発表者氏名	論文タイトル名	発表誌名	巻数	ページ	出版年
Yamashita, H., Wang, Z., Wang, Y., Furuyama, T., Kontani, Y., Sato, Y., and Mori, N.	Impaired basal thermal homeostasis in rats lacking capsaicin-sensitive peripheral small sensory neurons.	J. Biochem.	143	385-393	2008
Yamashita, H., Wang, Z., Wang, Y., Segawa, M., Kusudo, T., and Yasuhide Kontani.	Induction of fatty acid-binding protein 3 in brown adipose tissue correlates with increased demand for adaptive thermogenesis in rodents.	Biochem. Biophys. Res. Commun.	377	632-635	2008
山下　均	脂肪細胞の役割からみる肥満—新しい肥満予防法開発の試み一.	中部大学生命健康科学研究	4	47-53,	2008
服部恵美、安藤富士子、下方浩史	肥満と高感度C反応性蛋白(hs-CRP)— 地域住民における性・年代別の解析	日本未病システム学会雑誌			印刷中
Sugiura M, Nakamura M, Ogawa K, Ikoma Y, Matsumoto H, Ando F, Shimokata H, Yano M	Associations of serum carotenoid concentrations with metabolic syndrome: Interaction with smoking	Br J Clin Nutr			印刷中

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
山下　均	エネルギー代謝モデル動物実験ガイド.	日本基礎老化学会	老化・老年病研究のための動物実験ガイドブック	アドスリー	東京	52-57	2008
安藤富士子、今井具子、下方浩史	食事・栄養と中高年男性の健康 ―栄養疫学の立場から―.	熊本悦明、堀江重郎	更年期から熟年期までの男性医学―中高年のMen's Healthを支えるために―	ライフサイエンス社	東京		印刷中

Ⅲ. 研究成果の刊行物・別刷

ELSEVIER

Atherosclerosis 185 (2006) 183–190

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Effect of smoking habit on age-related changes in serum lipids: A cross-sectional and longitudinal analysis in a large Japanese cohort

Masafumi Kuzuya [a,*], Fujiko Ando [b], Akihisa Iguchi [a], Hiroshi Shimokata [b]

[a] *Department of Geriatrics, Nagoya University Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8550, Japan*
[b] *Department of Epidemiology, National Institute for Longevity Sciences, 36-3 Gengo, Morioka-cho, Obu-shi, Aich 474-8522, Japan*

Received 4 March 2005; received in revised form 19 May 2005; accepted 31 May 2005
Available online 26 July 2005

Abstract

To observe the effect of smoking habit on age-related serum lipid levels, we examined a large cohort of Japanese cross-sectionally and longitudinally. The participants included 103,648 Japanese men and women 17–94 years of age, who had received annual health examinations from 1989 to 2003. In cross-sectional analysis, total and LDL cholesterol levels of smokers were lower than those of nonsmokers up to an elderly age in men and up to middle age in women. Smoking was associated with decreased HDL cholesterol levels up to the 65–74 years age group in men and 55–64 years in women. The triglyceride levels were higher in smokers in both genders than those of nonsmokers below 55–64 years. In the longitudinal analysis, although smoking was associated with lower total and LDL cholesterol up to 60 years of age in women, beyond the sixties an inverted association was observed. The associations of smoking with lower LDL cholesterol levels in men and lower HDL cholesterol in both genders were fairly consistent at any given age. The increase of triglyceride levels in female smokers remained rather constant between 25 and 75 years, whereas the increase in triglyceride levels in male smokers was greater with older ages up to middle age. These results suggest that the effect of smoking on the serum lipid levels is dependent on age and gender.

Keywords: Smoking; Total cholesterol; Triglyceride; HDL cholesterol; LDL cholesterol; Longitudinal study; Ageing

Although smoking is well recognized as a risk factor for coronary artery disease and stroke [1,2], the underlying mechanisms and factors responsible for this association are complex and only partially understood [3]. One possible mechanism for the effect of smoking on cardiovascular disease risk is the atherogenic impact of tobacco smoke on serum lipids and lipoproteins. Previous observations suggest that smokers exhibit elevations of triglycerides, total and low-density lipoprotein (LDL) cholesterol, as well as decreases of high-density lipoprotein (HDL) cholesterol as compared with nonsmokers [4–6]. Most conclusions regarding these associations with smoking habit have been drawn from selected groups, including clinical trials or cross-sectional studies targeting adolescents, young adults, and adults. To our knowledge, no study has been done targeting the elderly.

We and other authors have demonstrated that serum lipid levels vary during the ageing process based on the longitudinal observations [7,8]. However, the effect of smoking habit on the age-related changes in serum lipid levels remains unknown, and to our knowledge, no study has examined the longitudinal changes in the smoking effect on serum lipid levels in individual across a broad age range over time.

In the present study, we examined the cross-sectional and longitudinal changes in serum lipid levels in a single cohort of individuals with or without smoking habit to observe the effect of the natural aging process on the effect of smoking on the age-related serum lipid levels.

1. Materials and methods

1.1. Study population

The study population was office workers and their families residing in Aichi Prefecture in the central region of Japan. The

* Corresponding author. Tel.: +81 52 744 2364; fax: +81 52 744 2371.
E-mail address: kuzuya@med.nagoya-u.ac.jp (M. Kuzuya).

0021-9150/$ – see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.atherosclerosis.2005.05.040

subjects included 103,648 Japanese (65,789 men and 37,859 women) with an average age of 44.7 years in men and 43.3 years in women, who had received annual examinations at a health examination center in Japan between 1989 and 2003 (Table 1). A total of 2030 subjects who were receiving medication for hyperlipidemia had already been excluded. Our cohort included more males than females, since the number of male workers is greater than the number of female workers in Japan. About 57% of the cohort attended at least one follow-up examination. Average visits for the follow-up examinations were 3.1 times for men and 2.7 times for women.

1.2. Procedures and laboratory methods

The examinations included a questionnaire, a physical examination, an anthropometric measurement, and laboratory analysis of blood samples, all taken on the same day. The anthropometric measurements included height and body weight, which were measured while the subject was wearing light clothing without shoes. The body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Information on smoking status (current cigarette smokers or not) was also recorded using a self-administered questionnaire.

All serum samples were obtained following a 12–14 h fast. Serum was separated promptly, and all lipid analyses were conducted at the clinical laboratory in the health examination center. Serum total cholesterol and triglycerides were measured by using enzymatic methods. HDL cholesterol was measured after dextran sulfate–magnesium precipitation. No differences were seen in the sample collection, laboratory apparatus, or techniques used between 1989 and 2003. LDL cholesterol was estimated by using the method of Friedewald et al. [9].

1.3. Data analysis

The data were analyzed with the Statistical Analysis System (SAS), release 8.2. Smoking status and age-related change of the serum lipids were quite different between men and women. Thus, the data were analyzed separately by gender. We previously demonstrated that there is a birth cohort effect on serum lipid levels based on a 10-year longitudinal analysis of the same cohort, which suggested that higher estimated total and LDL cholesterol levels were observed in younger birth cohorts than in older cohorts [7]. Average of total and LDL cholesterol levels increased with the year of the observation. Therefore, the cross-sectional data were adjusted for the year of the initial examination of each subject and BMI, and lipid levels were estimated for the examination in 1996 and at BMI = 22 (Table 3). The difference in serum lipid levels between smokers and nonsmokers was examined using Student's *t*-test in six age groups divided by decades ranging from less than 25–75 years and older.

Cross-sectional age-related changes in the lipid levels may represent cohort, period, and/or survivor ship effects rather than a true aging effect. Longitudinal data analysis is necessary to examine the effect of smoking habit on true age-related changes of serum lipid levels. Longitudinal changes in serum lipid levels were analyzed by a mixed effect model [10,11], which is a type of statistical analysis commonly used for repeated measurements. It is applied using the SAS procedure PROC MIXED, typically using the PEPEATED statement. Age-related changes of serum lipids were estimated by quadratic curve of age controlling for the observation year and BMI. Fixed effects for the observation year, BMI, age, age square, smoking status, smoking–age interaction, and smoking–age square interaction were included in the model, and random effect of subjects were also included in the model. Responses from points close in time are usually more highly correlated with each other than responses from points far apart in time. Therefore, special methods of analysis are usually needed to accommodate the correlation structure of the repeated measurements. This autoregression was controlled using the autoregressive covariance–structure in the mixed effect model. The least square means for serum lipid values at every age were determined in smokers and nonsmokers. The differences of the lipid levels between smokers and nonsmok-

Table 1
Characteristics of participants

	Men	Women
Number of subjects	65,789	37,859
Total no. of measurements for 14 years	204,064	103,244
No. of measurements per subject for 14 years, mean (S.D.)	3.1 (2.9)	2.7 (2.5)
Age (year), mean (S.D.)	44.7 (9.3)	43.3 (9.4)
Age range (year)	14–94	17–85
Height (cm) at initial measurement, mean (S.D.)	168.5 (6.0)	156.0 (5.4)
Body weight (kg) at initial measurement, mean (S.D.)	65.6 (9.3)	52.4 (7.3)
BMI (kg/m^2) at initial measurement, mean (S.D.)	23.1 (2.8)	21.6 (2.9)
Smoker (%) at initial examination	53.4	11.8
Serum lipid levels at initial measurement		
Total cholesterol (mM), mean (S.D.)	5.15 (0.90)	5.14 (0.94)
LDL cholesterol (mM), mean (S.D.)	3.02 (0.81)	2.94 (0.85)
HDL cholesterol (mM), mean (S.D.)	1.42 (0.34)	1.75 (0.37)
Triglyceride (mM), mean (S.D.)	1.60 (1.16)	0.98 (0.56)

