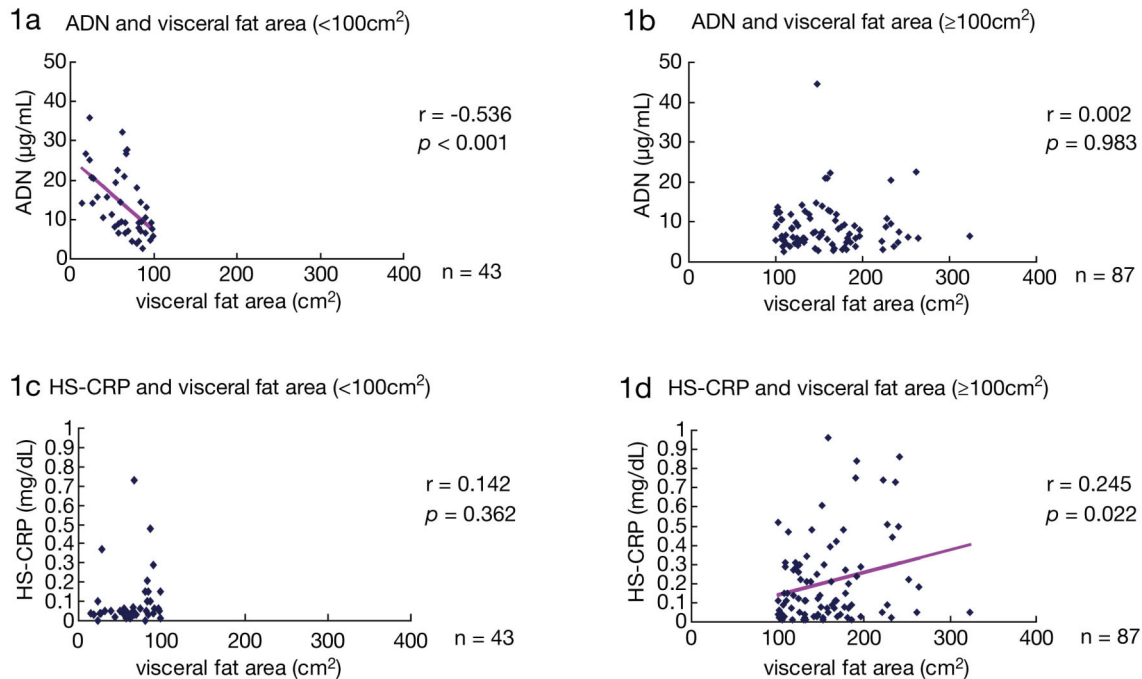


Fig. 1 Correlations of high sensitivity C-reactive protein (HS-CRP) and adiponectin (ADN) with visceral fat area in subjects with type 2 diabetes mellitus ($n = 130$)

of serum adiponectin and HMW adiponectin with all three obesity parameters remained significant in both men and women. The correlations of serum adiponectin and HMW adiponectin with the visceral fat area were strongest in men, while those with the subcutaneous fat area were strongest in women.

The subjects were divided into two groups with visceral fat areas $< 100 \text{ cm}^2$ and $\geq 100 \text{ cm}^2$. Serum adiponectin had a negative correlation with the visceral fat area in subjects with a visceral fat area $< 100 \text{ cm}^2$, while this correlation disappeared in those with a visceral fat area $\geq 100 \text{ cm}^2$ (Fig. 1a and 1b). Since there is a well known gender difference in serum adiponectin levels, the analysis was conducted separately for men and women. The correlation coefficients of serum adiponectin levels with visceral fat areas in men and women with a visceral fat area $< 100 \text{ cm}^2$ were both significant: $r = 0.426$ ($P < 0.05$, $n = 24$) and $r = 0.659$ ($P < 0.01$, $n = 19$), respectively. As expected, the correlation between serum adiponectin levels and visceral fat area disappeared in both men and women with a visceral fat area $\geq 100 \text{ cm}^2$. In contrast, there was a positive correlation between serum HS-CRP and visceral fat area in subjects with a visceral fat area $\geq 100 \text{ cm}^2$, but no correlation in those with a visceral fat area $< 100 \text{ cm}^2$ (Fig. 1c and 1d).

Discussion

The mean BMI of the subjects with type 2 diabetes was 24.6 ± 4.1 , which exceeded the ideal value of 22.0. The subcutaneous fat area and percentage body fat, which may also reflect subcutaneous fat, were significantly greater in women than in men. In contrast, the visceral/subcutaneous fat area ratio was significantly higher in men than in women. These findings are similar to those in healthy subjects. Based on the gender differences in the obesity profile, subgroup analyses of male and female subjects were performed, in addition to analysis of all subjects, to evaluate the relationship of adipokines with obesity. Bioelectrical impedance analysis and MRI can be used for measurement of subcutaneous and visceral fat areas. However, we chose abdominal CT for this purpose [13-15], since this method is widely used for measurement of fat areas due to its simplicity and the availability of facilities for measurement.

