Original article

The area of abdominal subcutaneous adipose tissue is independently correlated with C-peptide increment during glucagon load in Japanese patients with type 2 diabetes

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A short running title: Abdominal fat and glucagon test

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Abstract

Overall adiposity is associated with insulin resistance. Decline in insulin sensitivity induces a compensatory increase in insulin secretion from beta cells. The impact of adipose tissue distribution; visceral versus subcutaneous fat, on beta cell function remains to be elucidated. The aim of this study was to retrospectively investigate the relation between the areas of subcutaneous and visceral fat and the increment in C-peptide levels (CPR) during glucagon load (ΔCPR) in Japanese patients with type 2 diabetes. In 195 Japanese patients with type 2 diabetes, beta cell function was evaluated by ΔCPR, CPR index (100 x fasting CPR divided by fasting glucose) and 24-hour urinary C-peptide excretion (U-CPR) during the one-week hospitalization for diabetes education. Then, we investigated the relationships between markers of beta cell function and the areas of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) determined by abdominal computed tomography (CT). Stepwise multiple regression analyses identified SAT, but not VAT, as an independent variable of both ΔCPR and U-CPR [Stdβ (standard regression coefficient) = 0.332, $P < 0.01$ for ΔCPR, and Stdβ=0.181, $P < 0.05$ for U-CPR], and both SAT and VAT were identified as independent variables for CPR index (Stdβ= 0.253, $P < 0.01$ for SAT, and Stdβ=0.193, $P < 0.01$ for VAT). Our results indicated that SAT is independently correlated with beta cell function by glucagon test in Japanese patients with type 2 diabetes.

Key words: glucagon test, visceral fat, subcutaneous fat, C-peptide

Introduction

Adipose tissue plays important roles in the regulation of glucose metabolism. Overall adiposity is reported to be associated with a decrease in insulin sensitivity [1-3]. A decline in insulin sensitivity is associated with a compensatory increase in beta cell insulin secretion. Thus, beta cell function correlates with overall adiposity not only in healthy normoglycemic subjects. Previously, we [4] and others [5, 6] demonstrated that increments in C-peptide level during glucagon load (ΔCPR), which is widely used to evaluate pancreatic beta cell reserve in diabetic patients [7-9], correlate with body mass index (BMI) in Japanese patients with type 2 diabetes.

Several studies of adipose tissue distribution evaluated by computed tomography and magnetic resonance imaging have stressed the importance of visceral fat on insulin resistance [10-12]. Thus, accumulation of visceral adipose tissue (VAT) correlates with beta cell function [10, 12-14]. On the other hand, several studies have indicated that subcutaneous adipose tissue (SAT) correlates with insulin resistance, similar to VAT [15-17]. However, at present, it is still not clear whether VAT has a stronger effect on beta cell function compared with SAT.

Previous studies used fasting insulin level [2], 75g-oral glucose tolerance test (OGTT) [10, 12, 18] or intravenous glucose tolerance test (IVGTT) [13] to evaluate beta cell function in non-diabetic patients. However, these tests are known to be affected by glucose control [6, 19, 20] and are not always suitable for diabetic patients with glucose toxicity. Because of these limitations, there have been no studies to investigate the relationships between adiposity and beta cell function in diabetic patients already being treated with various medications including insulin injection so far. Therefore, in the present study, we investigated the relationships between ΔCPR during glucagon test and the abdominal adipose tissue distribution evaluated by computed tomography in order to evaluate the effect of adiposity on beta cell function in type 2 diabetic patients.

Materials and Methods

Study subjects

Subjects of the present retrospective study were 209 consecutive Japanese patients with type 2 diabetes mellitus who were admitted to Juntendo University Hospital between 2010 and 2012 for diabetes

education. Another 4 patients were excluded because of missing urinary C-protein (U-CPR) data. In addition, 10 patients were excluded because they were newly diagnosed with type 1 diabetes based on measurement of anti-GAD antibody. Thus, the remaining 195 diabetic patients were included for data analysis. In this group, patients with chronic pancreatitis, liver cirrhosis and severely reduced renal function [estimated glomerular filtration rate (eGFR) < 30 mL/min per 1.73 m^2] were not included.