Table 2
Characteristics of participants for longitudinal analysis

	Men	Women
Number of subjects	61,150	37,024
Total no. of measurements for 14 years	204,064	103,244
No. of measurements per subject for 14 years, mean (S.D.)	2.9 (2.8)	2.7 (2.5)
Age (year), mean (S.D.)	44.7 (9.3)	43.4 (9.4)
Age range (year)	14–94	17–85
Follow-up periods (year), mean (S.D.)	2.9 (3.9)	2.8 (3.8)
Height (cm) at initial measurement, mean (S.D.)	168.5 (6.0)	156.0 (5.4)
Body weight (kg) at initial measurement, mean (S.D.)	65.6 (9.3)	52.4 (7.3)
BMI (kg/m^2) at initial measurement, mean (S.D.)	23.1 (2.9)	21.6 (2.9)
Smoker (%) at initial examination	51.5	10.6
Serum lipid levels at initial measurement		
Total cholesterol (mM), mean (S.D.)	5.15 (0.90)	5.14 (0.94)
LDL cholesterol (mM), mean (S.D.)	3.03 (0.81)	2.95 (0.85)
HDL cholesterol (mM), mean (S.D.)	1.42 (0.34)	1.75 (0.37)
Triglyceride (mM), mean (S.D.)	1.60 (1.17)	0.98 (0.56)

ers at each age were obtained by the differences of estimated lipid levels based on the longitudinal analysis using a mixed effect model between smokers and nonsmokers at each age. In the longitudinal analysis, subjects who reported a nonsmoking status at least once during the repeated examinations over a 14-year period were excluded from the smoker group. In addition, subjects who reported current smoking status during at least one point of the repeated examinations were excluded from the nonsmoker group. As a result, all subjects who changed smoking habit as noted during the repeated measurements (4639 males and 835 females) were excluded from the longitudinal analysis. The characteristics of participants for the longitudinal analysis are summarized in Table 2.

2. Results

2.1. Cross-sectional analysis

Fig. 1 shows the smoking rate of the participants between 1989 and 2003. At the initial examination, the rate of smoking in men and women was 53.4 and 11.8%, respectively, which is similar to rates shown in a national survey. The rate of smoking decreased during the periods examined which is consistent with the observation of others [12].

Fig. 2 shows the age-specific means and 3-year moving average of serum lipid levels at initial measurement of each subject of men and women with or without smoking habit from 1989 through 2003 before including the effect of BMI and the time of examination. The age-related changes of serum lipid levels of both male and female smokers were similar to those of nonsmokers. In men, serum total cholesterol level gradually increased from 20–29 years up to 50–59 years, and no further increase was observed after 50–59 years. In women, serum total cholesterol level dramatically increased from 20–29 years up to 60–69 years and then subsequently decreased. These age-related changes were similar in LDL cholesterol levels in men and women. HDL cholesterol levels were rather constant up to 70–79 years in men. In women, HDL cholesterol levels were lower with increasing age. Serum triglyceride levels increased up to 40–49 years, followed by a decline above 50–59 years in men, whereas triglyceride levels in women increased up to 60–69 years and then decreased at 70–79 years.

Total and LDL cholesterol levels of smokers were somewhat lower than those of nonsmokers above middle age in men, but no obvious differences were observed between smokers and nonsmokers in women (Fig. 2). In HDL cholesterol and triglyceride, much lower and higher levels were observed, respectively, in smokers compared with those of nonsmokers at all ages of men and women (Fig. 2).

We previously demonstrated that there was a birth cohort effect on serum lipid levels in this large Japanese cohort [7]. BMI is also known to influence the serum lipid levels [13,14]. Therefore, the cross-sectional data of serum lipid levels at

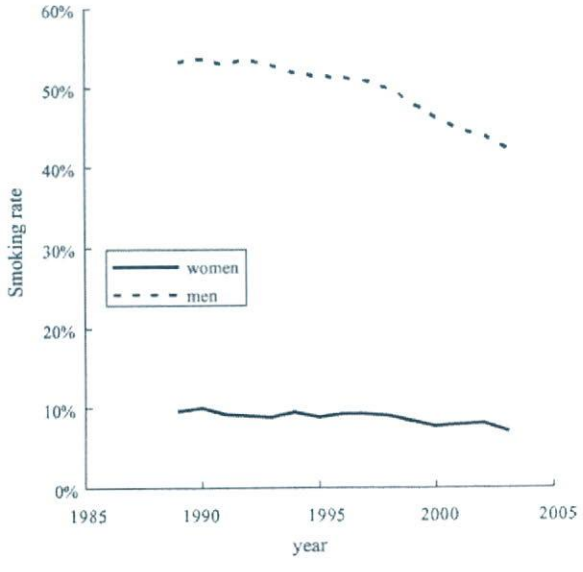

Fig. 1. Trends in smoking rate.

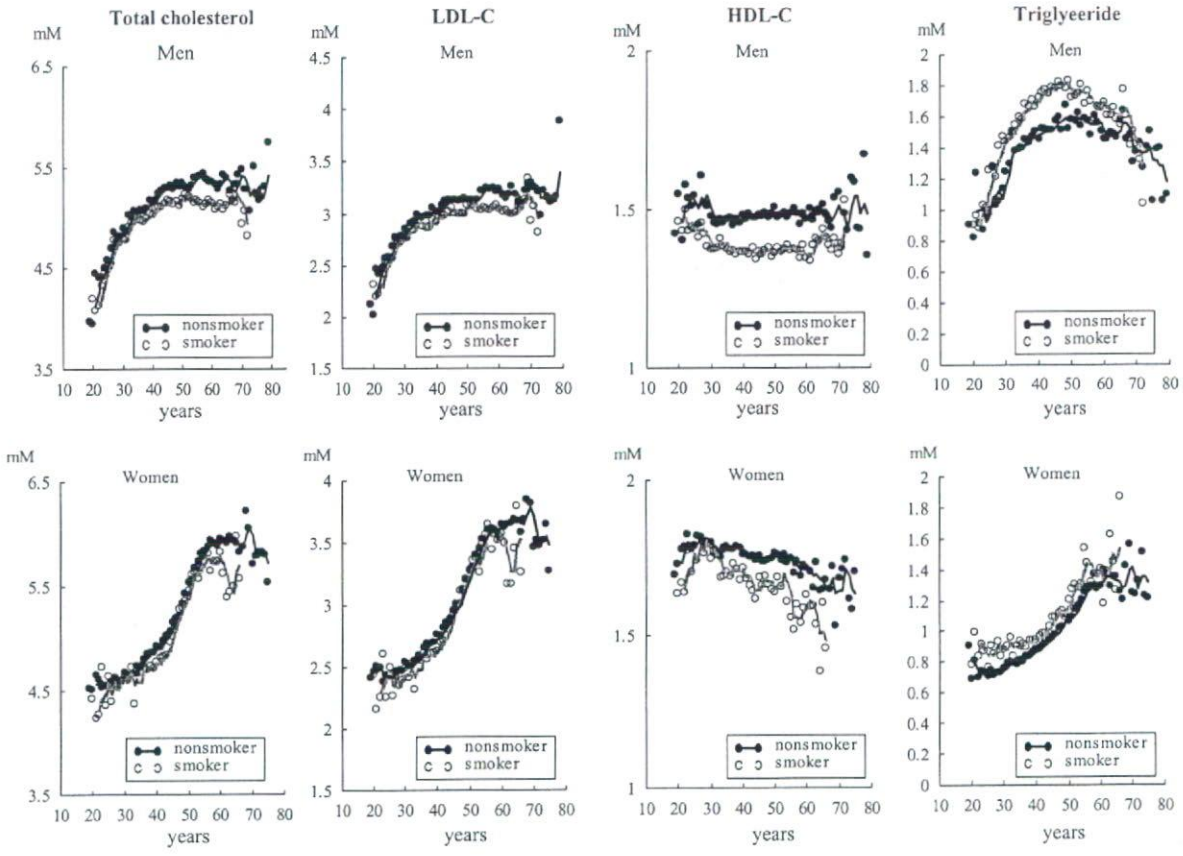

Fig. 2. Effect of aging on serum lipid levels in cross-sectional analysis. The age-specific means of serum lipid levels and a 3-year moving average of serum lipid levels are shown in smokers and nonsmokers at the initial examination.

initial examination of each subject from 1989 through 2003 were adjusted for the year of the individual examination and BMI. Mean values of serum lipid estimates for the examination in 1996 and at BMI = 22 are shown by age group and gender with or without smoking habit in Table 3. Significant differences existed in lipid levels between smokers and nonsmokers. Total and LDL cholesterol in male smokers were lower than those of nonsmokers from 25 to 34 years up to elderly age, while in women the effect of smoking on the total and LDL cholesterol lowering was observed from 35–44 years through 55–64 years and from 25–34 through 35–44 years, respectively. Smoking was associated with decreased HDL cholesterol levels between 25–34 years and 65–74 years in men, and from young adulthood up to 55–64 years in women. The triglyceride levels were higher in male and female smokers than those of nonsmokers below 55–64 years. However, after 65 years no difference in triglyceride levels was observed between male and female smokers and nonsmokers.

2.2. Longitudinal analysis

The serum lipid levels of smokers and nonsmokers from age 30 through age 70 at 10-year intervals were estimated for each age using the least square means method in the mixed effects model. These values were adjusted for the examination year in 1996 and BMI = 22. As shown in Table 4, male smokers exhibited lower total and LDL cholesterol levels than those of nonsmoker controls from age 30 through age 70. In women, a similar tendency toward lower total and LDL cholesterol levels in smokers was estimated at 40, 50, and 60 years, and 40 and 50 years, respectively. Both male and female smokers had lower HDL cholesterol levels at any of the 10-year intervals examined. In contrast, higher levels of triglyceride were estimated in smokers of both genders from age 30 to 70 years compared with those of nonsmokers.

