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ORIGINAL CONTRIBUTION

Self-reported rate of eating is associated with higher circulating ALT activity in middle-aged apparently healthy Japanese men

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Abstract
Purpose Elevated circulating activities of alanine aminotransferase (ALT), a marker for liver injury, and the lifestyle of a higher rate of eating in healthy and preclinical subjects are associated with increased risk of obesity and diabetes. In this study, we examined the associations between self-reported rate of eating and circulating ALT activity in middle-aged apparently healthy Japanese men.
Methods We conducted a cross-sectional study of 3,929 apparently healthy men aged 40–59 years (mean ± SD age, 49.2 ± 5.8 years; BMI, 23.5 ± 2.8 kg/m^2) who participated in health checkups in Japan. We analyzed their clinical serum parameters and lifestyle factors, including self-reported rate of eating. Associations between self-reported rate of eating and liver injury markers [ALT, γ-glutamyl transpeptidase (GTP), and aspartate aminotransferase (AST)], other clinical parameters or lifestyle factors were determined using analysis of variance followed by Tukey's test. Multivariate logistic regression analyses (MLRA) were performed with ALT activity as the dependent variable and independent variables that included self-reported rate of eating.
Results MLRA showed that ALT activity showed trends for higher self-reported rate of eating after adjustment for age, energy intake, and smoking status. The association between ALT activity and self-reported rate of eating disappeared after adjustment for BMI.
Conclusion The results of this study show that ALT activity is positively associated with self-reported rate of eating in middle-aged apparently healthy Japanese men.

Keywords Self-reported rate of eating · ALT activity · Japanese men · Apparently healthy subjects

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Introduction

Recent studies in Western and Asian countries, including Japan, have demonstrated that the risk of diabetes is greater in healthy and preclinical subjects with elevated circulating alanine aminotransferase (ALT) activity, a hepatic enzyme marker for liver injury, as compared with subjects with lower levels of these enzymes. This risk was also apparent in people with elevated ALT activity within the normal range and in people without fatty liver or hepatic dysfunction [1–5]. Recent studies have also demonstrated that circulating ALT activity is associated with hepatic insulin resistance, as assessed by measuring hepatic glucose output during insulin infusion, in subjects with/without impaired fasting glycemia, impaired glucose tolerance or type 2 diabetes mellitus in Western countries and in Japan [4, 6, 7]. These results indicate that ALT activity within the normal range is an early marker for the development of insulin resistance. Although the mechanism underlying the association between ALT activity and insulin resistance is unknown, a recent animal study showed that ALT activity facilitates hepatic gluconeogenesis by converting alanine to pyruvic acid [8]. It has been reported that insulin secretion insufficiency and hepatic insulin resistance both induce gluconeogenesis in the liver as a result of decreased insulin activity [9]. Accordingly, hepatic glucose release is increased, leading to elevated blood glucose levels. Thus, elevated ALT activity in subjects with hepatic insulin resistance may be due to increased hepatic gluconeogenesis in association with insulin resistance.

Recent epidemiological studies have demonstrated that the incidence of obesity and diabetes was higher in subjects with a higher self-reported rate of eating than in those with a lower self-reported rate of eating [10–12]. A higher rate of eating is thought to stimulate insulin secretion in association with postprandial hyperglycemia more than a lower rate of eating and that these factors enhance fatty acid synthesis in the liver and adipose tissue. Enhanced fatty acid synthesis in the liver and adipose tissue also leads to insulin resistance [13]. Thus, we hypothesized that a higher rate of eating will result in greater ALT activity in association with insulin resistance compared with a slower rate of eating in middle-aged subjects, resulting in the development of metabolic diseases. However, no studies have examined the associations between hepatic enzymes and the rate of eating in humans.

Therefore, in this study, we investigated the relationship between circulating ALT activity and other hepatic injury markers with the self-reported rate of eating in middle-aged apparently healthy men who were not taking any medications for metabolic diseases.