Study design

At Juntendo Hospital, one-week hospitalization for diabetes education program using the clinical pathway has been applied to diabetic patients. In the present study, patients underwent medical and physical examinations on the first day of hospitalization. On the third day, routine blood tests including glycosylated hemoglobin (HbA1c), and basal CPR were performed after an overnight fast. CPR was measured at 6 min after injection of 1 mg glucagon. ΔCPR was calculated using the following equation: ΔCPR = (CPR at 6 min - fasting CPR). In patients treated with oral antidiabetic drugs (OADs), the medication was stopped before the glucagon test. Urine was collected over a 24-hr period between second and third days of hospitalization for the measurement of U-CPR using a C-peptide stabilizer (Eiken Chemical Co., Tokyo, Japan) to prevent breakdown of C-peptide. On the sixth day of hospitalization, the areas of SAT and VAT were quantified by a single-slice CT scan at the level of the umbilicus [21]. The date of diagnosis of type 2 diabetes was determined from the medical records, medical history and other available clinical data. The eGFR was calculated using the equation proposed by the Japanese Society of Nephrology: eGFR (mL/min/1.73 m^2) = $194 \times \text{s-Cr}^{-1.094} \times \text{Age}^{-0.287}$ (× 0.739 for females) [22]. In the present study, the values of HbA1c were presented as National Glycohemoglobin Standardization Program (NGSP) values. Diabetic retinopathy was diagnosed by an ophthalmologist. The ethics committee of Juntendo University approved the study protocol.

Statistical analysis

All data were presented as median (IQR: interquartile range) and analyzed using StatFlex ver. 6 (Artech Co., Osaka, Japan) because the data distribution was non-parametric. The relationships between clinical variables and CPR index, ΔCPR and U-CPR were investigated by Spearman analysis and ρ value was presented as Spearman's rank correlation coefficient. Multiple regression analysis was performed

using the clinical parameters as the explanatory variables and CPR index, ΔCPR, and U-CPR as the dependent variable. These explanatory variables were selected because gender and age were used in general and eGFR, diabetes duration and fasting glucose levels affected ΔCPR as presented by our previous study [4]. The cutoff value of $P < 0.15$ was used for the stepwise procedure. For each multiple regression analysis, we calculated the value of standard regression coefficient (Stdβ). P values < 0.05 were considered statistically significant.

Results

Study subjects

Table 1 summarizes the clinical characteristics of the study subjects. Men constituted 69.2% of the study patients. The median age of the 195 patients was 62.0 (IQR: 53.0-70.0) years, and the duration of diabetes was 10.0 (IQR: 5.0-15.0) years. HbA1c at admission was 8.0% (IQR: 7.3-9.0), and fasting plasma glucose (Glu-0min) before glucagon test was 131 mg/dL (IQR: 113-159). With regard to medications before admission, 17.0% of the patients were not on any medications, 60.5% and 9.2% were being treated with OADs alone and insulin alone, respectively. Table 2 shows the clinical characteristics in male and female subjects. CPR-0min, U-CPR and VAT were significantly increased in the male subjects compared to the female subjects, and SAT was significantly increased in the female subjects compared to the male subjects.

Univariate and mutivariate regression analysis

As shown in Fig.1, BMI correlated significantly with both SAT (ρ =0.868, P<0.01) and VAT (ρ =0.685, P<0.01) and the correlation between VAT and SAT was also significant, but relatively weak, compared to the relation with BMI. Next, we assessed the correlation between adipose tissue distribution and ΔCPR in male and female. As shown in Fig.2A and 2B, ΔCPR correlated significantly with both SAT (ρ =0.337, P<0.01) and VAT (ρ =0.196, P<0.05) in male subjects. Additionally, ΔCPR in female subjects correlated significantly with SAT (ρ =0.329, P<0.05) and did not correlate significantly with VAT as presented by Fig. 3A and 3B.

Next, we assessed the correlation between adipose tissue distribution and two other markers in male and female. Both SAT and VAT correlated with CPR index and U-CPR in male subjects (SAT-

CPR index = 0.546, $P < 0.01$, VAT- CPR index: $\rho = 0.381$, $P < 0.01$, SAT-U-CPR: $\rho = 0.325$, $P < 0.01$, VAT-U-CPR: $\rho = 0.215$, $P < 0.05$). In female subjects, both SAT and VAT correlated with CPR index (SAT- CPR index: $\rho = 0.294$, $P < 0.05$, VAT- CPR index: $\rho = 0.347$, $P < 0.01$). Then, both SAT and VAT did not correlate with U-CPR in female subjects. Multiple regression analysis for all subjects identified the duration of diabetes, Glu-0min, SAT and insulin treatment before admission as independent and significant variables of ΔCPR (Table 3), accounting for 25.9% of the variability in ΔCPR. On the other hand, VAT was not identified as a significant factor associated with ΔCPR.

