The official journal of the Japan Atherosclerosis Society and the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Original Article

J Atheroscler Thromb, 2019; 26: 170-182. http://doi.org/10.5551/jat.43075

The Relationship of Dietary Cholesterol with Serum Low-Density Lipoprotein Cholesterol and Confounding by Reverse Causality:

The INTERLIPID Study

Yukiko Okami[1], Hirotsugu Ueshima[1, 2], Yasuyuki Nakamura[3], Nagako Okuda[4], Hideaki Nakagawa[5], Kiyomi Sakata[6], Shigeyuki Saitoh[7], Akira Okayama[8], Katsushi Yoshita[9], Sohel R. Choudhury[10], Queenie Chan[11], Paul Elliott[11], Jeremiah Stamler[12], Katsuyuki Miura[1, 2], for the INTERMAP and INTERLIPID Research Groups

[1]Center for Epidemiologic Research in Asia, Shiga University of Medical Science, Shiga, Japan
[2]Department of Public Health, Shiga University of Medical Science, Shiga, Japan
[3]Department of Food Science and Human Nutrition, Faculty of Agriculture, Ryukoku University, Kyoto, Japan
[4]Department of Health and Nutrition, University of Human Arts and Sciences, Saitama, Japan
[5]Department of Epidemiology and Public Health, Kanazawa Medical University, Kanazawa, Japan
[6]Department of Hygiene and Preventive Medicine, Iwate Medical University, Iwate, Japan
[7]School of Health Sciences, School of Medicine, Sapporo Medical University, Sapporo, Japan
[8]Research Institute of Strategy for Prevention, Tokyo, Japan
[9]Department of Food Science and Nutrition, Osaka City University, Osaka,Japan
[10]Department of Epidemiology and Research, National Heart Foundation Hospital and Research Institute, Dhaka, Bangladesh
[11]MRC-PHE Centre for Environment and Health, Department of Epidemiology and Biostatistics, Faculty of Medicine, School of Public Health, Imperial College London, London, UK
[12]Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Aim: The positive relationship between dietary cholesterol and serum cholesterol has been questioned by a set of recent cohort studies. This study aimed to investigate how employment status and education years relate to the association between dietary cholesterol and serum low-density lipoprotein cholesterol (LDL-C) in a Japanese population.

Methods: A population-based, random sample, cross-sectional study (INTERLIPID) was performed. Among 1,145 Japanese individuals aged 40–59 years, 106 were excluded because of special diets, use of lipid-lowering drugs, hormone replacement, and missing data, leaving 1,039 individuals (533 men and 506 women). Dietary cholesterol was assessed from four 24-h dietary recalls, and LDL-C was measured enzymatically with an auto-analyzer. A standard questionnaire inquired about employment status and education years.

Results: In men, a 1 standard deviation (SD) higher dietary cholesterol was associated with 3.16 mg/dL lower serum LDL-C (P=0.009; unadjusted model). After adjustment for covariates, higher serum LDL-C was estimated per 1 SD higher intake of dietary cholesterol in nonemployed men [self-employed, homemakers, farmers, fishermen, and retired employees; $\beta = +9.08$, 95% confidence interval (CI) = +0.90–+17.27] and less educated men ($\beta = +4.46$, 95% CI = −0.97–+9.90), whereas an inverse association was observed in employed men ($\beta = -3.02$, 95% CI = −5.49–−0.54) and more educated men ($\beta = -3.66$, 95% CI = −6.25–−1.07).

Conclusions: In men who were nonemployed and less educated, a higher intake of dietary cholesterol was associated with elevated concentrations of serum LDL-C, whereas an inverse association was observed in men who were employed and more educated.

Key words: Cholesterol, Employment, Education, Lifestyle modification, Reverse causality

Address for correspondence: Yukiko Okami, Center for Epidemiological Research in Asia, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu-shi, Shiga 520-2192, Japan E-mail: okami@belle.shiga-med.ac.jp

Received: October 15, 2017 Accepted for publication: April 22, 2018

Introduction

The previously established positive relationship between dietary cholesterol and serum cholesterol has been challenged by reported findings from large-scale observational studies[1)], and meta-analysis suggested that higher intake of cholesterol or eggs was not associated with an increased risk of incident cardiovascular disease (CVD)[2, 3)].

However, it is a well-known fact that dietary cholesterol intake influences the serum cholesterol level from human experimental studies[4-6)]. Keys *et al*. established an equation where the subduplicate power of dietary cholesterol multiplied by 1.5 is the most accurate number for predicating serum cholesterol following 39 human experimental studies[7)]. Other experimental studies confirmed that a 100 mg/day change in dietary cholesterol intake changes serum total cholesterol levels by 2.5 mg/dL on average[8)]. Because Japanese people eat as much as 350–450 mg/day of dietary cholesterol (equivalent of two eggs)[9)]; the effect of dietary cholesterol on serum cholesterol is certainly not negligible considering the population strategy in Japan.