Subjects and methods

Subjects

We conducted a cross-sectional study of 5,004 men aged 40–59 years who participated in health checkups at Shizuoka Medical Center (Shizuoka Prefecture, Japan) between July 2005 and March 2007. Of these, 1,301 subjects were excluded because they were under treatment for stroke, hypertension, cardiac disease, diabetes, hyperlipidemia, liver diseases, kidney diseases, bladder diseases, prostate gland diseases, gout, psychiatric diseases, cancer, ulcer connective tissue disease, adenoiditis, pancreatitis, rheumatism, accessory thyroid diseases, thyroid diseases, muscular atrophy, served muscular asthenia, fibroid lung, lung obstruction, lung emphysema, pneumonia, lung sarcoidosis, acute bronchitis, interstitial lung disease, Behçet's disease, Graves' disease, Parkinsonian tremor, nephrosis syndrome, epilepsia, thrombocytopenia purpura, cholelithiasis, cholecystitis, cingulum, multiple myositis, distention of the common bile duct, systemic erythematodes, hyperuricemia, pleurisy or Hashimoto's thyroiditis before the checkups. We also excluded 20 subjects who were missing data relating to anthropometric parameters and questionnaires. Therefore, we included 3,929 subjects in this analysis. Anthropometric data were recorded and blood samples were collected from each participant by trained medical staff. The participants were also asked about their smoking status and self-reported physical activity. Smoking status was recorded as the number of cigarettes per day and duration of smoking. Dietary habits, including self-reported rate of eating, energy intake, and alcohol intake during the previous month, were assessed using a brief-type self-administered diet history questionnaire (BDHQ) [14, 15]. The BDHQ consisted of a 4-page structured questionnaire with three sections: general dietary behavior and major cooking methods; consumption frequency and amount of 5 alcoholic beverage intakes, and consumption frequency of 50 selected foods and nonalcoholic beverages; and the rate of eating, graded on a 5-point scale (very fast, fast, normal, slow, and very slow). The food and beverage items and their standard portion sizes were primarily derived from those used in a previously developed self-administered diet history questionnaire (DHQ) [16–18], a 16-page structured questionnaire consisting of 7 sections. The estimated dietary intake of 48 food and beverage items, energy and 42 nutrients was calculated using an *ad hoc* computer algorithm developed for the BDHQ, which was mainly based on the Standard Tables of Food Composition in Japan (Science and Technology Agency 2000). Previous studies have satisfactorily validated the estimates of nutrient intake and self-reported rate of eating determined by the BDHQ compared with those determined by a DHQ [11, 19–21]. All subjects gave informed consent for the use of their personal information for this study. The study protocol was approved by the Ethics Committee of the University of Shizuoka (Shizuoka, Japan).

Measurements

Height, weight, fasting plasma glucose, plasma HbA1c, serum triacylglycerol, serum total cholesterol, and serum high-density lipoprotein (HDL) cholesterol, and serum liver injury markers [ALT, γ-glutamyl transpeptidase (GTP), and aspartate aminotransferase (AST)] were measured in the morning, after an overnight fast. Body mass index (BMI) was calculated as weight (kg)/height^2 (m). Plasma HbA1c was determined by the Japan Diabetes Society (JDS) method and values were converted to international units based on the National Glycohemoglobin Standardization Program reference as "JDS value plus 0.4 %".

Statistical analysis

The clinical and biochemical data are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) followed by Tukey's test was used to calculate differences among quartiles of subjects based on self-reported rate of eating. Multivariate logistic regression analysis (MLRA) was performed to identify the independent variables for the ALT activity. *P* for trend was assessed using multiple linear regression analysis with ordinal numbers 1–3 assigned to tertiles of ALT activity. For all analyses, a value of $p < 0.05$ was considered significant. All statistical analyses were performed using JMP version 9.0 (SAS Institute Japan Co., Ltd., Tokyo, Japan).

