Serum Butyrylcholinesterase is Strongly Associated with Adiposity, the Serum Lipid Profile and Insulin Resistance

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Abstract

Objective We investigated the association between the serum butyrylcholinesterase (BChE) activity and parameters of adiposity, insulin resistance and the serum lipid profile, including the serum levels of triglyceride (TG), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) in Japanese patients with type 2 diabetes and non-diabetic subjects.

Patients and Methods A total of 259 Japanese subjects [171 patients with type 2 diabetes (age 60.4±11.9 years, BMI 25.0±4.4) and 88 non-diabetic subjects (age 54.2±15.0 years, BMI 25.8±4.7)] were enrolled in the study. To assess the hepatic fat content, the ratio of the CT attenuation value of the liver to that of the spleen (L/S ratio) was calculated.

Results Serum BChE activity was significantly correlated with various indices of adiposity, including the waist circumference, L/S ratio, visceral fat area (VFA), subcutaneous fat area (SFA) and BMI, and also with the TG, HDL-C, LDL-C and HOMA-R, in the entire subject population. Even after adjustment for five variables, namely, age, gender, HbA1c, CRP and serum ferritin (the serum levels of ferritin and CRP have been reported to be associated with adiposity and insulin resistance), the serum BChE activity was still correlated with the waist circumference, L/S ratio, VFA, SFA, BMI, TG, HDL-C, LDL-C and HOMA-R. Furthermore, changes in the serum BChE activity were associated with changes in the L/S ratio, VFA and SFA at 1 year after the baseline.

Conclusions These results indicate that the serum BChE activity is associated with parameters of adiposity, the serum lipid profile and the degree of insulin resistance.

Key words: butyrylcholinesterase, adiposity, insulin resistance, visceral fat area, triglyceride

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Introduction

The enzyme cholinesterase is present in all mammals and two classes have been identified: acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8). AChE exists in the central nervous system, platelets and the erythrocyte membrane, while BChE is more abundant in the serum and is synthesized by the liver (1). It is now well known that BChE acts on hydrophilic and hydrophobic choline esters (2, 3), and that it hydrolyzes a variety of xenobiotics (4). BChE has attracted attention as a bioscavenger of drugs as well as of organophosphate and carbamate insecticides (5). Previous studies have reported a significant

association between the serum BChE activity and obesity, coronary artery disease, serum levels of triglycerides (TG), very low-density lipoprotein, low-density lipoprotein and apolipoprotein B, type 2 diabetes mellitus and the hepatic fat content (6-10). On the other hand, the relationship of radiologically measured abdominal fat accumulation and hepatic fat content to metabolic risk factors for atherosclerosis has been intensively investigated, and both the visceral fat area (VFA) and hepatic fat content are now recognized to be correlated with the plasma TG level and glucose utilization (11-15). In fact, we have recently demonstrated an association between the serum ferritin and serum C reactive protein (CRP) concentrations and parameters of adiposity, including VFA, and the degree of insulin resistance (16, 17). However,

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until now, the association between parameters of adiposity and the serum BChE activity has not been fully revealed. These findings prompted us to hypothesize that the body fat distribution and degree of insulin resistance may be significantly associated with the serum BChE activity. Therefore, we investigated the association between the serum BChE activity and the parameters of adiposity, insulin resistance and glucose metabolism, and also the serum lipid profile, including TG and HDL-C.

Patients and Methods

Patients

We evaluated the clinical characteristics of patients who were admitted to the Yokohama City University Hospital between 2003 and 2006. The protocol of this study was reviewed and approved by the Institutional Ethical Review Committee. Informed consent was obtained from all of the subjects prior to the examinations. The study was carried out in 259 subjects admitted to our hospital for evaluation of elevated serum transaminase levels or fatty liver, or stringent control of blood glucose levels. Of these, 171 were diabetic patients and 88 were non-diabetic subjects. The study was restricted to men and postmenopausal women to eliminate the influence of pregnancy and female hormone replacement therapy, and to reduce any possible confounding effect of iron deficiency/ iron supplementation. The study was also restricted to persons older than 20 years of age to reduce the possible confounding effect of growth and development. A total of 259 Japanese (141 men and 118 postmenopausal women; age, 57.8 ± 13.9 years; body mass index, 25.7 ± 4.6 kg/m²; 171 type 2 diabetes mellitus patients and 88 non-diabetic subjects) subjects were evaluated. Fifty-four of the diabetic patients were receiving diet therapy alone, 53 were receiving a sulfonylurea alone, 28 were receiving a sulfonylurea and an insulin sensitizer, 27 were receiving insulin therapy and 9 were receiving an insulin sensitizer alone. Persons with a history of alcohol intake of ≥ 20 gram/day were excluded from the study. Subjects with a positive serological test for HBs antigen, HCV antibody antinuclear antibody or antimitochondrial antibody were excluded from the study. Serum ferritin is an acute-phase reactant and does not accurately reflect the body iron stores in the presence of inflammation. We therefore measured the serum CRP in this study to exclude patients with obvious inflammation. The proportion of subjects with a history of smoking was 32.2% in the diabetic group and 15.9% in the non-diabetic group.