Fig. 3 demonstrated the difference of estimated lipid levels (the lipid levels of smokers–those of nonsmokers) between current smokers and nonsmokers at individual age from 25 through 75 years based on the longitudinal analysis. The estimates show that the effect of smoking on the decrease in total cholesterol levels is apparent from 30 up to 65 years in females, with a peak at 45 years old. However, beyond the sixth decades of life the effect of smoking on total cholesterol levels was inverted, showing higher cholesterol concentrations in female smokers than those of nonsmokers. In contrast, the smoking effect on male total cholesterol levels is rather consistent at any given age, although the decrement of

Table 3
The cross-sectional data of serum lipid levels at initial examination of each subject from 1989 through 2003

Age groups (years)	<25		25–34		35–44		45–54		55–64		65–74		75≤	
	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker
Male														
Number of subjects	154	229	3212	4119	11684	15293	9630	10704	5177	4363	739	366	94	24
Age (year)	21.9 (2.2)	22.5 (1.6)	31.4 (2.4)	31.4 (2.5)	39.4 (2.9)	39.4 (2.9)	49.3 (2.9)	49.0 (2.9)	58.4 (2.6)	58.1 (2.5)	67.7 (2.6)	67.4 (2.4)	78.1 (3.6)	77.8 (3.0)
Total cholesterol (mM)	4.38 (0.81)	4.29 (0.71)	4.95 (0.87)*	4.87 (0.89)*	5.16 (0.89)†	5.05 (0.90)†	5.32 (0.88)†	5.17 (0.90)†	5.38 (0.89)†	5.15 (0.88)†	5.33 (0.82)*	5.13 (0.87)*	5.57 (0.91)*	4.65 (1.04)*
LDL-cholesterol (mM)	2.44 (0.73)	2.39 (0.66)	2.88 (0.77)*	2.82 (0.79)*	3.03 (0.79)†	2.94 (0.81)†	3.14 (0.78)†	3.04 (0.83)†	3.22 (0.80)†	3.05 (0.81)†	3.18 (0.74)	3.06 (0.85)	3.19 (0.89)*	2.63 (0.77)*
HDL-cholesterol (mM)	1.51 (0.29)	1.45 (0.30)	1.48 (0.32)*	1.39 (0.32)†	1.47 (0.34)†	1.37 (0.32)†	1.49 (0.35)*	1.37 (0.33)†	1.48 (0.36)†	1.37 (0.35)†	1.49 (0.37)†	1.41 (0.39)†	1.50 (0.36)	1.43 (0.35)
Triglyceride (mM)	0.97 (0.51)	0.99 (0.49)	1.31 (0.97)†	1.49 (1.11)†	1.48 (1.03)†	1.71 (1.27)†	1.58 (1.13)†	1.78 (1.29)†	1.52 (1.01)†	1.67 (1.14)†	1.49 (0.98)	1.52 (0.95)	1.26 (0.60)	1.29 (0.60)
Female														
Number of subjects	499	131	4579	750	13803	1916	10172	1206	3802	403	501	45	49	4
Age (year)	22.5 (1.5)	22.2 (1.5)	30.9 (2.5)	30.6 (2.7)	39.3 (2.9)	39.3 (2.9)	49.1 (2.9)	48.8 (2.8)	58.0 (2.6)	58.0 (2.6)	67.9 (2.7)	67.7 (2.6)	77.9 (2.8)	76.5 (2.4)
Total cholesterol (mM)	4.57 (0.75)	4.43 (0.83)	4.66 (0.77)	4.59 (0.78)	4.90 (0.80)	4.77 (0.79)†	5.44 (0.92)	5.37 (0.93)*	5.90 (0.93)	5.72 (0.90)*	5.91 (0.94)	5.95 (1.05)	5.71 (0.89)	5.91 (1.60)
LDL-cholesterol (mM)	2.44 (0.66)	2.34 (0.77)	2.53 (0.67)	2.45 (0.70)*	2.73 (0.72)	2.66 (0.74)*	3.21 (0.84)	3.17 (0.87)	3.60 (0.85)	3.53 (0.87)	3.65 (0.85)	3.73 (0.92)	3.39 (0.73)	3.79 (1.59)
HDL-cholesterol (mM)	1.78 (0.32)	1.70 (0.32)*	1.78 (0.35)	1.74 (0.37)*	1.77 (0.36)	1.68 (0.37)†	1.75 (0.39)	1.67 (0.38)†	1.71 (0.41)	1.56 (0.38)†	1.66 (0.40)	1.54 (0.29)	1.75 (0.41)	1.33 (0.25)
Triglyceride (mM)	0.74 (0.34)	0.90 (0.65)*	0.77 (0.40)	0.89 (0.48)†	0.87 (0.43)	0.95 (0.49)†	1.07 (0.60)	1.17 (0.65)†	1.30 (0.73)	1.42 (0.75)*	1.33 (0.67)	1.49 (0.65)	1.25 (0.71)	1.74 (0.62)

Data were adjusted for BMI, and year of initial examination, and expressed at BMI = 22; values are mean (S.D.).
* $p < 0.05$ (nonsmoker vs. smoker).
† $p < 0.0001$ (nonsmoker vs. smoker).

the estimated total cholesterol for smokers is larger with age. The pattern of the difference of the estimated LDL cholesterol between smokers and nonsmokers was similar to the pattern for total cholesterol. The HDL cholesterol value declines constantly in smokers at all ages in both genders. The increase of the estimated triglyceride levels in female smokers is constant between 25 and 75 years, although there is a U shape with the bottom between 40 and 50 years. In males, the effect of smoking on the increase in triglyceride level was stronger with age up to middle age, with the peak between 45 and 50 years. Subsequently the effect decreased with age, and no difference of triglyceride levels was illustrated beyond 70 years.

3. Discussion

There has been debate as to whether the difference in serum lipid levels between smokers and nonsmokers is due to smoking itself or whether other confounding lifestyle factors, e.g., body weight, alcohol consumption, and diet, have a dominant influence. There is now evidence to suggest a causal relationship between smoking and serum lipid concentrations.

The meta-analysis of 54 published studies by Craig et al. shows an increase in plasma concentrations of total cholesterol (3%), triglyceride (9.1%), and LDL cholesterol (1.7%) and a reduction in the concentrations of HDL cholesterol (5.7%) in smokers as compared with nonsmokers [4]. However, as the authors described in the paper, in most of the previous studies lipid levels were not adjusted for age or BMI. Additionally, most studies have had only adolescent, young adult, or middle-aged subjects. To our knowledge no data were available to see the effect of smoking habit on the serum lipid levels in the elderly as well as age-related changes in various lipid levels in a large cohort.

In the present study, we demonstrated that the influence of smoking habit on serum lipid levels is dependent on the subject age based on the cross-sectional as well as longitudinal observation. Based on cross-sectional observation, we showed that there were no significant differences in serum lipid levels between smokers and nonsmokers in young adults (<25 years) in men and women except for HDL cholesterol and triglyceride in women. In addition, we observed that the effect of smoking on the total and LDL cholesterol lowering and the enhancing influence of smoking on triglyceride levels were not detected in the female elderly, although in male smokers, the total and LDL cholesterol levels were higher even at 75 years and older than those of nonsmokers. The result suggests that the effect of smoking on serum lipid levels is dependent on age.

We showed that the total and LDL cholesterol levels in female and male smokers are lower than those of nonsmokers at least in middle age, which is inconsistent with the most of the earlier observations that serum cholesterol concentrations were higher in smokers [4] In the meta-analysis

Table 4
The estimated serum lipid levels of smokers and nonsmokers from age of 30 years through 70 years at 10 years intervals

Age groups (years)	30		40		50		60		70	
	Nonsmokers	Smokers	Nonsmokers	Smokers	Nonsmokers	Smokers	Nonsmokers	Smokers	Nonsmokers	Smokers
Male										
Total cholesterol (mM)										
Mean	4.83	4.76	5.10	5.02	5.25	5.14	5.28	5.14	5.19	5.00
95%CI	4.80–4.85	4.74–4.78†	5.09–5.11	5.01–5.03†	5.24–5.26	5.13–5.15†	5.26–5.29	5.12–5.15†	5.16–5.22	4.96–5.04†
LDL-cholesterol (mM)										
Mean	2.78	2.73	2.98	2.91	3.10	3.02	3.14	3.04	3.10	2.99
95%CI	2.76–2.80	2.71–2.75*	2.97–2.99	2.90–2.92†	3.09–3.11	3.01–3.03†	3.13–3.15	3.02–3.06†	3.07–3.13	2.95–3.03†
HDL-cholesterol (mM)										
Mean	1.51	1.43	1.53	1.41	1.54	1.40	1.54	1.40	1.52	1.40
95%CI	1.50–1.52	1.42–1.44†	1.53–1.54	1.41–1.42†	1.54–1.54	1.40–1.41†	1.53–1.54	1.39–1.41†	1.51–1.53	1.39–1.42†
Triglyceride (mM)										
Mean	1.19	1.35	1.32	1.60	1.38	1.68	1.36	1.60	1.26	1.34
95%CI	1.16–1.22	1.32–1.38†	1.31–1.34	1.59–1.62†	1.36–1.39	1.67–1.70†	1.34–1.38	1.58–1.62†	1.22–1.30	1.29–1.40*
Female										
Total cholesterol (mM)										
Mean	4.63	4.60	5.06	4.96	5.47	5.35	5.86	5.79	6.23	6.28
95%CI	4.61–4.65	4.56–4.65	5.05–5.07	4.93–4.98†	5.46–5.48	5.32–5.38†	5.85–5.88	5.74–5.85*	6.20–6.27	6.15–6.40
LDL-cholesterol (mM)										
Mean	2.53	2.51	2.88	2.82	3.23	3.16	3.56	3.54	3.88	3.96
95%CI	2.51–2.54	2.47–2.55	2.88–2.89	2.79–2.84†	3.22–3.24	3.13–3.19†	3.55–3.58	3.49–3.59	3.85–3.92	3.85–4.08
HDL-cholesterol (mM)										
Mean	1.72	1.66	1.75	1.67	1.76	1.65	1.75	1.61	1.71	1.55
95%CI	1.71–1.73	1.64–1.68†	1.75–1.76	1.65–1.68†	1.76–1.77	1.64–1.67†	1.74–1.75	1.59–1.64†	1.69–1.72	1.50–1.61†
Triglyceride (mM)										
Mean	0.83	0.97	0.93	1.04	1.06	1.19	1.22	1.41	1.42	1.70
95%CI	0.82–0.84	0.94–1.00†	0.92–0.93	1.03–1.06†	1.05–1.06	1.17–1.21†	1.21–1.23	1.37–1.44†	1.40–1.44	1.62–1.78†

The values were estimated for each age using the least square means methods in the mixed effects model, and were adjusted for the examination year in 1996 and BMI = 22.

