

SERUM COMPONENTS AND LIFESTYLE FACTORS - V

Relationship of Soluble Fas with Body Mass Index in Healthy Japanese Adults

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Abstract

Recent studies have linked elevated serum sFas levels to atherosclerotic disease among patients. Confirming an association between obesity and serum sFas levels in healthy subjects would facilitate our understanding of obesity and its related disorders. We therefore analyzed serum sFas levels of 8,541 subjects selected as controls for a nested case-control study within the JACC Study. Body mass index (BMI) was calculated as the indicator of obesity based on self-reported height and weight. We found a statistically significant positive association between serum sFas levels and BMI among our apparently healthy subjects. Our result suggests that serum sFas rises to down-regulate increased apoptosis in atherogenesis processes caused by obesity.

Keywords: Japan Collaborative Cohort Study - sFas - BMI - healthy controls

Asian Pacific J Cancer Prev, **10**, JACC Serum Component Supplement, 41-44

Introduction

Obesity is reported to be highly associated with systemic oxidative stress (Keaney et al., 2003), which plays critical roles in the pathogenesis of various diseases, such as cancer, inflammation and atherosclerosis (Harrison et al., 2003). In atherogenic processes, reactive oxygen species (ROS) may initiate stimulation of vascular smooth muscle proliferation and migration, oxidation of lipids, and apoptosis in the endothelium (Harrison et al., 2003). Fas, an apoptosis inducing molecule expressed on cell membranes, interact with Fas ligand (FasL) in order to trigger cell death. Elevated levels of Fas expression on cells thus indicate increased sensitivity to apoptosis (Imirzalioglu et al., 2005). On the other hand, the contribution of sFas, a soluble isoform of Fas, might differ. It appears to play an important role in preventing the induction of apoptosis. For example, increased sFas levels in systemic lupus erythematosus patients has been shown to block apoptosis in cells by inhibiting the binding of FasL to Fas on cell membranes (Cheng et al., 1994).

Although elevated serum sFas levels have been linked to atherosclerotic diseases among patients in recent studies (Hebert et al., 2001; Masse et al., 2002), only few studies (Choi and Kim, 2005; Zahorska-Markiewicz et al., 2008)

have reported an association between sFas levels and obesity in healthy people. Elucidating any such link would facilitate understanding of obesity and its related disorders. Thus, the aim of the present study was to investigate association between body mass index (BMI), one of the indicators of obesity, and serum sFas levels in healthy subjects.

Materials and Methods

Study Population and Serum Samples

The study subjects here were controls of a nested case-control study within the Japan Collaborative Cohort (JACC) Study, a large scale cohort study. Details of the JACC Study have been already described elsewhere (Ohno et al., 2001; Tamakoshi et al., 2005). Briefly, it involved 110,792 subjects, aged 40 to 79 years at baseline living in 45 municipalities all over Japan. At the baseline, information on lifestyle factors were collected by using self-administered questionnaires. In 37 areas, blood samples were donated from a part of the cohort members at the same time. Serum samples were stored at -80°C until analyses. Informed consent of serum donation and its research use was obtained from each participant in 32 areas, though consent was given only by the leader of the

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area in 5 areas. The whole study design and use of serum was approved by the Ethical Board at Nagoya University School of Medicine, where the central office of JACC Study was located.

Body Mass Index

All information on lifestyle factors and medical history was queried by self-administered questionnaires. BMI was calculated based on self-reported height and weight ($BMI = \text{weight}(\text{kg})/\text{height}(\text{m})^2$). Subjects were grouped into the 5 BMI categories: BMIs <18.5, 18.5-21.9, 22.0-24.9, 25.0-29.9 and ≥ 30.0 .

Biochemical Assays of Sera

Serum levels of sFas were measured by enzyme-linked immuno-adsorbent assay (ELISA) in 1999 and 2000, using commercially available kits (MBL Co., Ltd., Nagoya). All samples were assayed at a single laboratory (SRL Inc., Hachioji) by trained staff. Assay methods have been described in detail elsewhere (Ito et al., 2005). The range of the assay for serum sFas levels was 1.0-10 ng/ml; the intra- and inter-assay precisions were 2.1-5.5% and 8.2-

12.3%, respectively. Since sFas levels were systematically low in one area, we excluded all sera from that area from the analysis. Those whose sFas levels exceeded 10 ng/ml were also excluded because of possibility of undetectable diseases. For the present analysis, 8,541 subjects (4,457 men and 4,084 women) were eligible as they had both data on serum sFas levels and BMI.