Table 1 Physical characteristics, anthropometric characteristics, alcohol and energy intake, and physical activity in middle-aged men

Characteristics ($N = 3{,}929$)	
Age (years)	49.2 ± 5.8
BMI (kg/m^2)	23.5 ± 2.8
Systolic blood pressure (mmHg)	117.9 ± 14.5
Diastolic blood pressure (mmHg)	76.1 ± 11.3
Fasting blood glucose (mg/dL)	98.0 ± 15.1
HbA1c (%)	5.57 ± 0.6
Total cholesterol (mg/dL)	213.9 ± 34.1
HDL cholesterol (mg/dL)	58.1 ± 16.2
LDL cholesterol (mg/dL)	131.2 ± 30.5
Triacylglycerol (mg/dL)	112 (79–167)
AST (U/L)	23.4 ± 9.0
ALT (U/L)	22 (17–32)
γ-GTP (U/L)	36 (25–61)
Alcohol intake (g/day)	23.0 (3.1–54.6)
Energy intake (kcal/day)	2,140 ± 517
Smoking	
Number of cigarettes (number/day)	15 (0–20)
Duration of smoking (years)	15 (0–26)
Physical activity (time/week)	0 (0–3)
Self-reported rate of eating (%)	
Very slow/slow	10.4
Normal	32.9
Fast	44.5
Very fast	12.1

Data are expressed as mean ± SD, median (lower quartile, upper quartile) or percentage

BMI body mass index, *HbA1c* hemoglobin A1c, *HDL cholesterol* high-density lipoprotein cholesterol, *AST* aspartate aminotransferase, *ALT* alanine transferase, *γ-GTP* γ-glutamyl transpeptidase

Results

The study subjects were all apparently healthy Japanese men. The characteristics of the subjects are shown in Table 1.

ANOVA followed by Tukey's test was used to assess associations between categories of self-reported rate of eating (very slow/slow, normal, fast, and very fast) and serum clinical characteristics. Age, BMI, blood pressure (systolic and diastolic), LDL cholesterol and triacylglycerol were higher in fast and/or very fast than in the very slow/slow and/or normal quartiles. HDL cholesterol was lower in the fast and very fast quartiles than in the very slow/slow and normal quartiles. ALT activity, but not AST or γ-GTP activities, was higher in the fast and very fast quartiles than in the very slow/slow quartile. Age, BMI and ALT activity were higher in the normal quartile than in the very slow/slow quartile. HDL cholesterol was lower in the normal quartile than in the very slow/slow quartile. No differences in serum parameters were found between the fast and very fast quartiles (Table 2).

We next performed MLRA for the tertiles of ALT activity using parameters such as self-reported rate of eating, age, energy intake, and smoking (number of cigarettes). Regarding the self-reported rate of eating, the odds ratios for tertiles 2 and 3 of ALT activity relative to tertile 1 were 1.18 and 1.33, respectively. The *p* for trend determined by MLRA between self-reported rate of eating and the ordinals 1–3 assigned to the tertiles of ALT activity was significant. However, the association disappeared after including BMI as an explanatory variable (Table 3).

Discussion

Recent studies have demonstrated that ALT activity is a marker for monitoring the risk of developing the diabetes [1–5]. In addition, self-reported rate of eating is closely associated with the development of obesity and diabetes [10–12, 19]. Thus, we focused on the associations between self-reported rate of eating and ALT activity in apparently middle-aged Japanese men in this study. Previous studies have demonstrated that BMI was positively associated with self-reported rate of eating in healthy subjects, overweight subjects and subjects with diabetes in Western countries and in Japan [10–12]. In this study, we also found a positive association between self-reported rate of eating and BMI, consistent with the earlier studies. In addition, we found positive associations between self-reported rate of eating and hypertension, LDL cholesterol and triacylglycerol levels, and a negative association between self-reported rate of eating and HDL cholesterol. Previous studies have demonstrated that BMI is closely and positively