* $p < 0.05$ (nonsmoker vs. smoker).

† $p < 0.0001$ (nonsmoker vs. smoker).

from Craig et al. [4], serum cholesterol concentrations were higher in smokers in all (22 studies) but one study. In addition, LDL cholesterol levels were higher in the smoking group by 1.7% from six studies compared with nonsmokers. Although the reason for this discrepancy of the effect of smoking in total and LDL cholesterol is not clear, some ethnic differences including dietary habits, physical activities, or life style as well as differences in public health awareness may have contributed to the inconsistency in observations between us and others. In fact, Halfon et al. found smoking to be associated positively with LDL cholesterol in males of European, but not of African descent [15]. Freedman et al. also reported in their longitudinal observation of early adulthood that although white male and female smokers had a larger increase in LDL cholesterol compared with nonsmokers, in black females smoking was inversely associated with LDL cholesterol [6].

We demonstrated in cross-sectional observation that HDL cholesterol levels were lower and triglyceride levels were higher in female as well as male smokers than in nonsmokers at most of the age groups examined, which was in agreement with other published results [4].

In longitudinal study, we observed apparent differences of smoking effect on serum lipid levels with age, except for HDL cholesterol levels, in which the effect of smoking is rather constant with age. The effect of smoking on the estimated total and LDL cholesterol in both genders is similar to the cross-sectional observation that total and LDL cholesterol decreased in male and female smoker up to elderly age and up to middle age, respectively. However, as shown in Fig. 3, the differences of the estimates of total and LDL cholesterol levels between smokers and nonsmokers based on the longitudinal observation suggest that there is an age effect on the influence of smoking on serum cholesterol concentrations. In addition, this analysis illustrated a gender difference with regard to this effect. In men, smoking is associated with lower total and LDL cholesterol at any given age, although there is an age effect in that the difference becomes larger with age. In women, the effect of smoking is not constant; an inverted influence on total and LDL cholesterol is detected, as in women younger than 60 years, the smoking is associated with lower cholesterol, but after 65 years smoking is associated with higher cholesterol levels. The reason for this remains unknown, although the life style changes or hor-

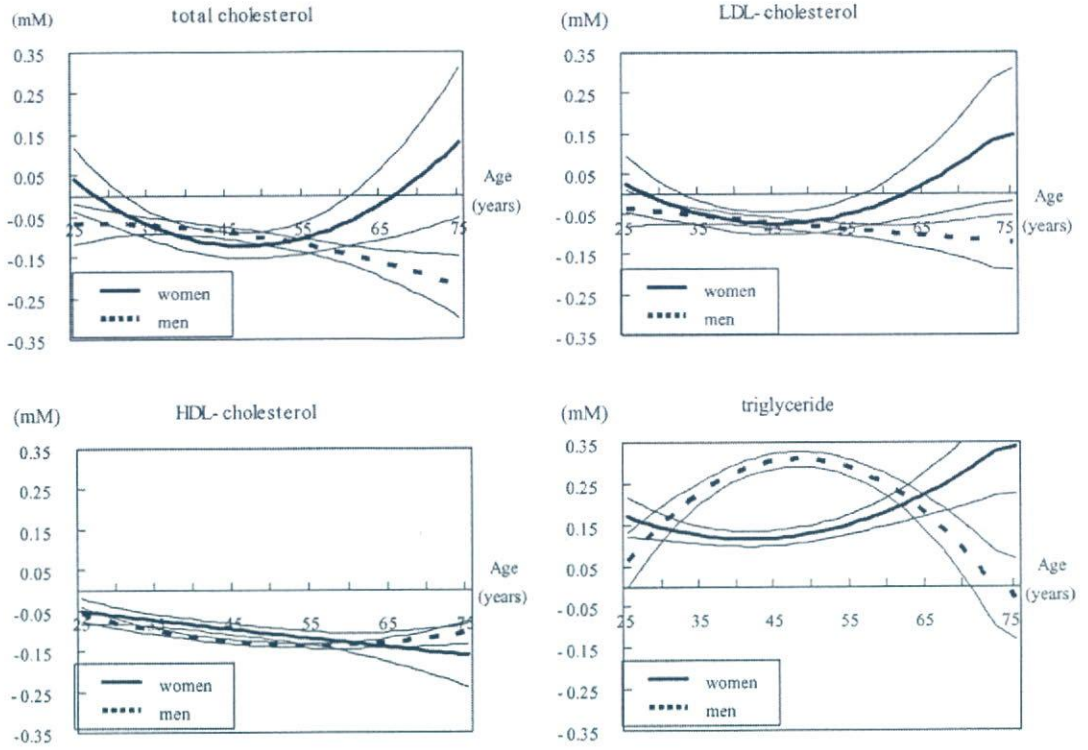

Fig. 3. The difference of estimated lipid levels (the lipid levels of smoker–those of nonsmokers) between current smokers and nonsmokers at individual age from 25 years through 75 years based on the longitudinal analysis. The curves show the average of the difference of estimated lipid levels based on the longitudinal analysis of mixed effect model between smokers and nonsmokers at each age. Thin curves indicate 95% CI.

monal changes in females after menopause might be involved in this inverted effect of smoking.

The effect of smoking on triglyceride levels also exhibits dynamic changes with age and gender difference. Based on longitudinal observation, smoking is associated with higher triglyceride levels at any age examined in both genders. In men, the strongest difference in triglyceride levels between smokers and nonsmokers is seen in middle age, and in women the stronger difference is seen after middle age. The reason for this gender difference and age-dependent effect of smoking on triglyceride levels remains unknown.

It seems that plasma enzymes involved in the metabolism of triglycerides and HDL cholesterol are potentially affected by smoking. However, there are conflicting observations. Some laboratories demonstrated that hepatic lipase is increased in smokers [16], and others demonstrated no difference between smokers and nonsmokers [17], or decreased hepatic lipase in smokers [18]. The hepatic lipase has been shown to be activated in smokers, and lectin:cholesterol acyl transferase activity has been shown to be unchanged [19] or decreased [17] compared with nonsmokers. Plasma cholesterol ester transfer protein activity has been shown to be marginally decreased in smokers in one study [17] and increased in another [19]. Plasma post-heparin lipoprotein lipase activity has been shown not to differ between smokers and nonsmokers in some studies [18,20] and to be increased in smokers in another study [17]. The reasons for these conflicting results on the effect of smoking on plasma enzymes regulating serum lipids and lipoproteins levels are not clear, but it is possible that the effect of smoking on these enzymes is dependent on the gender, age, genetic background, or ethnicity of the subjects.

It should be noted that some selection bias such as healthy worker bias may exist in our study, since most of the subjects were healthy office workers. In addition, the subjects may be aware of their lipid levels, since they had received annual examinations at a health examination center. There is another limitation of this study. Previous observations suggest that the effect of smoking on serum lipid levels is dose-dependent [4,6]. In this study, the data of smoking level in individuals were not available. In addition, alcohol consumption has an effect on serum lipid levels [21]. However, in the present study, the serum lipid levels were not adjusted to account for variations of alcohol consumption.

In the present study, we observed that the effect of smoking on serum lipid levels is age-dependent and that there is a gender difference based on the cross-sectional as well as longitudinal analysis. In men, smoking is associated with lower total and LDL cholesterol at any given age between 25 and 75 years. In women younger than 60 years, smoking is associated with lower cholesterol, but after 60–65 years smoking is associated with higher cholesterol levels. HDL cholesterol levels were lower in male and female smokers than in nonsmokers at most of the age groups examined. Smoking is

associated with higher triglyceride levels in any age examined in both genders except in males above 70 years. In men, the greatest difference in triglyceride levels between smokers and nonsmokers is seen in middle age, and in women, the greatest difference is seen after middle age.

Acknowledgments

This work was supported by a Grant-in Aid for the Comprehensive Research on Aging and Health from the Ministry of Health, Labor, and Welfare of Japan.