Blood sampling and biochemical assay

Blood samples were obtained before breakfast on the second day of hospitalization, for measurements of the plasma glucose and insulin levels. The insulin resistance was calculated by the homeostasis model assessment for insulin resistance (HOMA-R), using the following formula: [fasting serum insulin (μ U/ml) $\times$ fasting plasma glucose (mg/dl)/405].

However, the HOMA-R analysis and measurement of serum ferritin were performed in only 104 subjects in whom the fasting plasma glucose was less than 140 mg/dl, since HOMA-R has been reported to be an unreliable index of insulin resistance in type 2 diabetic patients with fasting glucose levels of over 140 mg/dl (18). Of the 104 subjects, 52 were diabetic patients and 52 were non-diabetic subjects. Of 52 diabetic patients, 23 were receiving diet therapy alone, 16 were receiving a sulfonylurea alone and 13 were receiving a sulfonylurea and an insulin sensitizer; none of the patients was receiving insulin therapy. BChE activity was measured at the Central Laboratories of Yokohama City University Hospital.

Determination of the visceral and subcutaneous fat areas

The abdominal fat distribution in the subjects was determined by CT with the subjects in the supine position, in accordance with a previously described procedure (19). The SFA and intra-abdominal VFA were measured at the level of the umbilicus using a standardized method, in terms of the CT number. In brief, a region of interest was defined in the subcutaneous fat layer by tracing its contour on each scan, and the attenuation range for fat tissue was measured in terms of the CT number (in Hounsfield units).

Assessment of hepatic fat content

The degree of liver steatosis was measured by CT. Previous studies have shown a strong correlation between the CT attenuation values of the liver and the extent of fatty infiltration as measured by biopsy (20, 21). The ratio of the CT attenuation value of the liver to that of the spleen (L/S ratio) was used for quantitative estimation of the hepatic fat content, with an L/S ratio of <1 being considered to represent fatty liver (20).

Data analysis

All the data were expressed as means $\pm$ SD. The relationship between any two variables was analyzed by standard correlation analysis conducted using StatView, ver. 5.0, software (SAS, Cary, NC, USA). Analysis of variance was applied for group comparisons, followed by Student's *t*-test for confirmation of the statistical associations. The relationship between the L/S ratio, VFA, SFA and other relevant covariates was determined by multiple regression analysis and calculation of the standardized correlation coefficients. Statistical significance was assumed at *p*-values of <0.05 .

Results

Relationship between the serum BChE activity and clinical parameters

The baseline characteristics of the 171 patients with type 2 diabetes mellitus and 88 non-diabetic subjects are shown in Table 1. There were no significant differences in the se-

Table 1. Clinical Characteristics of the Subjects

Variables	Diabetic	Non-diabetic
n	171	88
Age (years)	60.4 ± 11.9	54.2 ± 15.0†
Gender (male/female)	100/71	41/47
Body mass index (kg/m ²)	25.0 ± 4.4	25.8 ± 4.7
L/S ratio	1.088 ± 0.238	1.008 ± 0.278*
VFA (cm ²)	127 ± 72.0	117 ± 52.9
SFA (cm ²)	170 ± 88.5	181 ± 70.5
Ferritin (ng/ml)	189 ± 191	182 ± 157
Glucose (mg/dl)	138 ± 46.6	101 ± 9.32†
Insulin (μU/ml)	10.7 ± 19.9	8.50 ± 3.60
HOMA-R	2.26 ± 1.37	2.13 ± 0.932*
HbA1c (%)	7.61 ± 1.70	5.05 ± 0.33†
CRP (mg/dl)	0.093 ± 0.087	0.097 ± 0.011
AST (IU/l)	28.6 ± 20.8	36.4 ± 24.4†
ALT (IU/l)	35.4 ± 32.5	53.2 ± 44.9†
BChE	351 ± 74.9	370 ± 80.7
Smoking (%)	32.2	15.9

Data are means ± SD. *P<0.05, †P<0.01 Diabetic vs. Non-diabetic.