Previous studies reported that behavioral and environmental including lifestyle modifications in participants make the established positive relationship out to be reversed through epidemiological studies because many guidelines and mass media have recommended reduction of cholesterol intake for hypercholesterolemia over the past few decades[10, 11)]. Moreover, several historical events occurred in Japan in the late 20th century including the widespread use of statin drugs[12)]; the enactment of the Industrial Safety and Health Law in 1972, which mandated annual health check-ups including measurement of cardiovascular risk factors for employees in large-scale companies; and the enactment of the Law for the Welfare of the Elderly in 1983[13)].

Availability of health education with consequent lifestyle modifications particularly among the employed has markedly improved. Therefore, we hypothesized that individuals with higher serum cholesterol or those afraid of high-serum cholesterol are more likely to modify their diet (including reduction in dietary cholesterol intake) than those with lower serum cholesterol or those who do not take care of their lifestyles, particularly influenced by their education or employment in a company; if a sufficient number of individuals do this, dietary cholesterol will not relate to serum cholesterol or will inversely correlate with serum cholesterol as a whole. When the established exposure-outcome process is reversed in an epidemiological study, it is called reverse causality. In this situation, the opposite direction from serum cholesterol to dietary cholesterol would be called reverse causality under the condition of the established direction from dietary cholesterol to serum cholesterol as mentioned above.

We examined how the relationship and its reverse causality between dietary cholesterol intake and serum low-density lipoprotein cholesterol (LDL-C) are associated with employment status, education years, and other possible confounding factors.

Methods

Study Design and Participants

The INTERMAP study is an international, cooperative, cross-sectional study conducted on 4,680 participants aged 40–59 years old from 17 diverse population samples in China, Japan, the UK, and the US, with field work being conducted between 1996 and 1999. The INTERLIPID study is an INTERMAP ancillary study that focused on serum lipids and blood analytes as well as multiple dietary factors in Japanese participants in Japan and Hawaii[9, 14, 15)]. The methods employed in these two studies have been described elsewhere in detail[9, 16, 17)]. Participants in these studies were randomly recruited at five research centers: four in Japan (574 men and 571 women) and one in Hawaii (136 men and 131 women).

In the present study, Japanese participants from Hawaii were excluded because their lifestyles and environment markedly differed from those among Japanese participants from Japan. The four Japanese populations from Japan included Japanese factory workers in Toyama, central Japan (149 men and 150 women); Japanese factory workers in Sapporo, the capital city of Hokkaido, northern Japan (149 men and 148 women); Japanese factory workers in Wakayama, central Japan (146 men and 144 women); and Japanese residents in Aito town, a rural town in Shiga prefecture, central Japan (130 men and 129 women). Among the four research samples, 106 individuals were excluded because during the diet, they were on a special diet (n=50), weight loss diet (n=8), weight gain diet (n=1), vegetarian diet (n=8), diabetic diet (n=4), fat-modified diet (n=17), and other diets (n=23); were on lipid-lowering drugs (n=43) and hormone replacement (for women; n=10); and had missing data for serum LDL cholesterol (LDL-C; n=3), leaving 1,039 individuals (533 men and 506 women). The Ethics Committees of the Shiga University of Medical Science, the Sapporo Medical University, the Kanazawa Medical University, the Wakayama Medical University, Northwestern University, and the Pacific

Health Research Institute approved the study protocol. Written informed consent was obtained from all the participants.

Anthropometric and Lifestyle Assessments

Participants visited research centers four times on two pairs of consecutive days on an average of 3 weeks apart. Height and weight with light clothes were measured twice during the first and third visits (a total of four times); the four measurements of height and weight were averaged. Using a questionnaire, trained observers inquired about physical activity, smoking status, alcohol intake, special diets, previous medical history of CVDs/diabetes, and use of medication. Menopausal status and use of hormone replacement were also investigated among women. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

Employment Status and Education Years

Based on questionnaire data on employment status and years of education, these variables were dichotomized because they showed non-normal distributions. In the questionnaire, employment status was divided into “employed in a company,” “self-employed” and “nonemployed (i.e., homemakers, farmers, fishermen, and retired employees).” The self-employed and nonemployed were combined into one group called “the others” in the analysis. Education level was divided into more than or equal to high-school-educated level (years of education, ≥ 12) and less than high-school-educated level (years of education, ＜12). The cutoff point was identified from the results of previous studies[18, 19].

Dietary Assessment

Detailed, multipass, 24-h dietary recalls were conducted per participant four times during the four day visits by specially trained and certified dietary interviewers. Standardized quality control procedures were adopted to assess and maximize the quality of dietary data throughout data collection[17]. Although there were some participants whose energy intakes from any of the 24-h dietary recalls were ＞8,000 kcal/day for men and ＞5,000 kcal/day for women in the original INTERMAP study, all participants attended four study visits in Japan; their energy intakes were between 500 and 5,000 kcal/day for both men and women. The means of individual nutrients for each participant from the four 24-h dietary recalls were used in analyses.