References

[1] Willett WC, Green A, Stampfer MJ, et al. Relative and absolute excess risks of coronary heart disease among women who smoke cigarettes. N Engl J Med 1987;317:1303–9.
[2] Prescott E, Hippe M, Schnohr P, Hein HO, Vestbo J. Smoking and risk of myocardial infarction in women and men: longitudinal population study. BMJ 1998;316:1043–7.
[3] Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol 2004:431731–7.
[4] Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. BMJ 1989;298:784–8.
[5] Freeman DJ, Caslake MJ, Griffin BA, et al. The effect of smoking on post-heparin lipoprotein and hepatic lipase, cholesteryl ester transfer protein and lecithin:cholesterol acyl transferase activities in human plasma. Eur J Clin Invest 1998;28:584–91.
[6] Freedman DS, Srinivasan SR, Shear CL, et al. Cigarette smoking initiation and longitudinal changes in serum lipids and lipoproteins in early adulthood: the Bogalusa Heart Study. Am J Epidemiol 1986;124:207–19.
[7] Kuzuya M, Ando F, Iguchi A, et al. Changes in serum lipid levels during a 10 year period in a large Japanese population. A cross-sectional and longitudinal study. Atherosclerosis 2002;163:313–20.
[8] Porkka KV, Raitakari OT, Leino A, et al. Trends in serum lipid levels during 1980–1992 in children and young adults. The Cardiovascular Risk in Young Finns Study. Am J Epidemiol 1997;146:64–77.
[9] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[10] Ward MM, Leigh JP. Pooled time series regression analysis in longitudinal studies. J Clin Epidemiol 1993;46:645–59.
[11] Littell RC, Milliken GA, Stroup WW, Wolfinger RD. SAS System for Mixed Models. Cary, NC: SAS Institute Inc.; 1996 [1989 printing; Chapter 3].
[12] Hata Y, Nakajima K. Life-style and serum lipids and lipoproteins. J Atheroscler Thromb 2000;7:177–97.
[13] Wilsgaard T, Arnesen E. Change in serum lipids and body mass index by age, sex, and smoking status: The Tromsø Study 1986–1995. Ann Epidemiol 2004;14:265–73.
[14] Wilson PW, Anderson KM, Harris T, et al. Determinants of change in total cholesterol and HDL-C with age: The Framingham Study. J Gerontol 1994;49:M252–7.
[15] Halfon ST, Green MS, Heiss G. Smoking status and lipid levels in adults of different ethnic origins: The Jerusalem Lipid Research Clinic Program. Int J Epidemiol 1984;13:177–83.
[16] Kong C, Nimmo L, Elatrozy T, et al. Smoking is associated with increased hepatic lipase activity, insulin resistance, dyslipidaemia and early atherosclerosis in Type 2 diabetes. Atherosclerosis 2001;156:373–8.
[17] Freeman DJ, Griffin BA, Murray E, et al. Smoking and plasma lipoproteins in man: effects on low density lipoprotein cholesterol levels and high density lipoprotein subfraction distribution. Eur J Clin Invest 1993;23:630–40.
[18] Zaratin AC, Quintao EC, Sposito AC, et al. Smoking prevents the intravascular remodeling of high-density lipoprotein particles: implications for reverse cholesterol transport. Metabolism 2004;53:858–62.
[19] Dullaart RP, Hoogenberg K, Dikkeschei BD, van Tol A. Higher plasma lipid transfer protein activities and unfavorable lipoprotein changes in cigarette-smoking men. Arterioscler Thromb 1994;14:1581–5.
[20] Eliasson B, Mero N, Taskinen MR, Smith U. The insulin resistance syndrome and postprandial lipid intolerance in smokers. Atherosclerosis 1997;129:79–88.
[21] Gordon T, Doyle JT. Alcohol consumption and its relationship to smoking, weight, blood pressure and blood lipids. The Albany Study. Arch Intern Med 1986;146:262–5.

Original Article

Dietary Supplement Use by Community-living Population in Japan: Data from the National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA)

Tomoko Imai,[1] Mieko Nakamura,[1] Fujiko Ando,[1] and Hiroshi Shimokata.[1]

BACKGROUND: There are few studies about dietary supplement use and nutrient intake from these products in Japan. The purpose of this study was to clarify (1) the prevalence of dietary supplement use, (2) the characteristics of dietary supplement users, (3) nutrient intake from dietary supplements, and (4) the existence of dietary supplement users who took excessive nutrients from these products.
METHODS: To collect the information on dietary supplement use in the previous year and nutrient intake from these products, we conducted a self-administered dietary supplement frequency questionnaire. The subjects were 2,259 people aged 40-82 years. Dietary supplements were grouped into 8 major categories. A dietary supplement database was developed to estimate nutrient intake from these products. Excess users were defined as people who consumed more nutrient than the tolerable upper intake level of the Dietary Reference Intakes for Japanese.
RESULTS: In the previous year, 55 % of males and 61 % of females consumed dietary supplements. Dietary supplement use was especially prevalent in females, subjects who felt unhealthy, and subjects who were more careful of maintaining an appropriate weight, though the association was affected by the frequency of dietary supplement use. The most common dietary supplements were drink type in males and vitamins in females. Some nutrient values obtained from dietary supplements were higher than those from food. Excess users were found for intake of vitamin A, B_6, K, niacin, iron, and magnesium.
CONCLUSIONS: It is important to clarify dietary supplement use and to estimate nutrient intake from these products.
J Epidemiol 2006; 16:249-260.

Key words: Dietary Supplements, Nutrition Surveys, Cohort Studies, Minerals, Vitamins.

Because sales of dietary supplements have increased in Japan,[1] it is conceivable that striking growth in the use of dietary supplements will occur in Japan, as it has in the USA and other developed countries. Assessing nutrient intake from dietary supplements, especially micronutrient intake, is very important. Because the levels of some micronutrients contained in these products are much higher than those contained in food,[2-4] people can easily consume such nutrients at toxic levels.[5-9] To monitor nutrient intake from dietary supplements is an important issue for public health. Furthermore, to assess nutrient intake from dietary supplements is essential for the development of nutritional epidemiologic studies. Lack of inclusion of dietary supplements in nutrient intake could possibly cause misclassification of individuals with regard to their total nutrient intake.[2-4,10,11] However, there have been very few studies on dietary supplement use in Japan. There is still uncertainty about the prevalence of dietary supplement use, nutrient intake from these products, and existence of users who consume extremely high levels of nutrients. One reason for the delay in the study of dietary supplement use in Japan might be due to the lack of a dietary supplement database. An extensive database that includes nutrient contents of dietary supplements is necessary for evaluating nutrient intake from these products; how-

Received April 19, 2006, and accepted July 6, 2006.
This study was supported by a Grant-in-Aid for Comprehensive Research on Aging and Health from the Ministry of Health, Labour and Welfare of Japan.
[1] Department of Epidemiology, National Institute for Longevity Sciences, Japan.
Address for correspondence: Tomoko Imai, Department of Epidemiology, National Institute for Longevity Sciences, Japan, 36-3 Gengo, Morioka-cho, Obu, Aichi 474-8522, Japan. (e-mail: imai@nils.go.jp)

ever, such a database has not been established or distributed in Japan. In contrast, several studies have attempted to estimate quantitatively the amount of nutrient intake from these products in the United States and European countries.[2-11]

Therefore, we conducted a self-administered dietary supplement frequency questionnaire to collect information on dietary supplement use in the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA), and developed a database of dietary supplements to estimate the amount of nutrient intake from these products. The purpose of this study was to clarify the following four points: (1) the prevalence of dietary supplement use, (2) the characteristics of dietary supplement users, (3) nutrient intake from dietary supplements, and (4) the existence of dietary supplement users who took excessive nutrients from these products.

METHODS

Subjects

The subjects were 2,259 males and females aged 40 to 82 years who participated in the second wave examination of the NILS-LSA (from April 2000 through May, 2002). The NILS-LSA is a comprehensive population-based longitudinal study of aging, which started in 1997. The participants were stratified by both age and sex, and were randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan. The numbers of males and females recruited were similar and the baseline age was 40 to 79 years, with the similar numbers of participants in each decade of age (40s, 50s, 60s, 70s). At the first wave examination, we sent an invitation letter to 7,790 people and 3,434 people replied. A total of 2,267 people participated in the first wave examination. All participants gave their informed consent before they participated in the study. Details of the study purpose, design, and examination procedures have been described elsewhere.[12]

Definition and Categories of Dietary Supplements in the NILS-LSA

Dietary supplements were defined as supplements to meals containing any dietary ingredients from unnatural food forms such as capsules, tablets, powders, or liquid. Dietary supplements included vitamins, minerals, herbs, botanical products, and other sub-

Table 1. Categorization of dietary supplements by the National Institute for Longevity Sciences Longitudinal Study for Aging.

Category	Description or sub-category
1. Vitamin *	14 sub-categories Multivitamin, Vitamin A, Vitamin D, Vitamin E, Vitamin K, Vitamin B_1, Vitamin B_2, Vitamin B_6, Vitamin B_{12}, Niacin, Vitamin C, Folic acid, Biotin, and Pantothenic acid
2. Mineral *	4 sub-categories Calcium, Iron, Magnesium, and Other minerals
3. Fatty acid *	6 sub-categories Linoleic acid, Linolenic acid, Stearic acid, Docosahexaenoic acid, Eicosapentaenoic acid, and Other fatty acids
4. Amino acid	Formulations containing mainly of single amino acids and some proteins
5. Dietary fiber	Water soluble and water insoluble dietary fibers
6. Drink type	Liquid type dietary supplement for recovery from tiredness, or health promotion, etc. The amount consumed at one time is about 30 mL to 200 mL. Includes quasi-drugs and medicinal drugs but does not include beverages.
7. Medicine	Prescription and non-prescription medicines which contain some nutrients, except medicines which are classified into categories 1 to 6. Example : remedies for cold which contain vitamin C.
8. Others	These formulations included compounds that do not fit into any other category. Example: flavonoids, carotenoids other than beta-carotene, catechin, and herbal products (propolis, royal jelly, chlorella, garlic, etc.)

Dietary supplements were defined as supplements to meals including any dietary ingredients from unnatural food forms such as capsules, tablets, powders, or liquids. Dietary supplements included prescription medicine, and non-prescription medicine, but functional foods and modified foods were not included in the category of dietary supplement.

*: Further classified into sub-categories shown in the Table.

stances (e.g., enzymes, organ tissues, metabolites, concentrates, and constituent extracts of these substances). Dietary supplements also included prescription medicine and non-prescription medicine, but functional foods and modified foods were not included in the category of dietary supplements.