Stepwise multiple regression analysis identified gender, SAT and insulin treatment before admission as significant and independent variables of U-CPR, accounting for 19.6% of the variability of the dependent variable. VAT tended to correlate with U-CPR, although the correlation did not achieve statistical significance (Stdβ = 0.149, $P = 0.075$, Table 4). Forced entry multiple regression analysis identified gender, age, Glu-0min, VAT, SAT, eGFR and insulin treatment before admission as independent and significant variables of CPR index, accounting for 47.4% of the variability of the dependent variable (Table 5). Stepwise multiple regression analysis could not be performed because the *P* values of all variables for predicting CPR index was below 0.15 in the forced entry model.

Discussion

The present study is the first report to demonstrate that SAT, not VAT, is an independent variable of ΔCPR in Japanese patients with type 2 diabetes. Previous reports demonstrated the associations between adiposity and beta cell function evaluated by insulin response to oral or intravenous glucose loading and adiposity in diabetic [6] and non-diabetic patients [2, 10, 12, 13]. However, chronic hyperglycemia (i.e., glucose toxicity) in uncontrolled diabetic patients diminishes the insulin response to glucose loading [6, 19, 20], which is reversible after normalization of blood glucose. Thus, indices derived from glucose-inducible insulin secretion are not always valid for evaluating beta cell function especially in diabetic patients. In this study, the median of HbA1c at admission was 8.0%, indicating that glucagon test was not performed under severe chronic hyperglycemia. Furthermore, it has been reported that glucagon-inducible insulin secretion is little influenced by chronic hyperglycemia [4, 6, 20]. Therefore, we consider that beta cell function of each patient was appropriately assessed in this study.

During the past 10-20 years, studies on abdominal adipose tissue distribution by computed tomography and magnetic resonance imaging have identified that accumulation of visceral fat is strongly associated with insulin resistance [10-12]. Such association is true for both non-diabetic subjects [18, 23, 24] and patients with type 2 diabetes mellitus [25]. Based on these findings, VAT could be a strong predictor of ΔCPR in patients with type 2 diabetes. However, multiple regression analysis in this study identified SAT, not VAT, as an independent factor associated with ΔCPR. On the basis of recent findings [17, 26, 27], inflammation of the adipose tissue plays an important role in the pathophysiology of insulin resistance. Indeed, a human study reported that the expression of macrophage-specific genes in adipose tissue increases with the degree of adiposity and correlates with markers of insulin resistance to a similar degree in SAT and in VAT [16]. Because the lipolytic response to catecholamine is lower in subcutaneous adipocytes than in visceral adipocytes [28, 29], accumulation of subcutaneous fat is likely to reflect long-term overfeeding and show good correlation with BMI. On the other hand, visceral fat volume is relatively small and accounts for only 6-20% of total adipose tissue volume in obese individuals [30], thus unlikely to show good correlation with BMI. In fact, in this study, SAT correlated strongly with BMI. For these reasons, SAT might be detected as a strong variable for beta cell function in diabetic patients. In addition, we performed the multiple regression analysis with BMI instead of SAT. BMI, not VAT is an independent variable for ΔCPR, indicating that the absolute volume of fat is a strong factor for beta cell function (supplemental data). Interestingly, table 5 presented that CPR index was independently predicted by SAT, VAT and gender, which is inconsistent with the result of ΔCPR. These findings suggest the possibility that CPR index is significantly affected not only by SAT but also by VAT and gender difference unlike glucagon-inducible insulin secretion. This reason might be attributable to the differences in the mechanisms of insulin secretion by glucose and glucagon [31, 32], although the precise mechanisms remain unknown.

As presented by table 2, CPR index, U-CPR and VAT were significantly increased in male subjects compared to female subjects. However, there were no significant differences in BMI and ΔCPR between two groups. These findings indicate that the gender based differences in abdominal fat distribution need to be taken into account for evaluation of beta cell function.