Serum Lipid Measurements

In the INTERLIPID study, nonfasting blood was drawn after the last meal on the second day of the first two-day visit[9, 14, 15]. Serum and plasma were obtained by centrifugation within 30 min of blood drawing and were immediately refrigerated. Within 24 h, all specimens were frozen and locally stored at −70℃. Samples were shipped on dry ice to a central laboratory in Japan. Serum total cholesterol, serum high-density lipoprotein cholesterol (HDL-C), and serum LDL-C were directly measured enzymatically using an auto-analyzer (Hitachi 7107; Hitachi, Tokyo, Japan). Individual samples from the four centers were randomly allocated for analyses to avoid systematic measurement bias in the assigned order in the auto-analyzer.

Statistical Analyses

In the description of the characteristics of all participants for variables possibly related to serum LDL-C (mg/dl), means and standard deviations (SDs) or percentages were calculated with analysis of variance (ANOVA) or chi-squared tests for comparisons among the sex-specific quartiles of serum LDL-C. Unpaired *t*-tests or chi-squared tests compared each characteristic between men and women among participants used in the analyses. Based on a large number of significant differences in the individual dietary and nondietary variables between men and women, all analyses were stratified by sex. Multiple linear regression analysis was used to examine the relationship of dietary cholesterol intake with serum LDL-C with control for multiple possible cofounders (covariates included employment status and education years). Interactions were assessed with dietary cholesterol intake. Previous studies identified age, BMI, menopause, smoking, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and trans fatty acids (TFAs) as the factors that positively correlate with serum LDL-C[20-26]. Alcohol intake, polyunsaturated fatty acids (PUFAs), and dietary soluble fiber have been reported to decrease serum LDL-C[27-32]. Therefore, these variables were treated as covariates in multiple linear regression models. We then calculated the difference in serum LDL-C (mg/dl) per 1 SD higher intake of dietary cholesterol (mg/1,000 kcal), treating them as continuous variables. In regression models, the dependent variable was serum LDL-C. Model 1 had employment status (“employed in a company”/“the others”) or education years (≥ 12/＜12 years) and its interaction term with dietary cholesterol as independent variables. In the case of employment status, for example, the equation used in the regression model was as below: LDL-C = $\beta_0 + \beta_1 \times$ employment status (“Employed”/“the others”) + $\beta_2 \times$ dietary cholesterol + $\beta_3 \times$ employment status (“Employed”/

"the others") × dietary cholesterol. If "Employed" = 1 and "the others" = 0, LDL-C = ($\beta_0 + \beta_1$) + ($\beta_2 + \beta_3$) × dietary cholesterol using the model of "Employed" and LDL-C = $\beta_0 + \beta_2$ × dietary cholesterol using the model of "the others." Therefore, we showed the different slope (β) of dietary cholesterol among the employment statuses. The β in education years was calculated in the same way. Model 2 was adjusted for the established factors related to serum LDL-C as described above: age (year), BMI (kg/m^2), menopausal status for women (before/in the middle or after), number of cigarettes per day, alcohol intake (%kcal), SFA (%kcal), PUFA (%kcal), MUFA (%kcal), TFA (%kcal), soluble fiber (g/1,000kcal), and four sites. Model 3 was further adjusted for education years or employment status. Coefficients for dietary cholesterol in models were based on the use of indicator variables for employment status or education years. In addition, tests were performed to clarify whether the coefficients differed between the employment status or education years. Considering the different characteristics between factory workers in Toyama, Sapporo, and Wakayama and community-dwellers in Aito town, stratified analysis for Aito town was also conducted to examine the effect of employment status on the association between dietary cholesterol and serum LDL-C. Moreover, multiple linear regression analysis was conducted with serum LDL-C as a dependent variable and dietary cholesterol, dichotomized serum LDL-C (< 120/≥ 120 mg/dL), and its interaction between dietary cholesterol and dichotomized serum LDL-C to examine the difference in the coefficient values between higher serum LDL-C (≥ 120 mg/dL) and lower serum LDL-C (< 120 mg/dL). The model was adjusted for age, BMI, menopausal status (for women), number of cigarettes, alcohol intake, SFA, PUFA, MUFA, TFA, soluble fiber, and site. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC); two-tailed *P* values of < 0.05 were considered to be significant.