Dietary supplements were grouped into eight major categories on the basis of primary nutrient content or similarity in overall ingredients and rationale for use.[13] In addition, we defined "drink type" separately, because Hakura et al[14] reported that "drink type" dietary supplements were widely consumed in Japan. The major categories of dietary supplements used in the NILS-LSA were (1) vitamin, (2) mineral, (3) fatty acid, (4) amino acid, (5) dietary fiber, (6) drink type (liquid type dietary supplement for recovery from tiredness, health promotion, etc., with a serving size of about 30 mL to 200 mL. The drink type category included quasi-drugs, but did not include beverages), (7) medicine (prescription and non-prescription medicines which contained some nutrients, except medicines which were classified into categories 1 to 6, e.g. cold remedies with vitamin C), and (8) others (These formulations included compounds that did not fit into any other category and were not described in the Standard Tables of Food Composition in Japan, Fifth Revised Edition,[15] for example, flavonoids, carotenoids expect beta-carotene, propolis, and so on) (Table 1). In addition, vitamins, minerals, and fatty acids were further classified into sub-categories. We use the term "all" dietary supplements which consisted of eight categories, when we did not consider the categories of the dietary supplements.

Assessment of Dietary Supplement Intake

A self-administered questionnaire was used to assess dietary supplement intake. First, it was mailed to the participants and the participants were asked to record it by themselves at home before the study examination. Then, the participants came to our center to get the study examination. At the examination, the questionnaire was reviewed by trained dietitians through an interview that took approximately 10 minutes. In the questionnaire, participants were asked whether they had taken any dietary supplement in the previous year. In case they had taken any dietary supplement, the name of the product, manufacturer or distributor, serving size and frequency of intake in the previous year (6 categories, i.e., less than once per week, 1-2 times per week, 3-6 times per week, one per day, 2 times per day, and 3 or more times per day) were also recorded.

Definition of Dietary Supplement Users

Dietary supplement users in the present study were defined as persons who took any dietary supplement at least once in the previous year. Users of dietary supplements were categorized into three groups: "daily users": those who reported any dietary supplement use once a day or more for the past 12 months, "weekly users": those who reported any dietary supplement use once a week or more but less than once a day for the past 12 months, and "seldom users": those who reported any dietary supplement use once a year or more but less than once a week for the past 12 months. When a participant had taken multiple dietary supplements in a major category or in a sub-category, the user category was defined based on the dietary supplement with the highest frequency of use. We used the term "any users" when we did not consider the frequency of use. "Weekly users" and "daily users" were considered to be "regular users". "Seldom users" were excluded when we calculated the amount of nutrient intake from dietary supplements.

Development of Dietary Supplement Database

A new dietary supplement database was developed for the NILS-LSA based on information obtained from the study participants and additional intensive investigation. We asked dietary supplement users to bring the products to the study visit. Then, the labels of the products were transcribed or photocopied to get information on the nutrient contents. In case dietary supplement users did not bring the products or could not provide enough information about the products at the visit, we asked them to send the labels of the products by mail. In addition, when information on nutrient content was not available from users, we tried to get it directly from the manufacturer or distributor of the products. We created a database of dietary supplements that included the names of products, manufacturer and/or distributor and nutrient contents in standardized units such as a tablet or a capsule.

Some products in which nutrient content was not described were excluded when we developed the database, and we did not calculate the nutrient intake from these products (62 products). Finally, we succeeded in constructing a database of 902 dietary supplement products in May 2002.

Assessment of Nutrient Intake from Dietary Supplements

Energy and nutrient intake from "all" dietary supplements among "regular users" was estimated using the frequency, amount of intake and nutrient contents in the dietary supplement database. The frequency of dietary supplement intake per day was quantified during the calculation (0.2 for 1-2 per week, 0.6 for 3-6 per week). If a participant reported uncertainty about the information on dietary supplement intake, that dietary supplement was excluded from the calculation of nutrient intake (7 males and 22 females were excluded from the analysis because they reported uncertainty about the information on dietary supplement intake). When a participant had taken various kinds of dietary supplements, energy and nutrient intake were summed across all dietary supplements. Nutrient intakes from "all" dietary supplements were compared with those from food according to the results of the National Nutrition Survey in Japan 2002.[16]

Participants who daily consumed some nutrients at more than the tolerable upper intake level (UL) in the 6th Edition[17] or 2005 Edition[18] of Nutrient-Based Dietary Reference Intakes (DRIs) in Japan were defined as "excess users". The ULs for adults in the 6th Edition of DRIs were as follows: 5,000 IU for vitamin A, 2,000 IU for vitamin D, 600 mg α -TE for vitamin E, 30,000 μg

for vitamin K, 30 mgNE for niacin, 100 mg for vitamin B_6, 2,500 mg for calcium (under 70 years old), 40 mg for iron, and 650 mg (50 years old and over) or 700 mg (40 to 49 years old) for magnesium. The UL for adults in the 2005 Edition of DRIs were as follows: 3,000 μgRE (10,000 IU) for vitamin A, 50 μg (2,000 IU) for vitamin D, 600 mg (70 years old and over for females) to 800 mg (40 to 69 years old for males) for vitamin E, 300 mgNE for niacin (the amount of mg of nicotinic acid amide was used), 60 mg for vitamin B_6, 2,300 mg for calcium, and 40 mg (40-49 and 70 years old and over for females) to 55 mg (40 to 49 years old for males) for iron.

Other Variables

Sociodemographic and lifestyle characteristic data, such as smoking habits, subjective health status, total family annual income, education, marriage status, and care of maintaining appropriate weight, were collected using a questionnaire. The body mass index (BMI) was calculated using the formula (weight (kg)/height $(m)^2$). Energy intake from food, energy intake from fat, and total alcohol intake were assessed through 3-day weighed dietary records (3DR). 3DR was carried out on three continuous days (two weekdays and one weekend day). The average intakes of nutrient per day were calculated according to the 5th Edition Standard Tables of Foods Consumption and other resources.[19]

Statistical Analysis

The prevalences of "all" dietary supplement users among males and females were compared by the chi-squared test by user category (any, seldom, weekly, and daily). Sociodemographic and lifestyle characteristics of "all" dietary supplement users and nonusers were compared by the Cocran-Mantel-Haenszel test adjusted for sex and age by user category. The prevalences of dietary supplement use of each major category of users and of the main sub-categories of users among males and females were compared by the chi-squared test by user category. Energy and nutrient intake from "all" dietary supplements among "regular users" (by sex), and major categories of dietary supplements, including (1) vitamin, (2) mineral, (6) drink type, and (8) others among "regular users" were expressed as percentiles, maximum values, and number of "excess users". All the statistical analyses were performed using the Statistical Analysis System, release 8.2.[20] Differences with p value less than 0.05 were considered significant.

RESULTS

The prevalence of "all" dietary supplement users in each user category and sociodemographic and lifestyle characteristics by user categories are shown in Table 2. In this study, 55 % of males and 61 % of females consumed some kind of dietary supplement ("all" dietary supplements) in the previous year. Among these subjects, females were more likely to take dietary supplements than males ($p<0.01$). "Seldom users" constituted about 20 % of the subjects (males: 23%, females: 19%). "Regular users" constituted about 30 % of males ("weekly users": 14 %, "daily users": 18 %) and 40% of females ("weekly users": 16 %, "daily users": 26 %). "Seldom users" were predominant among males ($p<0.05$) while "daily users" were predominant among females ($p<0.001$). The prevalence of "all" dietary supplement users in each user category varied depending on the age group ($p<0.05$, adjusted for sex). "Seldom users" ($p<0.001$) and "weekly users" ($p<0.05$) were prevalent among middle-aged people, while "daily users" were prevalent among older people ($p<0.001$). "All" dietary supplement users were subjectively less healthy than nonusers after adjustment for sex and age ("any users": $p<0.01$). However, the association was influenced by the frequency of use ("seldom users": not significant, "weekly users": $p<0.01$, and "daily users": $p<0.05$). When dietary supplement use was limited to use without all prescription and non-prescription medicine, subjective health status was significantly associated with the use of dietary supplements in "any users" and "weekly users" ("any users": $p<0.01$, "seldom users": $p=0.57$, "weekly users": $p<0.01$, "daily users": $p=0.07$). "All" dietary supplement users were more careful of maintaining appropriate weight than nonusers in the "any users"category ($p<0.05$); however, the associations of "all" dietary supplements with other characteristics were not significant (i.e., smoking, education, marriage status, BMI, energy intake from food, alcohol intake, etc.) in all user categories.

The prevalence of dietary supplement users by major category and sub-category by user categories are shown in Table 3. Among major categories of dietary supplements, the most widely consumed dietary supplement was drink type (27.0%), the second was vitamin (23.1%), the third was "others" (18.3%) and the fourth was medicine (12.0%) in males. On the other hand, the most widely consumed dietary supplement was vitamin (30.2%), the second was "others" (26.9%), the third was drink type (24.8%), and the fourth was medicine (9.7%) in females. The prevalence of vitamin, "others", and mineral dietary supplement use in females was significantly higher than that in males; however, drink type dietary supplement use in males was significantly higher than that in females in "any users". The prevalence of amino acid, fatty acid, and dietary fiber use was only about 1 % or less in "any users".

About a half of vitamin, "others", and mineral users consumed their respective supplements daily, whereas 60 % of drink type dietary supplement users and most medicine users consumed these supplements less frequently than once a week.

With regard to the prevalence in the sub-category of vitamin users, the prevalence of multivitamin was the highest, the second highest was vitamin C, and the third highest was vitamin E for both sexes. Calcium was the most popular nutrient in the mineral sub-category for both sexes.

Energy and nutrient intake from "all" dietary supplements among "regular users" are shown in Table 4. Median values of energy, macronutrients, minerals, and some fat-soluble vitamins (vitamin A, vitamin D, vitamin E, and vitamin K) intake from

Table 2. Prevalence of "all" dietary supplement users in each user category and sociodemographic and lifestyle characteristics by user categories.