From these results and considerations, we would like to hypothesize that beta cell function evaluated by glucagon test is likely to be affected by accumulation of subcutaneous fat, namely, which

well reflects the increased BMI caused by long-term overfeeding. Conversely, beta cell function evaluated by CPR index is likely to be affected by both subcutaneous and visceral fat, suggesting that ΔCPR and CPR index might be affected by the distribution of abdominal adipose tissue.

Finally, our study has certain limitations. First, we were not able to confirm a causal relationship between adiposity and ΔCPR because our study was retrospective in nature. Second, unknown confounding factors such as changes in body weight and life style before admission might have affected our findings and such information was not available in this study. Third, the effects of ethnic difference on the relations between adiposity and ΔCPR remain unknown. Japanese patients with type 2 diabetes have genetically reduced insulin secretion capacity [33] and eventually have lower BMI compared to Westerners [34], indicating that beta cell function and insulin resistance due to adiposity greatly differ by ethnicity. Therefore, interpretation of the present findings is limited and the results should be viewed to apply to Japanese patients with type 2 diabetes until they are confirmed in other races.

In conclusion, our study has demonstrated the importance of SAT, quantified by abdominal CT, in affecting beta cell function in Japanese patients with type 2 diabetes and distribution of abdominal adipose tissue might affect CPR after glucagon stimulation and CPR index.

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Conflict of Interest

The authors declare no conflict of interest.

Abbreviations

BMI: body mass index

CPR: C-peptide levels

eGFR: estimated glomerular filtration rate

IQR: interquartile rage

OADs: oral antidiabetic drugs

R: multiple correlation coefficient

R^2: adjusted coefficient of determination

SAT: subcutaneous adipose tissue

VAT: visceral adipose tissue

Stdβ: standard regression coefficient

U-CPR: 24-hour urinary CPR excretion

Figure legends

Fig. 1: Correlation between (A) VAT and BMI (ρ = 0.685, P < 0.01, n = 195), (B) SAT and BMI (ρ = 0.868, P < 0.01, n = 195) and (C) SAT and VAT (ρ = 0.517, P < 0.01, n = 195).

Fig. 2: Correlation between (A) VAT and ΔCPR (ρ = 0.196, P < 0.05, n = 135), (B) SAT and ΔCPR (ρ = 0.337, P < 0.01, n = 135) in male subjects.

Fig. 3: Correlation between (A) VAT and ΔCPR (ρ = 0.144, NS, n = 60), (B) SAT and ΔCPR (ρ = 0.329, P < 0.05, n = 60) in female subjects.

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Table 1. Clinical characteristics of the study subjects.

n	195
Age (years)	62.0 (53.0-70.0)
Male/Female	135/60
Body mass index (kg/m^2)	25.3 (22.8-29.5)
Duration of diabetes (years)	10.0 (5.0-15.0)
HbA1c at admission (%)	8.0 (7.3-9.0)
Glucagon test	
CPR-0min (ng/mL)	2.1 (1.5-2.8)
CPR-6min (ng/mL)	4.1 (2.9-5.8)
Glu-0min (mg/dL)	131 (113-159)
ΔCPR (ng/mL)	2.1 (1.3-3.1)
U-CPR (μg/day)	74.0 (45.0-101.0)
CPR index	1.45 (1.09-2.07)
eGFR (mL/min per 1.73 m^2)	80.0 (68.2-97.8)
Serum creatinine (mg/dL)	0.59 (0.39-070)
Abdominal CT	
VAT (cm^2)	124.6 (89.1-170.1)
SAT (cm^2)	165.4 (111.0-233.4)
Waist circumference (cm)	80.7 (67.2-88.5)
Hypertension (%)	56.4
Dyslipidemia (%)	66.7
Non-Smoker (%)	74.8
Medications before admission	
None	17.0% (33/195)
OADs alone	60.5% (118/195)
SU	48.2% (94/195)
α-GI	24.6% (48/195)
metformin	32.3% (63/195)
thiazolidine	13.8% (27/195)
DPP-4 inhibitor	25.1% (49/195)
glinide	7.6% (15/195)
insulin + OADs	11.8% (23/195)
insulin alone	9.2% (18/195)
GLP-1 agonist	1.5% (3/195)

Data are median (25th–75th interquartiles). Glu-0 min and CPR-0 min: fasting plasma glucose and C-peptide levels before glucagon stimulation, CPR-6min: C-peptide levels at 6 min after glucagon stimulation, OADs: oral antidiabetic drugs, CPR index: 100 x fasting CPR divided by fasting glucose, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue

Table 2. Clinical characteristics of the male and female subjects

	Male	Female	*P*
N	135	60	
Age (years)	61.0 (53.0-69.0)	64.5 (54.0-71.0)	0.292
Body mass index (kg/m^2)	25.6 (22.9-29.8)	24.3 (22.3-28.8)	0.138
Duration of diabetes (years)	8.0 (5.0-15.0)	10.0 (5.5-15.0)	0.513
HbA1c at admission (%)	8.0 (7.4-8.9)	8.0 (7.2-9.2)	0.886
Glucagon test			
CPR-0min (ng/ml)	2.2 (1.6-2.9)	1.7 (1.4-2.5)	0.016
CPR-6min (ng/ml)	4.3 (3.1-5.9)	4.1 (2.7-5.1)	0.183
Glu-0min (mg/dl)	131.0 (114.0-155.5)	139.5 (113.0-165.0)	0.543
ΔCPR (ng/ml)	2.1 (1.3-3.2)	2.1 (1.3-2.8)	0.799
U-CPR (μg/day)	78.0 (48.3-116.0)	64.5 (36.5-82.5)	<0.01
CPR index	1.6 (1.2-2.2)	1.3 (0.9-1.7)	<0.01
eGFR (ml/min per 1.73 m^2)	77.5 (67.1-96.9)	85.3 (70.3-102.4)	0.142
Abdominal CT			
VAT (cm^2)	141.5 (105.1-230.8)	107.3 (82.1-134.3)	<0.01
SAT (cm^2)	141.0 (105.1-230.8)	193.6 (144.1-450.8)	<0.01

See Table 1 for abbreviations and definition of the variables.

Table 3. Multiple regression analysis for ΔCPR

	Forced entry model		Stepwise method		
	Stdβ	*P*	Stdβ	*P*	R^2 (R)
Male (reference: female)	0.123	0.087	0.108	0.09	0.259 (0.53)
Age (years)	-0.078	0.361			
Duration of diabetes (years)	-0.150	0.036	-0.179	<0.01	
Glu-0 min (mg/dL)	0.238	<0.01	0.229	<0.01	
VAT (cm^2)	-0.081	0.328			
SAT (cm^2)	0.355	<0.01	0.332	<0.01	
eGFR (mL/min per 1.73 m^2)	-0164	0.020	-0.124	0.052	
Insulin treatment before admission (reference: none)	-0.179	<0.01	-0.163	<0.01	

See Table 1 for abbreviations and definition of the variables.

Table 4. Multiple regression analysis for U-CPR

	Forced entry model		Stepwise method		
	Stdβ	*P*	Stdβ	*P*	R^2(R)
Male (reference: female)	0.231	<0.01	0.236	<0.01	0.196 (0.46)
Age (years)	-0.041	0.647			
Duration of diabetes (years)	-0.029	0.366			
Glu-0 min (mg/dL)	0.060	0.366			
VAT (cm^2)	0.148	0.091	0.149	0.075	
SAT (cm^2)	0.154	0.099	0.181	0.031	
eGFR (mL/min per 1.73 m^2)	0.099	0.178	0.123	0.066	
Insulin treatment before admission (reference: none)	-0.216	<0.01	-0.224	<0.01	

See Table 1 for abbreviations and definition of the variables.

Table 5. Multiple regression analysis for CPR index

	Forced entry model		
	Stdβ	*P*	R^2(R)
Male (reference: female)	0.119	0.048	0.474 (0.70)
Age (years)	-0.206	<0.01	
Duration of diabetes (years)	-0.097	0.105	
Glu-0 min (mg/dL)	-0.364	<0.01	
VAT (cm^2)	0.193	<0.01	
SAT (cm^2)	0.253	<0.01	
eGFR (mL/min per 1.73 m^2)	-0.281	<0.01	
Insulin treatment before admission (reference: none)	-0.305	<0.01	

See Table 1 for abbreviations and definition of the variables.