Results

The characteristics of all participants (n = 1,142) after excluding missing data by sex-specific quartiles of serum LDL-C are shown in **Table 1**. In men, the BMI, serum total cholesterol, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were higher, and the intake of alcohol and dietary cholesterol and number of drinkers (%), were lower across the quartiles of serum LDL-C. In women, age, BMI, menopause, serum total cholesterol, serum triglycerides (TGs), SBP, and DBP were higher, but alcohol intake, total energy intake, and serum HDL-C were lower; dietary cholesterol intake tended to be lower across the quartiles of serum LDL-C. For both men and women, other dietary factors including SFA, PUFA, MUFA, and Keys score had similar means across the quartiles of serum LDL-C. Aito town had the smallest number of employed men (34.8%) among four sites (data not shown).The characteristics of participants used in analyses (n = 1,039) are shown in **Table 2**. This analysis included 533 men and 506 women. The mean (SD) intakes of dietary cholesterol (mg/1,000 kcal) were not significantly different between men and women (P = 0.939). The mean (SD) intakes of SFA (%kcal), PUFA (%kcal), MUFA (%kcal), and TFA (%kcal) and Keys score were significantly higher in women than in men. Serum total cholesterol (mg/dL), serum HDL-C (mg/dL), and serum LDL-C (mg/dL) were also significantly higher in women than in men. Dietary cholesterol intake did not significantly differ with regard to employment status ("employed in a company"/"the others") or education years (≥ 12/< 12 years) in both men and women (data not shown).

Table 3 shows estimated differences in serum LDL-C per 1 SD higher dietary cholesterol intake according to employment status. In men, a 1 SD greater dietary cholesterol was associated with 3.16 mg/dL lower serum LDL-C (P = 0.009; unadjusted model). In Model 1, 4.13 mg/dL lower serum LDL-C was estimated per 1 SD greater dietary cholesterol intake in men "employed in a company" (P = 0.001) but was associated with 8.56 mg/dL higher serum LDL-C in "the others" (P = 0.050). In Model 2 that was adjusted for covariates, 3.03 mg/dL lower serum LDL-C was estimated per 1 SD higher dietary cholesterol intake in men "employed in a company" (P = 0.017), whereas this was 9.13 mg/dL higher serum LDL-C in "the others" (P = 0.029). In Model 3 that was further adjusted for education years, 3.02 mg/dL lower serum LDL-C was estimated per 1 SD higher dietary cholesterol intake in the employed men (P = 0.017), but 9.08 mg/dL higher serum LDL-C was observed in "the others" (P = 0.030). These significant relationships were not observed in women. Among the men in Aito town, there was a positive tendency in the association between dietary cholesterol and serum LDL-C in "the others" [β = + 7.87, 95% confidence interval (CI) = − 1.12– + 16.85, P = 0.085] but no significant association between dietary cholesterol and serum LDL-C in men "employed in a company" (β = + 2.40, 95% CI = − 4.99– + 9.78, P = 0.521; Model 3).

Table 4 shows estimated differences in the serum LDL-C per 1 SD higher dietary cholesterol intake according to education years. In men, Model 1 estimated 4.83 mg/dL lower serum LDL-C per 1 SD higher dietary cholesterol intake in more educated

Table 1. Characteristics of all participants (n = 1,142; age, 40–59 years)* by sex-specific quartiles of serum LDL cholesterol in the INTERLIPID study in Japan, 1996–1999