Table 2 Subject characteristics according to category of self-reported rate of eating

n	Very slow/slow	Normal	Fast	Very fast	ANOVA	
	410	1,293	1,750	476	*F* value	*p* value
Age (years)	50.4 ± 5.9 A	49.5 ± 5.7 B	48.9 ± 5.7 B	48.7 ± 5.9 B	9.58	<0.0001
BMI (kg/m^2)	22.3 ± 2.8 C	23.1 ± 2.6 B	23.9 ± 2.8 A	24.1 ± 2.9 A	55.53	<0.0001
Systolic blood pressure (mmHg)	116.4 ± 15.0 B	117.5 ± 14.1 AB	118.6 ± 14.8 A	118.2 ± 14.1 AB	3.05	0.0276
Diastolic blood pressure (mmHg)	74.6 ± 10.9 B	75.9 ± 11.4 AB	76.6 ± 11.2 A	76.6 ± 11.3 A	4.08	0.0067
Fasting blood glucose (mg/dL)	97.0 ± 15.6 A	97.4 ± 14.5 A	98.7 ± 16.4 A	97.9 ± 10.9 A	2.36	0.0695
HbA1c (%)	5.55 ± 0.6 A	5.56 ± 0.6 A	5.58 ± 0.6 A	5.57 ± 0.4 A	0.42	0.7373
Total cholesterol (mg/dL)	211.8 ± 33.5 AB	212.0 ± 32.5 B	215.6 ± 35.1 A	214.8 ± 34.8 AB	3.55	0.0139
LDL cholesterol (mg/dL)	126.8 ± 29.2 B	128.9 ± 29.4 B	133.1 ± 31.4 A	133.8 ± 30.1 A	8.74	<0.0001
HDL cholesterol (mg/dL)	62.2 ± 18.2 A	59.3 ± 16.2 B	56.7 ± 15.5 C	56.3 ± 16.0 C	17.39	<0.0001
Triacylglycerol (mg/dL)	126.9 ± 93.5 B	132.6 ± 95.2 B	147.1 ± 112.3 A	137.9 ± 79.9 AB	7.34	<0.0001
AST (U/L)	23.0 ± 11.2 A	23.0 ± 8.6 A	23.7 ± 9.1 A	23.5 ± 7.9 A	1.86	0.1348
ALT (U/L)	23.4 ± 12.1 C	25.9 ± 16.2 B	28.4 ± 17.2 A	28.6 ± 16.6 A	14.23	<0.0001
γ-GTP (U/L)	49.9 ± 48.9 A	51.2 ± 49.3 A	54.4 ± 55.7 A	50.8 ± 47.8 A	1.51	0.2097
Alcohol intake (g/day)	34.0 ± 37.1 A	33.8 ± 34.5 A	33.4 ± 35.7 A	31.1 ± 35.0 A	0.76	0.5191
Energy intake (kcal/day)	2,131 ± 528 AB	2,131 ± 494 AB	2,132 ± 526 B	2,201 ± 531 A	2.53	0.0552
Smoking						
Number of cigarettes (number/day)	12.0 ± 12.3 AB	12.0 ± 11.9 B	13.1 ± 12.4 AB	13.8 ± 12.9 A	3.85	0.0091
Duration of smoking (years)	14.5 ± 14.3 A	13.5 ± 13.5 A	14.2 ± 13.5 A	13.8 ± 13.1 A	0.89	0.4445
Physical activity (times/a week)	1.89 ± 2.4 A	1.80 ± 2.4 A	1.71 ± 2.3 A	1.64 ± 2.4 A	1.11	0.3454

Post hoc analyses of the ANOVA were performed by Tukey's multiple range test. (A, B, C) Different letters denote significant differences among groups

BMI body mass index, *HbA1c* hemoglobin A1c, *HDL cholesterol* high-density lipoprotein cholesterol, *AST* aspartate aminotransferase, *ALT* alanine amino transferase, *γ-GTP* γ-glutamyl transpeptidase