CRP is a typical inflammatory cytokine that is secreted by the liver and by macrophages and adipocytes [16]. The HS-CRP level in all subjects was $0.17 \pm 0.21 \text{ mg/dL}$, with no significant sex difference (data not shown). HS-CRP showed the strongest cor-

relation with visceral fat area, indicating that HS-CRP reflected visceral fat accumulation regardless of gender. Visceral fat is related to liver fat [17] and the association of CRP with visceral fat may reflect an association with liver fat accumulation. Fatty liver has been related to an increase in CRP levels and insulin resistance [18, 19], and the serum CRP level may change in association with a change in visceral fat accumulation, and probably with a change in liver fat accumulation. The increase in CRP level can be reduced by improvement of lifestyle [16, 20].

Adiponectin is an abundant plasma protein that is secreted by adipocytes and has an anti-inflammatory function. In our subjects, the serum adiponectin levels were 9.1 ± 6.1 and 11.5 ± 8.2 $\mu\text{g/mL}$ in men and women, respectively. This gender difference is consistent with previous studies. Serum adiponectin was negatively correlated with subcutaneous and visceral fat areas in all subjects. Similar results were obtained in subgroup analyses of male and female subjects. The correlation with the visceral fat area was the strongest in all subjects and in male subjects, which may suggest that reduction of serum adiponectin reflects an increase in visceral fat accumulation. However, we also found a significant association of adiponectin with subcutaneous fat. It remains uncertain whether adiponectin is predominantly produced by visceral or subcutaneous fat, and some reports indicate no difference in insulin resistance between visceral fat and subcutaneous fat [21]. Therefore, we are unable to clarify whether visceral fat is the main source of adiponectin, based on our current results.

In the systemic circulation, adiponectin is present as 3 multimers that are referred to as the high-, middle-, and low-molecular-weight forms [22]. Recent studies have suggested that reduction of HMW adiponectin, rather than total adiponectin, has pronounced involvement in development of diabetes mellitus and insulin resistance [23, 24]. In the present study, serum HMW adiponectin was negatively correlated with percentage body fat and subcutaneous and visceral fat areas, which suggests that there was no difference in the changes in serum adiponectin and HMW adiponectin. Similar findings were obtained in other studies [25], in contrast to the strong association of HMW adiponectin with insulin sensitivity or metabolic syndrome [24].

IL-18 is an inflammatory cytokine produced by various tissues [26]. As for other adipokines, serum IL-18 had a significant correlation with the visceral fat area in

all subjects. An increase in serum IL-18 may be linked to inflammatory changes in visceral adipocytes, and IL-18 is also involved in cardiovascular derangement [26]. RBP4 is also produced by adipocytes, as well as by hepatocytes, but serum RBP4 did not show a significant relationship with adipose tissue accumulation in the current study. Thus, RBP4 production might be associated more strongly with the liver, rather than adipose tissues, in patients with diabetes.

Glycemic control (HbA1c) showed no significant correlations with any adipokines or with the levels of visceral and subcutaneous fat accumulation. The time courses of HbA1c with those of several adipokines and visceral fat accumulation in individual patients have been shown to be correlated. However, the present cross-sectional analysis was performed in 130 subjects with a wide distribution of glycemic conditions at the time of sampling, and this accounts for the absence of correlations of adipokines and fat levels with glycemic control.

We found that an increase in visceral fat area was associated with serum CRP, adiponectin and HMW adiponectin levels. In Japan, a visceral fat area < 100 cm^2 at the navel level is a cut-off value for high and low visceral fat accumulation [27], and therefore we evaluated the adipokine levels in two groups of subjects based on this value. In subjects with a visceral fat area < 100 cm^2 , serum adiponectin had a negative correlation with the visceral fat area, but there was no correlation between serum CRP and the visceral fat area. In contrast, in subjects with a visceral fat area ≥ 100 cm^2 , the relationship of serum adiponectin with visceral fat area disappeared. However, a positive correlation between serum CRP and visceral fat area became significant in these patients. Thus, in patients with type 2 diabetes who are obese, serum adiponectin levels are strongly suppressed and display low variability, and the association with visceral fat area is rendered non-significant. As serum CRP gradually increases, an "inflammatory change" may occur in the adipose tissue. These findings indicate that an increase in visceral fat tissue can alter the properties of adipose tissues. Adiponectin inhibits macrophage infiltration in the vascular endothelium [28, 29] and macrophage infiltration increases in adipose tissues in obesity [30, 31] because of decreased adiponectin secretion due to enlargement and alteration of the properties of visceral adipocytes. These changes may cause inflammation of adipose tissues. Increased visceral fat may diminish

adiponectin production, and the reduced level of adiponectin might be linked to an increase in CRP, inducing inflammation of fat tissue *per se*.

In conclusion, our results demonstrate that the visceral fat area has a positive correlation with HS-CRP and negative correlations with adiponectin and HMW

adiponectin in patients with type 2 diabetes. Regulation of adipokine synthesis and release may be altered in an area with increased visceral fat, thus causing a change in the functional properties of adipose tissues in type 2 diabetes mellitus.

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