Multiple regression analysis for ΔCPR with BMI instead of SAT

	Forced entry model		Stepwise method		
	Stdβ	*P*	Stdβ	*P*	R^2(R)
Male (reference: female)	0.033	0.615			0.254 (11.9)
Age (years)	-0.074	0.398			
Duration of diabetes (years)	-0.131	0.067	-0.155	0.018	
Glu-0 min (mg/dL)	0.234	<0.01	0.232	<0.01	
VAT (cm^2)	-0.125	0.192	-0.138	0.117	
BMI(kg/m^2)	0.366	<0.01	0.403	<0.01	
eGFR (mL/min per 1.73 m^2)	-0.158	0.026	-0.137	0.035	
Insulin treatment before admission (reference: none)	-0.179	<0.01	-0.170	<0.01	

Figure 1

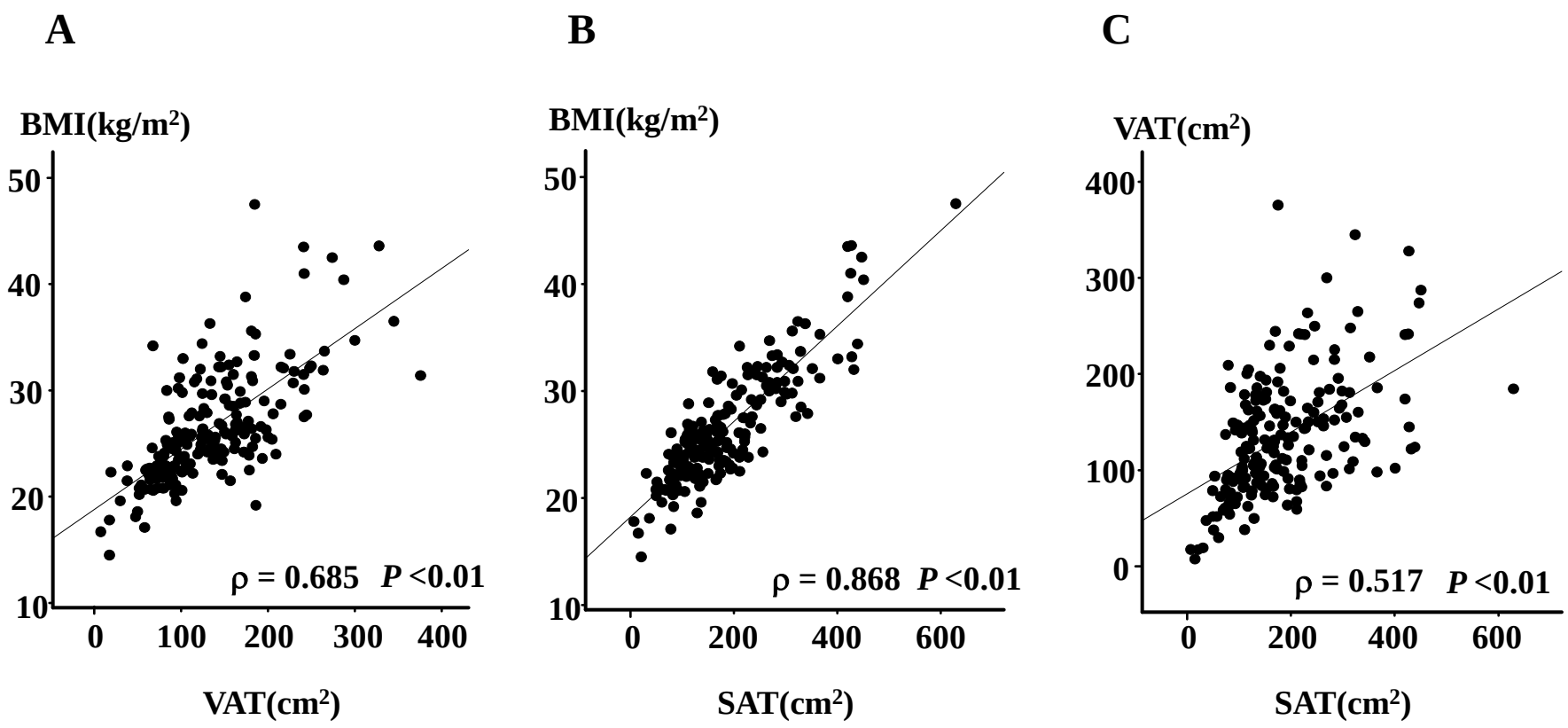

A
BMI(kg/m2)
50
40
30
20
10
0
100
200
300
400
ρ = 0.685 P <0.01
VAT(cm2)
B
BMI(kg/m2)
50
40
30
20
10
0
200
400
600
ρ = 0.868 P <0.01
SAT(cm2)
C
VAT(cm2)
400
300
200
100
0
0
200
400
600
ρ = 0.517 P <0.01
SAT(cm2)