Table 3 Multivariable logistic regression analysis for tertiles of ALT activity

	Variables	T2		T3		*p* for trend
		OR	CI	OR	CI	
Without BMI	Self-reported rate of eating[a]	1.18	(1.08, 1.29)	1.33	(1.21, 1.46)	<0.0001
	Age	0.99	(0.98, 1.00)	0.96	(0.94, 0.97)	<0.0001
	Energy intake	1.00	(1.00, 1.00)	1.00	(1.00, 1.00)	0.0055
	Number of cigarettes	0.99	(0.99, 1.00)	1.00	(0.99, 1.01)	0.8540
With BMI	Self-reported rate of eating[a]	1.06	(0.96, 1.16)	1.06	(0.95, 1.18)	0.1916
	Age	0.99	(0.97, 1.00)	0.96	(0.95, 0.98)	<0.0001
	BMI	1.24	(1.20, 1.28)	1.53	(1.47, 1.59)	<0.0001
	Energy intake	1.00	(1.00, 1.00)	1.00	(1.00, 1.00)	0.0702
	Number of cigarettes	0.99	(0.99, 1.00)	1.00	(0.99, 1.00)	0.3481

Tertiles for ALT activity were divided into T1 (range 4–19 U/L, $n = 1{,}478$), T2 (range 20–29 U/L, $n = 1{,}313$) and T3 (range 30–165 U/L, $n = 1{,}138$)

T1 of ALT was coded as 0, T2 or T3 was coded as 1 in multivariate logistic regression analyses

p for trend is assessed using multiple linear regression analysis with ordinal number 1–3 assigned to the tertile categories of ALT activity

OR odds ratio, *CI* confidence interval, *BMI* body mass index

[a] Self-reported rate of eating: 1 = very slow/slow, 2 = normal, 3 = very fast/fast, 4 = very fast

associated with hypertension, LDL cholesterol, triacylglycerol and negatively associated with HDL cholesterol [22, 23]. Our results are consistent with these earlier studies. Interestingly, we found an association between self-reported rate of eating and ALT activity, but not with AST or γ-GTP activities. Previous studies in Western countries and Japan have demonstrated that circulating ALT activity is associated with hepatic insulin resistance, assessed by measuring hepatic glucose output during insulin infusion [4, 6, 7]. Thus, we hypothesized that self-reported rate of eating is associated with elevated ALT activity in middle-aged apparently healthy Japanese men.

To examine this hypothesis, we carried out MLRA to determine associations between self-reported rate of eating and ALT activity. As shown in Table 3, ALT activity was strongly positively associated with higher self-reported rate of eating. However, the association between self-reported rate of eating and ALT activity disappeared after adjusting for BMI, indicating that this association may be dependent on BMI. It is still unclear whether the association between self-reported rate of eating and ALT activity in this study is dependent on BMI. Therefore, future cohort studies should examine the causal associations among rate of eating, BMI and ALT activity. These results also suggest that ALT activity is physiologically variable within the normal range in subjects without fatty liver or hepatic dysfunction. This notion is supported by previous studies in Western and Asian countries, including Japan, showing that the risk of diabetes was greater in healthy and preclinical subjects with elevated circulating ALT activity within the normal range as compared with subjects with lower levels of these enzymes [1–5]. Those studies also showed that the risk was higher in subjects without fatty liver or hepatic dysfunction. Further studies are needed to identify which lifestyle factors and food intake characteristics, other than the self-reported rate of eating, influence physiologic ALT activity within the normal range in subjects without fatty liver or hepatic dysfunction. Such studies should also examine whether these lifestyle factors increase the risk of obesity and diabetes. The association between self-reported rate of eating and ALT activity should also be investigated in other groups of subjects, including other age ranges and in subjects with metabolic diseases.

It should be noted that the validity of energy intake assessed by questionnaires, such as the BDHQ, is quite low [24]. Thus, it is debatable whether subjects reporting a higher rate of eating have a higher energy intake. Accordingly, studies should examine the association between the rate of eating and ALT activity independently of energy intake using more valid methods of assessing food intake, such as the food-weighing method.

In summary, the results of this study suggest that the self-reported rate of eating is associated with elevated circulating ALT activity in apparently healthy Japanese men.

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Conflict of interest The authors declare no conflicts of interest.

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