Table 2. Correlation Coefficients by Simple Linear Regression Analyses between BChE and Other Associated Variables for the Entire Group

	r value	P value
waist circumference	0.326	<0.0001
L/S ratio	-0.315	<0.0001
VFA	0.242	0.0001
SFA	0.309	<0.0001
BMI	0.300	<0.0001
TG	0.404	<0.0001
HDL-C	-0.211	0.0008
LDL-C	0.216	0.0006
HOMA-R	0.396	<0.0001

rum BChE activity, VFA or SFA between the two groups (type 2 diabetes mellitus patients and non-diabetic subjects). The relationships between the serum BChE activity and the other factors examined in this study are shown in Table 2. The serum BChE activity was inversely correlated with the L/S ratio ($r=-0.315$, $p<0.0001$); in other words, it was positively correlated with the degree of liver steatosis. The serum BChE activity was also significantly correlated with various indices of adiposity, including the waist circumference ($r=0.326$, $p<0.0001$), VFA ($r=0.242$, $p=0.0001$), SFA ($r=0.309$, $p<0.0001$) and BMI ($r=0.300$, $p<0.0001$), the serum lipid profile, including TG ($r=0.404$, $p<0.0001$), HDL-C ($r=-0.211$, $p=0.0008$) and LDL-C ($r=0.216$, $p=0.0006$), and the degree of insulin resistance, namely, HOMA-R ($r=0.396$, $p<0.0001$) in the entire subject population. The association between the serum BChE activity and the waist circumference, L/S ratio, VFA, SFA, BMI, TG, HDL-C and HOMA-R was observed in both the diabetic patients and the non-

diabetic subjects (data not shown). In contrast, the serum BChE activity was significantly correlated with the LDL-C in the diabetic patients, but not in the non-diabetic subjects.

Multiple regression analysis

Multiple regression analysis was conducted to quantify the impact of the measured variables (age, gender, HbA1c and serum BChE activity) on the waist circumference, L/S ratio, VFA, SFA, BMI, HDL-C, TG, LDL-C and HOMA-R. The results shown in Table 3 indicate that even after adjustment for age, gender and HbA1c, the serum BChE activity was still correlated significantly with the waist circumference, L/S ratio, VFA, SFA, BMI, HDL-C, TG, LDL-C and HOMA-R.

We had previously shown an association between the serum ferritin and serum CRP concentrations and parameters of adiposity (16, 17). Therefore, we carried out multiple regression analysis to quantify the impact of the measured

Table 3. Relationships between Adiposity, Serum Lipids, Insulin Resistance and Clinical Background Factors Evaluated by Multiple Regression Analysis

Dependent variables	Independent variables				
	Standard regression coefficient (P value)				
	Regression analysis R ² (P value)	Age	Gender	HbA1c	BChE
Waist circumference	0.147 (<0.0001)	-0.211 (0.0013)	-0.003 (0.9557)	0.082 (0.1737)	0.254 (<0.0001)
L/S ratio	0.154 (<0.0001)	0.213 (0.0012)	-0.068 (0.2740)	0.066 (0.2712)	-0.254 (<0.0001)
VFA	0.127 (<0.0001)	-0.034 (0.6005)	0.233 (0.0003)	0.101 (0.0985)	0.253 (0.0001)
SFA	0.211 (<0.0001)	-0.286 (<0.0001)	-0.292 (<0.0001)	0.024 (0.6745)	0.188 (0.0024)
BMI	0.195 (<0.0001)	-0.345 (<0.0001)	-0.005 (0.9310)	0.025 (0.6814)	0.195 (0.0026)
TG	0.213 (<0.0001)	-0.098 (0.1165)	0.135 (0.0244)	0.103 (0.0764)	0.395 (<0.0001)
HDL-C	0.165 (<0.0001)	0.246 (0.0002)	-0.195 (0.0017)	-0.089 (0.1347)	-0.156 (0.0139)
LDL-C	0.065 (0.0027)	-0.015 (0.8250)	-0.156 (0.0172)	-0.020 (0.7515)	0.183 (0.0063)
HOMA-R	0.220 (<0.0001)	-0.080 (0.3708)	0.136 (0.1111)	0.096 (0.2260)	0.399 (<0.0001)

variables (age, gender, HbA1c, serum CRP concentration, serum ferritin concentration and serum BChE activity) on the waist circumference, L/S ratio, VFA, SFA, BMI, HDL-C, TG, LDL-C and HOMA-R. Even after adjustment for age, gender, HbA1c, serum CRP concentration and serum ferritin concentration, the serum BChE activity was still correlated significantly with the waist circumference, L/S ratio, VFA, SFA, BMI, TG, HDL-C, LDL-C and HOMA-R (Table 4). Thus, the serum BChE activity was significantly associated with not only parameters of adiposity, but also the serum lipid profile and the degree of insulin resistance.