	Men (n = 572)				
	Q1 (n = 137)	Q2 (n = 147)	Q3 (n = 144)	Q4 (n = 144)	P value
Serum LDL cholesterol, median (range), mg/dL	88.0 (45–99)	110 (100–119)	129 (120–141)	155 (142–201)	
Serum LDL cholesterol, mean (SD), mg/dL	84.0 (13.1)	109.3 (5.9)	129.7 (6.4)	157.3 (12.3)	
Age, mean (SD), years	49.9 (5.3)	49.3 (5.5)	49.7 (5.5)	49.2 (4.9)	0.605
BMI, mean (SD), kg/m^2	23.0 (2.4)	23.5 (2.9)	23.7 (2.8)	24.5 (2.5)	< 0.001
Employed in a company***, no. (%)	129 (94.2)	131 (89.1)	130 (90.3)	136 (94.4)	0.235
Education***, mean (SD), years	12.3 (2.3)	12.1 (2.1)	12.5 (2.2)	12.4 (1.9)	0.342
Education years ≥ 12 years (finished high school)***, no. (%)	111 (81.0)	113 (76.9)	122 (84.7)	127 (88.2)	0.066
Site, no. (%)					0.003
Toyama	34 (24.8)	34 (23.5)	36 (25.4)	44 (30.8)	
Sapporo	53 (38.7)	39 (26.9)	35 (24.7)	20 (14.0)	
Aito	22 (16.1)	33 (22.8)	37 (26.1)	36 (25.2)	
Wakayama	28 (20.4)	39 (26.9)	34 (23.9)	43 (30.1)	
Smokers***, no. (%)	69 (50.4)	77 (52.4)	65 (45.1)	85 (59.0)	0.127
Numbers of cigarettes, mean (SD), n/day	10.6 (11.9)	11.8 (13.4)	11.0 (13.8)	13.6 (13.5)	0.222
Numbers of cigarettes in smokers, mean (SD), n/day	21.0 (7.8)	22.4 (10.2)	24.3 (9.8)	23.0 (9.6)	0.175
Drinkers***, no. (%)	134 (97.8)	145 (98.6)	137 (95.1)	138 (95.8)	0.283**
Alcohol intake, mean (SD), %kcal	10.5 (7.5)	9.6 (7.5)	7.9 (7.2)	8.6 (6.7)	0.017
Alcohol intake in drinkers, mean (SD), %kcal	10.7 (7.5)	9.8 (7.5)	8.3 (7.2)	8.9 (6.6)	0.038
Alcohol intake, mean (SD), g/day	34.7 (24.9)	31.4 (24.7)	26.0 (25.1)	28.4 (23.8)	0.025
Alcohol intake in drinkers, mean (SD), g/day	35.4 (24.6)	31.8 (24.6)	27.3 (25.0)	29.7 (23.6)	0.051
Dietary energy, mean (SD), kcal/day	2317 (371)	2270 (429)	2263 (448)	2268 (455)	0.633
Dietary total carbohydrate, mean (SD), %kcal	51.1 (8.1)	52.1 (7.9)	52.9 (7.2)	52.9 (7.7)	0.187
Dietary total protein, mean (SD), %kcal	15.8 (2.4)	15.8 (2.3)	15.9 (2.2)	15.7 (2.2)	0.785
Dietary total fat, mean (SD), %kcal	23.8 (5.0)	23.4 (4.6)	23.9 (4.9)	23.5 (4.7)	0.819
Dietary cholesterol, mean (SD), mg/1000 kcal	200.6 (72.7)	201.9 (67.1)	198.4 (66.7)	181.2 (61.2)	0.020
SFA, mean (SD), %kcal	6.0 (1.7)	6.1 (1.6)	6.0 (1.5)	6.1 (1.6)	0.945
PUFA, mean (SD), %kcal	6.4 (1.6)	6.0 (1.4)	6.3 (1.6)	6.1 (1.4)	0.136
MUFA, mean (SD), %kcal	8.6 (2.1)	8.5 (2.0)	8.7 (2.2)	8.5 (2.0)	0.928
TFA, mean (SD), %kcal	0.3 (0.2)	0.4 (0.2)	0.4 (0.2)	0.3 (0.2)	0.018
Total fiber, mean (SD), g/1000 kcal	7.0 (2.0)	6.4 (1.8)	7.2 (1.9)	6.8 (1.7)	0.002
Soluble fiber, mean (SD), g/1000 kcal	1.1 (0.4)	1.1 (0.4)	1.2 (0.5)	1.1 (0.4)	0.008
Insoluble fiber, mean (SD), g/1000kcal	5.8 (1.7)	5.3 (1.4)	5.9 (1.5)	5.6 (1.4)	0.003
Keys score, mean (SD)	28.6 (6.1)	29.3 (5.8)	28.7 (6.0)	28.3 (5.8)	0.565
Serum total cholesterol, mean (SD), mg/dL	169.2 (18.8)	189.3 (17.6)	206.5 (16.7)	230.8 (19.0)	< 0.001
Serum HDL cholesterol, mean (SD), mg/dL	56.9 (16.9)	52.1 (13.6)	53.5 (12.1)	52.3 (11.2)	0.040
Serum TG, mean (SD), mg/dL	155.2 (142.8)	666.9 (127.2)	155.5 (86.3)	166.5 (80.4)	0.619
SBP, mean (SD), mmHg	119.7 (11.1)	118.1 (12.6)	120.5 (13.0)	123.2 (14.4)	0.017
DBP, mean (SD), mmHg	75.3 (8.5)	75.7 (9.8)	76.3 (9.4)	79.9 (11.4)	< 0.001
On special diet***, no. (%)	7 (5.1)	7 (4.8)	11 (7.6)	6 (4.2)	0.578
On lipid-lowering drugs***, no. (%)	3 (2.2)	7 (4.8)	2 (1.4)	6 (4.2)	0.308**

(Cont Table 1)