			User category (%)†			
		n	Any‡	Seldom§	Weekly‖	Daily¶
Sex	Males	1,152	55	23	14	18
	Females	1,107	61**	19*	16	26***
Age (year)	40-49	534	65	33	17	14
	50-59	580	55	23	15	17
	60-69	562	55	17	15	24
	70-	583	57*	11***	13*	33***
Smoking	Never	1,268	61	20	15	25
	Past	524	55	21	13	21
	Current	462	54	22	16	17
Subjective health status	Excellent/Good	573	55	26	12	18
	Usual	1,433	58	19	16	23
	Bad/Very bad	244	66**	20	18**	28*
Total sum of family annual income, million yen	-4.49	668	57	15	14	28
	4.50-9.99	1,012	57	24	14	20
	10.00-	513	61	24	18	19
Education	Less than high school	671	58	15	14	28
	High school or equivalent	923	57	20	16	22
	More than high school	655	60	28	15	17
Marriage status	Unmarried	58	50	24	14	12
	Married	1,944	57	21	15	22
	Separated/Divorced	51	67	31	20	16
	Widowed	202	63	13	17	33
Body mass index (kg/m^2)	<18.5	123	56	16	11	29
	18.5-24.9	1,588	59	21	16	22
	25.0-	547	56	21	14	21
Care of maintaining appropriate weight	Yes	1,375	60	20	16	24
	No	876	55*	22	14	20
Energy intake (kcal/day) ††	-1500	201	58	19	13	26
	1500-1999	926	60	19	15	26
	2000-2499	759	56	22	15	20
	2500-	225	58	27	16	15
Energy intake from fat (%) ††	<20	203	59	15	16	28
	20-24	639	56	19	14	24
	25-29	792	58	22	15	21
	30-	477	61	25	15	21
Total alcohol intake (g etanol/day) ††	<10	1,500	60	20	16	24
	10-19	265	56	21	12	23
	20-29	139	52	24	13	15
	30-	207	51	23	12	16

Participants using any dietary supplements were defined as any dietary supplement users during the previous year.

†: Dietary supplement users were categorized into three user groups:
Seldom; seldom users those who reported any dietary supplement use once a year or more but less than once a week for the past 12 months.
Weekly; weekly uses those who reported any dietary supplement use once a week or more but less than once a day for the past 12 months.
Daily; daily users those who reported any dietary supplement use once a day or more for the past 12 months.

‡: n=1,306 (628 males and 678 females)

§: n=470 (260 males and 210 females)

‖: n=335 (158 males and 177 females)

¶: n=501 (210 males and 291 females)

*p<0.05, **p<0.01, ***p<0.001: Sex distribution was tested by chi-squared test. Age distribution was tested by Cocran-Mantel-Haenszel chi-squared test adjusted for sex. Other variables were tested by Cocran-Mantel-Haenszel chi-squared test adjusted for sex and age

††: Intake was settled using 3-day diet record.

"all" dietary supplements were very few in both sexes. On the other hand, 90th percentile value of vitamin E, vitamin B group, vitamin C, and niacin intake exceeded respective nutrient intake from diet shown in the National Nutrition Survey; i.e. about 10 % or more of dietary supplement users took large amount of such nutrient from dietary supplement. "Excess users" existed for iron, magnesium (only the 6th Ed.), vitamin A, vitamin K (only the 6th Ed.), vitamin B_6, and niacin (only the 6th Ed.).

Energy and nutrient intake from dietary supplement by major category among "regular users" is shown in Table 5. Individuals with intake of some nutrients at the 90th percentile value were larger amount than that from diet by the National Nutrition Survey (vitamin category: vitamin E, vitamin B group, niacin, and vitamin C; Mineral category: calcium; Drink type category: vitamin B_1, vitamin B_2, vitamin B_6, and niacin; "other" category: vitamin E and vitamin B group). "Excess users" existed in vitamin

Table 3. Prevalence of dietary supplement users by major category and sub-category by user category (%) (1,152 males and 1,107 females)

		User category†							
		Any		Seldom		Weekly		Daily	
Category	Sub-category	Males	Females	Males	Females	Males	Females	Males	Females
1. Vitamin		23.1	30.2*	6.2	6.8	6.9	7.2	10.0	16.2*
	Multivitamin	14.6	15.5	4.4	4.4	5.4	4.4	4.8	6.6
	Vitamin C	4.7	8.0*	1.1	1.4	1.5	2.1	2.1	4.6*
	Vitamin E	4.0	6.8*	0.7	1.3	0.5	1.0	2.8	4.5*
	Vitamin B_2	2.0	2.8	0.8	1.1	0.4	0.5	0.9	1.2
	Vitamin B_{12}	2.3	2.4	0.6	0.6	0	0.4*	1.7	1.4
	Vitamin D	0.3	2.8*	0	0.1	0	0.5*	0.3	2.2*
	Vitamin A	0.4	1.1*	0.1	0.2	0.1	0	0.2	0.9*
	Vitamin B_1	0.5	0.8	0.2	0.1	0.2	0.2	0.2	0.5
	Pantothenic acid	0.2	0.8*	0	0.1	0.1	0.1	0.1	0.6*
	Vitamin B_6	0.4	0.3	0.1	0	0	0	0.3	0.3
	Vitamin K	0.1	0.5	0	0	0	0	0.1	0.5
	Folate	0.1	0	0	0	0	0	0.1	0
2. Mineral		2.7	7.6*	0.8	1.4	0.4	2.1*	1.5	4.2*
	Calcium	1.7	5.2*	0.4	0.6	0.3	1.3*	1.0	3.3*
	Iron	0.2	2.4*	0.2	0.6	0	1.0*	0	0.7*
	Magnesium	0.4	0.5	0.1	0.2	0.1	0	0.2	0.3
	Other minerals	0.5	0.5	0.2	0.1	0.1	0	0.3	0.4
3. Fatty acid		1.0	1.2	0.1	0.3	0.1	0.2	0.7	0.8
4. Amino acid		1.1	1.5	0.1	0.4	0.4	0*	0.6	1.2
5. Dietary fiber		0.1	0.5	0	0.1	0	0	0.1	0.5
6. Drink type		27.0	24.8*	17.5	14.0*	7.4	8.0	2.2	2.9
7. Medicine		12.0	9.7	10.0	8.2	1.6	0.9	0.4	0.5
8. Others		18.3	26.9*	3.0	4.6*	4.1	6.0*	11.3	16.4*

†: Dietary supplement users were categorized into three user groups:
Seldom; seldom users those who reported any dietary supplement use once a year or more but less than once a week for the past 12 months.
Weekly; weekly uses those who reported any dietary supplement use once a week or more but less than once a day for the past 12 months.
Daily; daily users those who reported any dietary supplement use once a day or more for the past 12 months.
Any; combined three groups.

*: $p<0.05$ by Chi square test

No subject used niacin or biotin sub-category dietary supplement.

Table 4. Energy and nutrient intake per day from "all" dietary supplements among "regular users".

		Males (n=361)								Females (n=446)							
	National Nutrition		90th per-	95th per-		Tolerable upper intake level †		Excess Users ‡			90th per-	95th per-		Tolerable upper intake level †		Excess Users ‡	
Nutrient	Survey *	Median	centile	centile	Max.	6th edition	2005 edition	6th edition	2005 edition	Median	centile	centile	Max.	6th edition	2005 edition	6th edition	2005 edition
Energy (kcal)	1930	0	16	30	363	-	-	-	-	0	30	60	237	-	-	-	-
Protein (g)	72.2	0	1	2	80	-	-	-	-	0	1	2	35	-	-	-	-
Fat (g)	54.4	0	trace §	1	20	-	-	-	-	0	1	1	17	-	-	-	-
Carbohydrate (g)	271.2	0	trace §	1	21	-	-	-	-	0	trace §	1	15	-	-	-	-
Calcium (mg)	546	0	126	256	1320	2500 (40-69 y.o.) - (70+ y.o.)	2300	0	0	0	226	400	2123	2500 (40-69 y.o.) - (70+ y.o.)	2300	0	0
Iron (mg)	8.1	0	trace §	2	129	40	55 (40-49 y.o.) 50 (50-69 y.o.) 45 (70+ y.o.)	1	1	0	1	5	93	40	40 (40-49 y.o.) 45 (50-69 y.o.) 40 (70+ y.o.)	1	1
Magnesium (mg)	259	0	9	30	808	700 (40-49 y.o.) 650 (50+ y.o.)	-	1	-	0	7	48	906	700 (40-49 y.o.) 650 (50+ y.o.)	-	1	-
Vitamin A (IU)	3130	0	1200	2900	10200	5000	10000	8	4	0	800	1500	11000	5000	10000	12	4
Vitamin D (IU)	328	0	26	120	726	2000	2000	0	0	0	40	140	678	2000	2000	0	0
Vitamin E (mg)	8.2	0	91	198	483	600	800 (40-69 y.o.) 700 (70+ y.o.)	0	0	0	112	210	483	600	700 (40-69 y.o.) 600 (70+ y.o.)	0	0
Vitamin K (μ g)	260	0	0	0	30000	30000	-	1	-	0	0	4	45000	30000	-	6	-
Vitamin B_1 (mg)	0.87	2	38	55	280	-	-	-	-	1	43	72	144	-	-	-	-
Vitamin B_2 (mg)	1.21	1	6	10	68	-	-	-	-	1	8	16	64	-	-	-	-
Vitamin B_6 (mg)	1.17	1	16	41	185	100	60	3	9	1	30	66	106	100	60	3	26
Vitamin B_{12} (μ g)	7.4	0	250	1000	2340	-	-	-	-	0	500	1044	1566	-	-	-	-
Niacin (mg)	14.8	2	34	43	128	30	300 ‖	41	0	0	26	44	140	30	300 ‖	43	0
Vitamin C (mg)	101	0	210	668	6482	-	-	-	-	0	500	1100	4400	-	-	-	-

'Weekly users" plus "daily users" were defined as "regular users".