As independent factors related to deterioration of waist circumference, the following were identified: age, serum CRP concentration and serum BChE activity. As independent factors related to deterioration of VFA, the following were identified: gender, serum CRP concentration and serum BChE activity. Calculation of the standard regression coefficients revealed that the degree of association of the serum BChE with parameters of adiposity, including the waist circumference and VFA, was weaker than that of the serum CRP concentration with these parameters, while the degree of association of the serum BChE with the serum lipid profile and insulin resistance was stronger than that of the serum CRP or serum ferritin concentrations with these parameters.

Analysis of the relationship between the Δ BChE and Δ body composition at 1 year after the initial hospitalization in the diabetic patients

We next examined whether the changes in the serum BChE activity (Δ BChE) were associated with changes in the body composition, as measured by changes in the L/S ratio, VFA and SFA; that is, the correlation between Δ BChE and the Δ indices of body composition, including the Δ L/S ratio, Δ VFA and Δ SFA. The Δ BChE was significantly correlated with the Δ L/S ratio ($r=-0.560$, $p=0.0010$), Δ VFA ($r=0.629$, $p=0.0001$) and Δ SFA ($r=0.502$, $p=0.0041$) (Table 5). These results suggest that the serum BChE may be a clinically useful marker to assess the degree of liver steatosis and visceral fat accumulation.

Discussion

In this study, we investigated the association between the serum BChE activity and the waist circumference, hepatic fat content, VFA, SFA, BMI, serum lipid profile and degree of insulin resistance. We demonstrated that the serum BChE activity was significantly associated with the waist circumference, CT-estimated L/S ratio, VFA, SFA, BMI, HDL-C,

Table 4. Relationships between Adiposity, Serum Lipids, Insulin Resistance, and Clinical Background Factors Evaluated by Multiple Regression Analysis

Dependent variables	Independent variables						
	Standard regression coefficient (P value)						
	Regression analysis R ² (P value)	Age	Gender	HbA1c	CRP	Ferritin	BChE
Waist circumference	0.271 (<0.0001)	-0.157 (0.0148)	-0.014 (0.8201)	0.042 (0.4824)	0.347 (<0.0001)	0.082 (0.1933)	0.225 (0.0004)
L/S ratio	0.282 (<0.0001)	0.197 (0.0023)	-0.061 (0.3329)	0.133 (0.0265)	-0.251 (<0.0001)	-0.210 (0.0010)	-0.194 (0.0022)
VFA	0.237 (<0.0001)	0.016 (0.8021)	0.225 (0.0006)	0.036 (0.5561)	0.3041 (<0.0001)	0.107 (0.0989)	0.216 (0.0009)
SFA	0.304 (<0.0001)	-0.248 (<0.0001)	-0.333 (<0.0001)	0.015 (0.8002)	0.264 (<0.0001)	0.110 (0.0740)	0.165 (0.0076)
BMI	0.416 (<0.0001)	-0.286 (<0.0001)	0.021 (0.7251)	-0.039 (0.4787)	0.445 (<0.0001)	0.076 (0.1943)	0.203 (0.0006)
TG	0.229 (<0.0001)	-0.053 (0.4202)	0.104 (0.1103)	0.049 (0.4289)	0.042 (0.4862)	0.100 (0.1260)	0.406 (<0.0001)
HDL-C	0.179 (<0.0001)	0.240 (0.0005)	-0.195 (0.0041)	-0.049 (0.4382)	-0.143 (0.0240)	0.012 (0.8558)	-0.146 (0.0296)
LDL-C	0.085 (0.0032)	-0.002 (0.9794)	-0.146 (0.0396)	-0.068 (0.3106)	0.138 (0.0389)	-0.007 (0.9246)	0.172 (0.0153)
HOMA-R	0.352 (<0.0001)	-0.006 (0.9466)	0.030 (0.7284)	0.034 (0.6614)	0.245 (0.0015)	0.185 (0.0277)	0.425 (<0.0001)