	Women ($n=570$)				
	Q1 ($n=141$)	Q2 ($n=139$)	Q3 ($n=148$)	Q4 ($n=142$)	*P* value
Serum LDL cholesterol, median (range), mg/dL	91 (39–101)	113 (102–122)	132 (123–142)	160 (143–213)	
Serum LDL cholesterol, mean (SD), mg/dL	86.8 (11.6)	112.5 (5.8)	132.1 (5.5)	163.0 (17.4)	
Age, mean (SD), years	47.4 (5.2)	48.7 (5.2)	49.5 (5.3)	51.4 (4.7)	<0.001
BMI, mean (SD), kg/m^2	22.3 (2.8)	22.9 (2.8)	23.3 (3.1)	24.1 (3.4)	<0.001
Employed in a company***, no. (%)	110 (78.0)	107 (77.0)	103 (69.6)	99 (69.7)	0.209
Education***, mean (SD), years	12.3 (2.1)	11.8 (1.8)	11.5 (1.9)	10.9 (1.9)	<0.001
Education years ≥ 12 years (finished high school)***, no. (%)	116 (82.3)	104 (74.8)	103 (69.6)	73 (51.4)	<0.001
Site, no. (%)					0.029
Toyama	35 (25.0)	32 (23.0)	43 (29.1)	39 (28.1)	
Sapporo	52 (37.1)	37 (26.6)	27 (18.2)	30 (21.6)	
Aito	25 (17.9)	38 (27.3)	36 (24.3)	30 (21.6)	
Wakayama	28 (20.0)	32 (23.0)	42 (28.4)	40 (28.8)	
Before menopause (women)***, no. (%)	85 (60.3)	68 (48.9)	59 (39.9)	41 (28.9)	<0.001
Smokers***, no. (%)	14 (9.9)	11 (7.9)	10 (6.8)	14 (9.9)	0.723
Numbers of cigarettes, mean (SD), *n*/day	1.2 (4.4)	0.9 (3.5)	0.6 (2.7)	1.3 (4.4)	0.329
Numbers of cigarettes in smokers, mean (SD), *n*/day	12.2 (7.7)	11.1 (6.8)	8.9 (6.3)	12.9 (7.0)	0.538
Drinkers***, no. (%)	124 (87.9)	113 (81.3)	118 (79.7)	114 (80.3)	0.236
Alcohol intake, mean (SD), %kcal	2.3 (4.4)	1.2 (2.8)	1.4 (2.9)	1.1 (2.0)	0.032
Alcohol intake in drinkers, mean (SD), %kcal	2.6 (4.6)	1.6 (3.1)	1.7 (3.1)	1.4 (2.1)	0.057
Alcohol intake, mean (SD), g/day	6.1 (11.2)	3.3 (7.1)	3.5 (7.4)	2.9 (5.3)	0.028
Alcohol intake in drinkers, mean (SD), g/day	6.9 (11.7)	4.1 (7.7)	4.4 (8.0)	3.6 (5.7)	0.053
Dietary energy, mean (SD), kcal/day	1848 (367)	1823 (310)	1754 (296)	1764 (308)	0.041
Dietary total carbohydrate, mean (SD), %kcal	55.3 (7.4)	56.5 (6.1)	56.2 (6.2)	56.7 (5.7)	0.323
Dietary total protein, mean (SD), %kcal	16.1 (2.5)	16.1 (2.1)	16.2 (2.5)	16.1 (2.0)	0.938
Dietary total fat, mean (SD), %kcal	26.4 (5.3)	26.1 (4.9)	26.0 (4.5)	26.0 (4.8)	0.907
Dietary cholesterol, mean (SD), mg/1000 kcal	211.1 (75.4)	195.2 (58.4)	190.3 (61.8)	198.9 (68.1)	0.079
SFA, mean (SD), %kcal	7.0 (1.9)	7.2 (1.8)	7.0 (1.7)	7.1 (1.8)	0.880
PUFA, mean (SD), %kcal	6.7 (1.5)	6.6 (1.5)	6.6 (1.3)	6.6 (1.4)	0.854
MUFA, mean (SD), %kcal	9.6 (2.3)	9.3 (2.2)	9.4 (2.1)	9.3 (2.1)	0.741
TFA, mean (SD), %kcal	0.5 (0.4)	0.5 (0.3)	0.5 (0.3)	0.5 (0.3)	0.993
Total fiber, mean (SD), g/1000 kcal	8.5 (2.2)	9.0 (2.2)	9.1 (2.5)	8.8 (2.2)	0.146
Soluble fiber, mean (SD), g/1000 kcal	1.5 (0.5)	1.6 (0.5)	1.6 (0.6)	1.5 (0.5)	0.201
Insoluble fiber, mean (SD), g/1000 kcal	7.0 (1.7)	7.4 (1.7)	7.4 (2.0)	7.2 (1.8)	0.126
Keys score, mean (SD)	31.1 (6.7)	31.2 (6.0)	30.5 (6.5)	31.1 (6.7)	0.704
Serum total cholesterol, mean (SD), mg/dL	169.9 (21.5)	191.6 (14.5)	210.0 (16.0)	237.0 (21.9)	<0.001
Serum HDL cholesterol, mean (SD), mg/dL	62.1 (17.1)	62.2 (14.3)	60.3 (13.3)	55.6 (10.7)	<0.001
Serum TG, mean (SD), mg/dL	97.4 (69.8)	100.9 (50.3)	110.8 (52.0)	133.2 (63.7)	<0.001
SBP, mean (SD), mmHg	110.8 (13.0)	113.0 (11.5)	114.4 (14.5)	118.2 (15.4)	<0.001
DBP, mean (SD), mmHg	67.7 (8.8)	70.1 (8.6)	70.9 (9.7)	73.2 (10.5)	<0.001
On special diet***, no. (%)	9 (6.4)	10 (7.2)	13 (8.8)	13 (9.2)	0.800
Taking lipid-lowering drugs***, no. (%)	4 (2.8)	5 (3.6)	3 (2.0)	7 (4.9)	0.562**

*Excluded missing data for serum LDL cholesterol ($n=3$). *P* values were obtained using Welch's ANOVA test or chi-squared test (**Fisher's exact test). ***Self-reported. SI conversion factors: to convert serum cholesterol to mmol/L, multiply values by 0.0259.