Seven males and 22 females were excluded from the analysis because they reported uncertainty about the information on dietary supplement intake.

Some products in which nutrient content was not described were excluded when we developed the database and we did not calculate nutrient intake from these products.

* : Results from the National Nutrition Survey in Japan, 2002 (mean of the total).

† : Tolerable upper intake level of adults in 6th edition or 2005 edition of Nutrient-Based Dietary Reference Intakes in Japan.

- : Tolerable upper intake level of adults in 6th edition or 2005 edition of Nutrient-Based Dietary Reference Intakes in Japan was not shown.

‡ : Number of participants who daily consumed some nutrients at more than the tolerable upper intake level in the 6th Edition or 2005 Edition of Nutrient-Based Dietary Reference Intakes (DRIs) in Japan.

§ : Below display limit

‖ : The amount of mg of nicotinic acid amide was used.

Table 5. Energy and nutrient intake per day from dietary supplements by major category among "regular users".

	1. Vitamin†						2. Mineral‡						6. Drink type§						8. Others‖					
		90th	95th		Excess Users *			90th	95th		Excess Users *			90th	95th		Excess Users *			90th	95th		Excess Users *	
		per-	per-		6th	2005		per-	per-		6th	2005		per-	per-		6th	2005		per-	per-		6th	2005
Nutrient	Median	centile	centile	Max	edition	edition	Median	centile	centile	Max	edition	edition	Median	centile	centile	Max	edition	edition	Median	centile	centile	Max	edition	edition
Energy (kcal)	0	4	12	80	-	-	0	8	10	57	-	-	0	16	30	237	-	-	5	27	54	145	-	-
Protein (g)	0	0	0	2	-	-	0	0	0	1	-	-	0	0	trace¶	28	-	-	0	2	3	36	-	-
Fat (g)	0	0	0	2	-	-	0	0	0	trace¶	-	-	0	0	0	0	-	-	0	1	3	20	-	-
Carbohydrate (g)	0	0	0	9	-	-	0	0	1	2	-	-	0	0	0	13	-	-	0	1	2	21	-	-
Calcium (mg)	0	7	130	1040	0	0	126	600	920	1833	0	0	0	0	9	54	0	0	0	106	320	1200	0	0
Iron (mg)	0	0	0	12	0	0	0	4	5	10	0	0	0	0	0	4	0	0	0	4	6	129	2	2
Magnesium (mg)	0	0	0	36	0	-	0	125	300	906	1	-	0	0	0	42	0	-	0	20	60	205	0	-
Vitamin A (IU)	0	1,000	2400	8000	3	0	0	0	0	200	0	0	0	0	0	0	0	0	0	1,000	3,600	11000	12	8
Vitamin D (IU)	0	30	40	600	0	0	0	80	159	396	0	0	0	0	0	0	0	0	0	40	200	726	0	0
Vitamin E (mg)	2	182	285	483	0	0	0	0	0	10	0	0	0	0	trace¶	27	0	0	0	30	100	280	0	0
Vitamin K (μ g)	0	0	0	45000	7	-	0	6	8	66	0	-	0	0	0	11	0	-	0	4	48	400	0	-
Vitamin B1 (mg)	2	58	78	280	-	-	0	0	0	6	-	-	1	5	6	20	-	-	0	10	20	68	-	-
Vitamin B2 (mg)	trace¶	8	12	64	-	-	0	0	trace¶	6	-	-	1	4	5	10	-	-	0	3	14	68	-	-
Vitamin B6 (mg)	1	48	66	185	5	34	0	0	0	4	0	0	1	5	6	25	0	0	0	2	10	61	0	1
Vitamin B12 (μ g)	0	1008	1500	2340	-	-	0	0	0	4	-	-	0	0	0	10	-	-	0	9	30	62	-	-
Niacin (mg)	0	39	60	140	62	0	0	0	0	15	0	0	4	20	20	100	6	0	0	7	18	128	10	0
Vitamin C (mg)	0	700	1336	4400	-	-	0	40	50	1000	-	-	0	44	50	2500	-	-	0	90	360	1620	-	-

'Weekly users" plus "daily users" were defined as "regular users".

Because there were few "regular users", 3.fatty acid, 4.amino acid, 5.dietary fiber, and 8.medicine were omitted.

Seven males and 22 females were excluded from the analysis because they reported uncertainty about the information on dietary supplement intake.

Some products for which nutrient content was not described were excluded when we developed the database and we did not calculate nutrient intake from these products.

* : Number of participants who daily consumed some nutrients at more than the tolerable upper intake level (UL) in the 6th Edition or 2005 Edition of Nutrient-Based Dietary Reference Intakes (DRIs) in Japan.

† : n=451 (191 males and 260 females)

‡ : n=85 (21 males and 64 females)

§ : n=232 (110 males and 122 females)

‖ : n=302 (129 males and 173 females)

- : Tolerable upper intake limit of adults in 6th edition or 2005 edition of Nutrient-Based Dietary Reference Intakes in Japan was not shown.

¶ : Below display limit

category (vitamin A, vitamin K, vitamin B6, and niacin), in the mineral category (magnesium), in drink type (niacin), and in the "others" category (iron, vitamin A, vitamin B_6, and niacin). In the other major categories, there were no "excess users" for any nutrients.

According to the 6th Ed. UL, 20 people were "excess users" of vitamin A among "all" dietary supplement users (Table 4), 12 among the "others" category and 3 among vitamin category (Table 5). This indicates that 5 people consumed excess doses of vitamin A from more than one dietary supplement category. Some people consumed excess doses of magnesium (one participant), vitamin B_6 (one participant), and niacin (six participants) from more than one dietary supplement category.

DISCUSSION

We conducted this study to evaluate the information on dietary supplement use and nutrient intake from these products in a random sample of a community-living population. Dietary supplements were used by more than half of the respondents in the previous year. The intake of some minerals and vitamins from these products were equal or more than the daily intake from food in the National Nutrition Survey.[16] Some users were found to take excess doses of minerals or vitamins from these products.

The prevalence of "all" dietary supplement use among "any users" in the previous year in our study was more than 50 % among both sexes. This is relatively high in comparison to those reported from Japan,[21,23] but it is almost the same as the prevalence found in studies that were conducted in the US.[2,20,22] However, differences in the definition of dietary supplements, dietary supplement users, duration of the study period (e.g., not specified or previous one year), and survey method (e.g., questionnaire only or including interview) among these studies make direct comparisons difficult.

In the National Nutrition Survey in Japan (J-NNS) in 2001,[23] dietary supplements were defined only as products which contained vitamins and minerals, and a concrete study period was not specified. Under this condition, 17.0 % of males and 23.6 % of females reported usual use of dietary supplements. In the subgroup of the Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Disease Cohort II,[21] dietary supplement was investigated in a questionnaire survey. In this study, dietary supplement was classified into multivitamins, beta-carotene, vitamin C, vitamin E, and others, and dietary supplement users were defined as subjects who used a dietary supplement one or more times a week for a year or longer. In this situation, the prevalence of dietary supplement use was 10.9 %.

Survey method (e.g., questionnaire only or including interview) may be another methodological factor to affect the prevalence of the dietary supplement intake. Third National Health and Nutrition Examination Survey in 1999-2000 (NHANES III)[22] in the US was conducted by household interviews. In NHANES III, dietary supplements included non-vitamin and non-mineral products, the duration of the study period was the previous month. Under this condition, the prevalence of dietary supplement use was 52 %, and it was similar to our results. It is possible that relatively high prevalence of dietary supplement use found in NHANES III and our study may result from the use of survey methods including interview.

At present, there have been a few studies on dietary supplement assessment methodology.[5-7,11,21,24-27] It is important to develop generally accepted assessment method in the dietary supplement study to make direct comparison.

We clarified the characteristics of dietary supplement users for the first time in Japan. Many studies conducted in the US and European countries reported that dietary supplement use was related to many aspects of appropriate lifestyles and a high health status.[6,9,13,28-33] In contrast, dietary supplement users in this study were likely to feel less healthy than nonusers. Dietary supplement users might have been more careful of maintaining an appropriate weight than nonusers, whereas other characteristics (i.e., smoking, education, marriage status, BMI, energy intake from food, and alcohol intake) were not significantly associated with dietary supplement use or nonuse in this study. The characteristics of dietary supplement users in our study might have been different from the characteristics of dietary supplement users in other countries. Such characteristics may depend on sex, age, and ethnicity.[7-9,28] Furthermore, some characteristics were different between frequencies of use of dietary supplements. For example, "seldom users" were prevalent among middle-aged subjects and were more likely to be males, whereas "daily users" were prevalent among older people and more likely to be females in our study. The association between dietary supplement use and other characteristics may be affected by the frequency of use of dietary supplements.

Multivitamin, vitamin C, and vitamin E were the popular dietary supplements in the vitamin category, and calcium was the most popular dietary supplement in the mineral category in our study. In the US, approximately 40% of subjects was reported to be users of some vitamin or mineral supplements in the NHANES III.[8] About 40 to 80% of adults was reported to be users of some vitamin or mineral supplements. Multivitamins were the most popular dietary supplements, and vitamin C, vitamin E, vitamin A, and calcium were commonly used in vitamins and mineral supplements.[8,9,13,28,29,31,34-36] Many studies reported the prevalence of combined dietary supplement use (vitamins and mineral). The prevalence of use of each dietary supplement was not determined; however, our results (vitamin plus mineral: males 25.8%, females; 37.8%) would be broadly comparable to the results of those studies.

Schaffer et al[33] reported that the prevalence of non-vitamin and non-mineral dietary supplement use was 32.7% (participants were the members of a large group in a model health plan, the duration of the study period was the past 12 months, and dietary supplement use was assessed by a questionnaire). The prevalence of non-vitamin and non-mineral dietary supplement users in our study ("all" - vitamin - mineral: males; 28.7%, females; 23.5%)