Table 5. Correlation Coefficients Determined by Simple Linear Regression Analyses between Δ BChE and Indices of Body Composition in 30 Subjects

	r value	p value
Δ L/S ratio	-0.560	0.0010
Δ VFA	0.629	0.0001
Δ SFA	0.502	0.0041

TG, LDL-C and HOMA-R (Table 2). We were able to further show that even after adjustment for age, gender and HbA1c, the serum BChE activity was still correlated significantly with the HDL-C, TG and LDL-C (Table 3), confirming the previously reported association between the serum BChE activity and the lipid profile and hepatic fat content (6, 7, 9). We also examined whether the changes in the serum BChE activity were associated with changes in the L/S ratio, VFA and SFA at 1 year after the initial hospitalization in the patients (Table 5). Our results revealed that the Δ BChE was significantly correlated with the Δ L/S ratio, Δ VFA and Δ SFA in the patients. The serum BChE activity has been reported previously to be associated with the waist circumference and BMI (22, 23), however, there are few reports on the direct association between the serum BChE ac-

tivity and body fat accumulation. Based upon the results of our cross-sectional and longitudinal examinations, we report that the serum BChE activity is also closely related to the degree of liver steatosis and of visceral and subcutaneous fat accumulation.

We had previously demonstrated an association of the serum ferritin and serum CRP concentrations with the parameters of adiposity and degree of insulin resistance (16, 17). In this study, the results of multiple regression analysis demonstrated that the serum BChE activity was significantly associated with parameters of adiposity, but that the serum CRP concentration was even more strongly associated with the parameters of adiposity than the serum BChE. Interestingly, however, the association of the serum BChE activity with the serum lipid profile and degree of insulin resistance was

even stronger than that of the serum ferritin and serum CRP concentrations with these parameters (Table 4).

Although the serum BChE activity is clinically used as an indicator of the nutritional status, its biological roles and the molecular mechanisms in which it is involved are surprisingly still unknown. In the present study, we demonstrated that in addition to the parameters of adiposity, the serum BChE activity was also associated with the serum lipid profile, including TG. As a possible explanation for this, it has been proposed that insulin resistance and an incremental flux of free fatty acids from adipose tissue to the liver might stimulate the hepatic synthesis of serum BChE (24). We speculate that BChE activity may be involved in TG metabolism and the flux of free fatty acids between adipose tissue and liver, to regulate adiposity. Interestingly, it was shown that adipose tissue also possessed BChE activity, at about 40% to 50% of the level found in the liver (25). Potential involvement of BChE activity in lipid metabolism in the adipose tissue may affect systemic insulin resistance and the lipid profile. Consistent with this hypothesis, it has been reported that the administration of tetraisopropylpyrophosphoramidate (iso-OMPA), a specific BChE inhibitor, caused a significant decrease of the TG, LDL-C and body weight (25). Therefore, we administered iso-OMPA to db/db mice, a mouse model of type 2 diabetes with obesity, and, consistent with the previous report, noted a decrease in the fat mass, body weight, serum lipids and food intake (data not shown) following its administration. These experimental data support the role of BChE in the regulation of the serum lipid profile and fat accumulation (25), however, the mechanisms underlying the regulation of the serum lipid profile and adiposity by BChE remain to be investigated in the future.

Cholinesterase inhibitors are now clinically used for both

Alzheimer's-type dementia and dementia associated with Parkinson's disease. No changes in the serum lipid profile or degree of insulin resistance have been shown in these patients receiving the medication, but this can perhaps be explained by the fact that these agents do not possess pharmacological BChE activity (26). Interestingly, rivastigmine, an acetylcholinesterase and BChE inhibitor, has been reported to decrease body weight (<http://www.fda.gov/cder/foi/label/2000/210251bl.pdf>). This may be due to inhibition of BChE activity; it raises the possibility of the potential use of BChE inhibitors for the treatment for obesity. However, it should be noted that the gastrointestinal side effects of rivastigmine, such as nausea, vomiting and diarrhea, may also be responsible for the reduction in body weight. The relationship between the gastrointestinal side effects and body weight reduction associated with this drug should be further investigated.

In conclusion, a direct association was demonstrated between the serum BChE activity and parameters of adiposity, the serum lipid profile and insulin resistance. Furthermore, the association of the serum BChE with the serum lipid profile and degree of insulin resistance was even stronger than that of the serum ferritin and serum CRP concentrations with these parameters. Thus, BChE inhibition might represent a novel strategy for the treatment of obesity with dyslipidemia and insulin resistance.

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