Figure 2

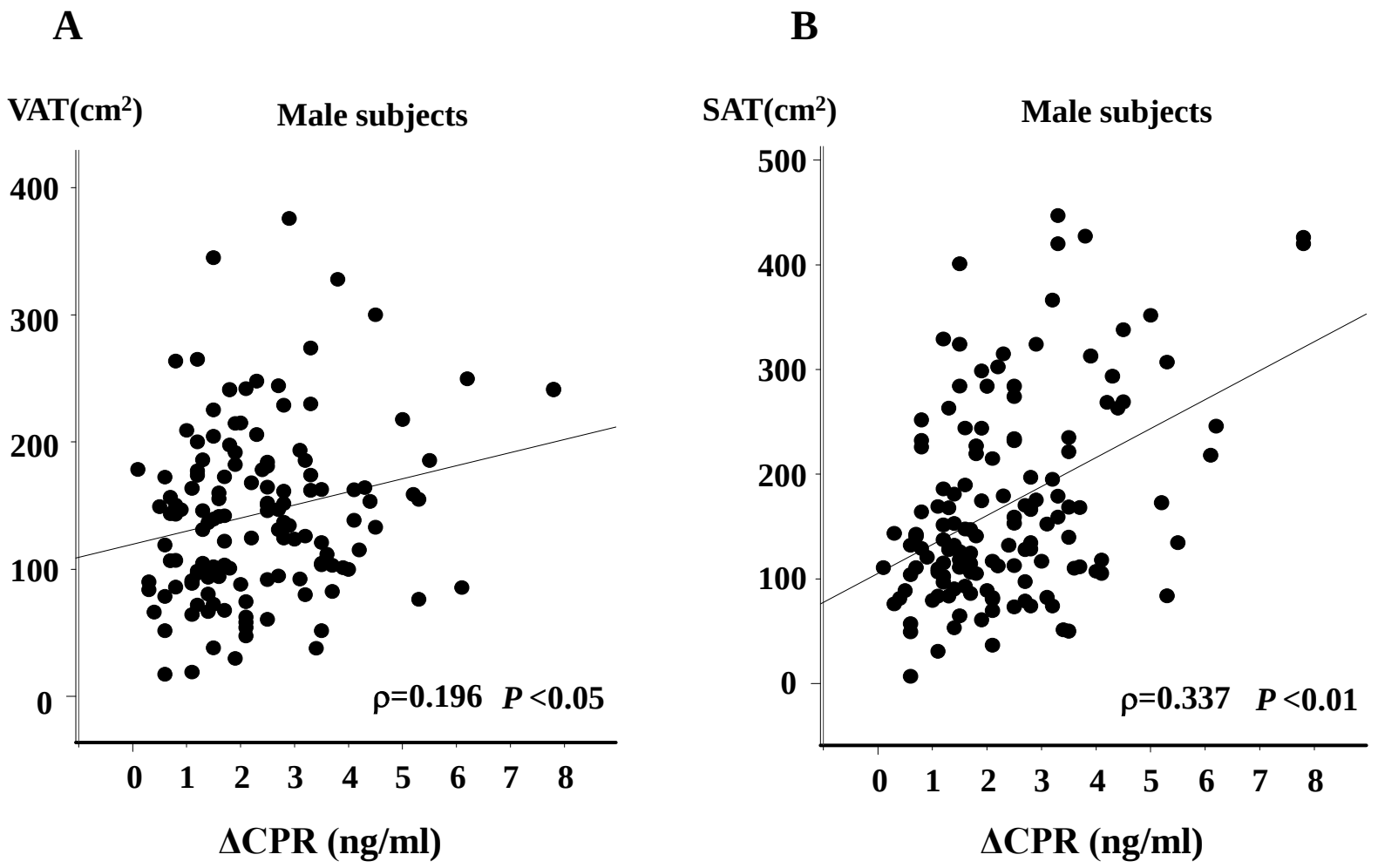
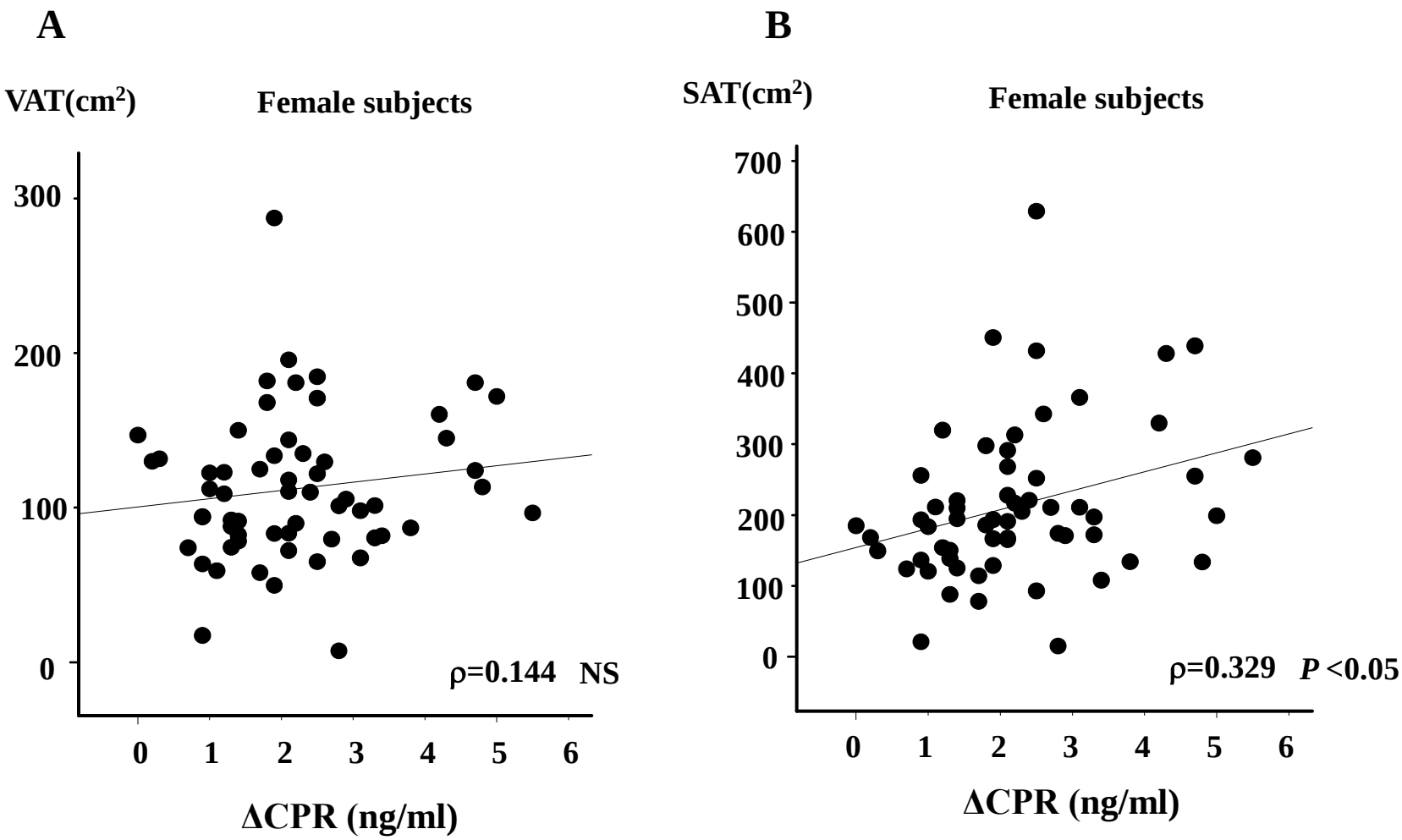

A
VAT(cm2)
Male subjects
400
300
200
100
0
ρ=0.196 P <0.05
0 1 2 3 4 5 6 7 8
ΔCPR (ng/ml)
B
SAT(cm2)
Male subjects
500
400
300
200
100
0
ρ=0.337 P <0.01
0 1 2 3 4 5 6 7 8
ΔCPR (ng/ml)

Figure 3


A
VAT(cm2)
Female subjects
300
200
100
0
ρ=0.144 NS
0 1 2 3 4 5 6
ΔCPR (ng/ml)
B
SAT(cm2)
Female subjects
700
600
500
400
300
200
100
0
ρ=0.329 P <0.05
0 1 2 3 4 5 6
ΔCPR (ng/ml)