Analytical method

Distributions of some baseline characteristics were compared according to BMI categories using the Mantel-Haenszel test. Least square means of serum sFas according to BMI categories were estimated while controlling for possible confounding factors. Since sFas levels had logarithmic distributions, all tests and estimations were conducted using log-transformed levels. Variables adjusted for multivariate analysis were age group at baseline, area, smoking status (current smoker, ex-smoker, non-smoker and unknown), alcohol consumption (current drinkers, quitters, non-drinkers, and unknown), walking (≥ 1 hour per day, < 1 hour per day, and unknown) and consumption of green leaf vegetables (within 1-2 times per week, 3-4 times per week, almost everyday, and unknown). All p values were two-sided, and all statistical analyses were performed using the Statistical Analysis System (SAS 9.1, Cary, NC).

Table 1. Characteristics of Subjects by BMI Category

	<18.5	18.5-21.9	22-24.9	25-29.9	≥ 30.0	p value
Men						
Number	248	1,769	1,724	680	36	
Age Mean	65.4	63.3	62.4	60.7	60.1	<0.0001
SD	6.8	7.5	7.6	8.2	9.6	
Smoker*	56.9	52.9	44.6	38.4	22.2	<0.0001
Drinker*	65.3	71.4	73.6	71.5	69.4	0.18
Walker**	44.4	44.2	37.9	34.0	44.4	0.01
Eating green leaf vegetables almost daily*	28.2	26.9	27.4	23.8	22.2	0.98
Women						
Number	284	1,372	1,522	844	62	
Age Mean	66.1	62.9	62.4	62.1	61.5	<0.0001
SD	8.9	9.5	8.7	8.3	8.5	
Smoker*	2.1	2.6	2.5	3.0	3.2	0.88
Drinker*	15.1	20.3	21.2	21.2	14.5	0.13
Walker**	43.0	41.0	41.1	37.2	40.3	0.20
Eating green leaf vegetables almost daily*	29.2	29.6	28.1	26.8	35.5	0.37

%; * ≥ 1 hr/day; p, performed by Mantel Haenszel test adjusted for area, or for area and age category

Results

Table 1 shows distribution of baseline characteristics according to BMI categories. Those with higher BMI were younger among both men and women. Among men, they were less likely to be a current smoker and less likely to walk, however, no such trend was found among women. Alcohol drinking and consumption of green leaf vegetables had no association with BMI categories among both men and women.

Serum sFas levels were positively associated with BMI among both men and women (Table 2). Least square means adjusted for possible confounding factors was 2.23 (95% CI: 2.15-2.31) ng/ml among men with lowest BMI category and increased up to 2.49 (2.28-2.71) ng/ml among the highest. Among women, the lowest and the 2nd lowest BMI categories showed similar sFas levels

Table 2. BMI categories and sFas levels

	<18.5	18.5-21.9	22.0-24.9	25.0-29.9	≥ 30.0
Men					
Number	248	1,769	1,724	680	36
Means (95%CI)*	2.23 (2.15-2.31)	2.24 (2.19-2.29)	2.29 (2.24-2.34)	2.38 (2.31-2.44)	2.49 (2.28-2.71)
P value**	0.700	ref	0.022	<0.0001	0.019 Trend <0.0001
Women					
Number	284	1,372	1,522	844	62
Means (95%CI)*	2.21 (2.12-2.31)	2.18 (2.11-2.26)	2.24 (2.16-2.32)	2.31 (2.23-2.39)	2.40 (2.23-2.57)
P value**	0.433	ref	0.010	<0.0001	0.005 Trend <0.0001
Men & Women					
Number	532	3,141	3,246	1,524	98
Means (95%CI)***	2.22 (2.16-2.27)	2.20 (2.17-2.24)	2.26 (2.22-2.29)	2.34 (2.30-2.38)	2.44 (2.31-2.57)
P value**	0.677	ref	<0.001	<0.0001	<0.001 Trend <0.0001

*; adjusted for area, age category, smoking status, drinking status, walking, consumption of green leaf vegetables; **; compared with BMI 18.5-21.9; ***; Least square means were adjusted for area, age category, gender, smoking status, drinking status, walking, consumption of green leaf vegetables

(2.21 ng/ml and 2.18 ng/ml), and going up with increasing BMI categories (2.40 ng/ml for the highest BMI category). The analysis of men and women combined showed similar results.