Keys score $=2.7\times S-1.35\times P+1.5\times\sqrt{C}$; S, saturated fatty acid (%kcal); P, polyunsaturated fatty acid (%kcal); C, dietary cholesterol (mg/1,000 kcal).

Table 2. Characteristics of participants who were analyzed (n = 1,039; age, 40–59 years, in the INTERLIPID study in Japan, 1996–1999

	Men	Women	*P* value
	(n = 533)	(n = 506)	
Age, mean (SD), years	49.5 (5.3)	49.0 (5.3)	0.189
BMI, mean (SD), kg/m2	23.6 (2.7)	23.0 (2.9)	0.001
Employed in a company*, no. (%)	490 (91.9)	374 (73.9)	< 0.001
Education*, mean (SD), years	12.4 (2.1)	11.6 (1.9)	< 0.001
Education years ≥ 12 years (finished high school)*, no. (%)	443 (83.1)	355 (70.2)	< 0.001
Site, no. (%)			0.926
Toyama	139 (26.2)	135 (26.9)	
Sapporo	139 (26.2)	123 (24.5)	
Aito	118 (22.3)	117 (23.3)	
Wakayama	134 (25.3)	127 (25.3)	
Before menopause (women)*, no. (%)	—	239 (47.2)	—
Smokers*, no. (%)	277 (52.0)	43 (8.5)	< 0.001
Numbers of cigarettes, mean (SD), *n*/day	11.6 (13.0)	1.0 (3.7)	< 0.001
Numbers of cigarettes in smokers, mean (SD), *n*/day	22.3 (9.2)	11.2 (6.6)	< 0.001
Drinkers*, no. (%)	516 (96.8)	415 (82.0)	< 0.001
Alcohol intake, mean (SD), %kcal	9.2 (7.3)	1.6 (3.3)	< 0.001
Alcohol intake in drinkers, mean (SD), %kcal	9.5 (7.2)	2.0 (3.6)	< 0.001
Alcohol intake, mean (SD), g/day	30.1 (24.6)	4.1 (8.4)	< 0.001
Alcohol intake in drinkers, mean (SD), g/day	31.1 (24.4)	5.0 (9.0)	< 0.001
Dietary energy, mean (SD), kcal/day	2279 (423)	1808 (325)	< 0.001
Dietary total carbohydrates, mean (SD), %kcal	52.4 (7.7)	56.2 (6.3)	< 0.001
Dietary total protein, mean (SD), %kcal	15.7 (2.2)	16.0 (2.2)	0.032
Dietary total fat, mean (SD), %kcal	23.6 (4.7)	26.1 (4.7)	< 0.001
Dietary cholesterol, mean (SD), mg/1000 kcal	197.3 (67.5)	197.6 (65.9)	0.939
SFA, mean (SD), %kcal	6.1 (1.6)	7.1 (1.8)	< 0.001
PUFA, mean (SD), %kcal	6.2 (1.5)	6.6 (1.4)	< 0.001
MUFA, mean (SD), %kcal	8.6 (2.1)	9.4 (2.1)	< 0.001
TFA, mean (SD), %kcal	0.3 (0.2)	0.5 (0.3)	< 0.001
Total fiber, mean (SD), g/1000 kcal	6.8 (1.9)	8.8 (2.3)	< 0.001
Soluble fiber, mean (SD), g/1000 kcal	1.1 (0.4)	1.5 (0.5)	< 0.001
Insoluble fiber, mean (SD), g/1000 kcal	5.7 (1.5)	7.2 (1.8)	< 0.001
Keys score, mean (SD)	28.8 (5.9)	31.1 (6.5)	< 0.001
Serum total cholesterol, mean (SD), mg/dL	198.3 (28.2)	201.9 (31.0)	0.050
Serum LDL cholesterol, mean (SD), mg/dL	120.2 (28.3)	123.8 (30.0)	0.045
Serum HDL cholesterol, mean (SD), mg/dL	54.0 (13.8)	60.3 (14.2)	< 0.001
Serum TG, mean (SD), mg/dL	153.5 (98.0)	108.9 (60.4)	< 0.001
SBP, mean (SD), mmHg	120.2 (13.0)	113.7 (13.7)	< 0.001
DBP, mean (SD), mmHg	76.7 (10.1)	70.3 (9.6)	< 0.001

SI conversion factors: to convert serum cholesterol to mmol/L, multiply values by 0.0259. *P* values were obtained using the unpaired *t*-test or chi-squared testKeys score = $2.7 \times S - 1.35 \times P + 1.5 \times \sqrt{C}$; S, saturated fatty acid (%kcal); P, polyunsaturated fatty acid (%kcal); C, dietary cholesterol (mg/1,000 kcal). *Self-reported.