Discussion

Using data of 8,541 apparently healthy subjects, we found that serum sFas levels were statistically significantly associated with BMI among both men and women.

One previous study showed no significant differences in serum sFas levels between the subjects with obesity and those with a healthy weight (n=176), although men with higher sFas levels displayed significantly higher total body fat and waist-hip-ratio (n=91) (Choi and Kim, 2005). Another study examined the effect of weight loss on sFas levels in obese women, and though no change was found after weight loss, there was a positive association between BMI and sFas levels before treatment (n=23) (Zahorska-Markiewicz et al., 2008). These 2 studies were relatively small compared with ours, but obesity, even if measured by BMI or not, was found to have some correlation with sFas levels, as in our study.

Obesity is known to generate ROS which induces apoptosis (Keaney et al., 2003), and the Fas-FasL system is a well-known death factor causing apoptosis to cells (Nagata, 1997). In contrast, sFas, splicing variant of Fas, binds FasL and act as competitive antagonists of Fas apoptotic signaling (Owen-Schaub et al., 2000). Therefore, considering our results in light of previous knowledge, levels of serum sFas might rise to down-regulate increased apoptosis in atherogenesis process caused by obesity.

We have to take caution regarding some limitations of our study when interpreting the results. First, since not all the cohort participants provided blood samples, there was the possibility of a selection bias. However, donation depended solely on the subject's intention, and control selection in nested case-control study was only based on matching information, age, area and gender. Thus, any bias due to blood donation or selection would not seriously affect our results. Second, serum samples were stored at -80°C for approximately 10 years. The stability of sFas in these cohort samples could not be determined because their levels were not measured at baseline. However, Ito et al compared newly collected sera and frozen specimens stored for 9 years gathered from a variety of different individuals, and found no statistically significant difference in the distributions of sFas levels (Ito et al., 2005), indicating that the serum sFas levels remained stable after long-term storage at -80°C. Third, our data for BMI were based on self-reported rather than measured heights and weights. According to the National Nutrition Survey in 1986-90, the prevalence of overweight/ obese (BMI \geq 25) were 24.2%, 27.1%, 20.0% and 15.3% among men aged 40-49, 50-59, 60-69 and \geq 70, and 21.3%, 28.7%, 30.3% and 24.6% among women, respectively (Yoshiike et al., 2002). In our study, the comparative numbers were relatively small, 29.8%, 19.3%, 14.8% and 11.2% among men and 20.5%, 24.1%, 24.7% and 16.4% among women, respectively. Thus, we could not exclude

the possibility of some misclassifications due to underestimation of overweight/ obesity subjects. However, such misclassification might diminish the differences between normal and overweight/ obese groups at baseline, and the differences of least square means of sFas might be diminished accordingly.

In conclusion, serum sFas levels appear to be associated with obesity among healthy men and women.

Member list of the JACC Study Group

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Acknowledgements

The JACC Study has been supported by Grants-in-Aid for Scientific Research (Nos. 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, 11181101, 17015022, 18014011, 20014026) from MEXT, Japan.

The authors express their sincere appreciation to Dr. Kunio Aoki, Professor Emeritus, Nagoya University School of Medicine and a former chairperson of the JACC Study, to Dr. Haruo Sugano, the former Director of the

Cancer Institute, Tokyo, who greatly contributed to the initiation of the JACC Study, and to Dr. Yoshiyuki Ohno, Professor Emeritus, Nagoya University School of Medicine, who was also a former chairperson of the study. The authors also wish to thank Dr. Tomoyuki Kitagawa, Director Emeritus of the Cancer Institute of the Japanese Foundation for Cancer Research and a former chairperson of a Grant-in-Aid for Scientific Research on Priority Area 'Cancer', for his valuable support of this study.

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