Table 3. Estimated mean difference in the serum LDL cholesterol (mg/dL) and dietary cholesterol (mg/1,000 kcal) higher by 1 SD* by employment status and sequential regression models in the INTERLIPID study in Japan, 1996–1999

Model	Men (n = 533)				Women (n = 506)			
	LDL-C (mg/dL)	95% CI		P value	LDL-C (mg/dL)	95% CI		P value
Unadjusted	− 3.16	− 5.52	− 0.79	0.009	− 1.64	− 4.30	+ 1.02	0.226
Model 1								
Employed in a company	− 4.13	− 6.59	− 1.68	0.001	− 3.05	− 6.12	+ 0.03	0.052
The others**	+ 8.56	0.00	+ 17.11	0.050	+ 1.71	− 3.55	+ 6.98	0.523
Model 2								
Employed in a company	− 3.03	− 5.51	− 0.56	0.017	− 1.00	− 4.27	+ 2.28	0.551
The others**	+ 9.13	+ 0.94	+ 17.31	0.029	+ 0.98	− 3.98	+ 5.94	0.699
Model 3								
Employed in a company	− 3.02	− 5.49	− 0.54	0.017	− 1.07	− 4.34	+ 2.19	0.518
The others**	+ 9.08	+ 0.90	+ 17.27	0.030	+ 1.09	− 3.86	+ 6.04	0.665

*1 SD difference for dietary cholesterol is 67.5 mg/1,000 kcal for men and 65.9 mg/1,000 kcal for women. **"The others" includes self-employed, homemakers, farmers, fishermen, and retired employees. Coefficients for dietary cholesterol were based on the use of indicator variables for the employment status. Tests were also performed to clarify whether the coefficients differed between the employed and the others.
Model 1 = dietary cholesterol + employment status ("employed in a company"/ "the others") + dietary cholesterol × employment status
Model 2 = Model 1 + age, BMI, menopausal status for women (before/in the middle or after), number of cigarettes, alcohol intake, SFA, PUFA, MUFA, TFA, soluble fiber, and site
Model 3 = Model 2 + education years
P values for the interaction term between dietary cholesterol and employment status (dietary cholesterol × employment status) are as follows: P = 0.005 for men and P = 0.126 for women in Model 1, P = 0.005 for men and P = 0.494 for women in Model 2, and P = 0.005 for men and P = 0.451 for women in Model 3.

men (education years, ≥ 12 years; P < 0.001). In Model 2 adjusted for covariates, 3.65 mg/dL lower serum LDL-C was estimated per 1 SD higher dietary cholesterol in more educated men (P = 0.006). In Model 3 that was further adjusted for employment status, 3.66 mg/dL lower serum LDL-C was estimated per 1 SD higher dietary cholesterol in more educated men (P = 0.006). These significant relationships were not observed in women.

Both men and women could be divided into two groups on a one-to-one basis when the cutoff point was 120 mg/dL. Among all men, there were 268 participants (50.3%) with ≥ 120 mg/dL of serum LDL-C and 265 participants (49.7%) with < 120 mg/dL. Among all women, there were 230 participants (45.5%) with ≥ 120 mg/dL of serum LDL-C and 276 participants (54.5%) with < 120 mg/dL. In men, 3.03 mg/dL lower serum LDL-C was estimated per 1 SD greater dietary cholesterol intake in men with higher serum LDL-C (≥ 120 mg/dL) but was associated with 0.55 mg/dL higher serum LDL-C in men with lower serum LDL-C (< 120 mg/dL; P = 0.010). These significant relationships were not observed in women.

Discussion

The established positive relationship in human experimental studies was confirmed only in nonemployed ("the others") and less educated men. A possible explanation for the inverse association with employed and more educated men might be the reverse causality; a direction from serum LDL-C to the dietary cholesterol intake was stronger among the employed and more educated men rather than nonemployed and less educated.

Employed and more educated men having inverse associations probably modified their dietary habits, i.e., participants with higher serum LDL-C or those afraid of high-serum LDL-C avoided dietary cholesterol intake, following health information disseminated through mass media since around 1970s[33]. In the present study, participants with higher serum LDL-C had a lower intake of dietary cholesterol. The result leads to the assumption that people, especially those with high serum cholesterol, have a lot of health information and take care about their food and diet both consciously or unconsciously. Because the number of participants consciously taking care about their diets was low (< 10%) in this study, we assumed that relatively large numbers of participants modify their diets unconsciously, or they just did not recognize their diets as "on special diet" in the questionnaire.

Not only the general knowledge though mass media but also appropriate health education and med-

Table 4. Estimated mean difference in serum LDL cholesterol (mg/dL) and dietary cholesterol (mg/1,000 kcal) higher by 1 SD* by education years and sequential regression models in the INTERLIPID study in Japan, 1996–1999

Model	Men (n = 533)				Women (n = 506)			
	LDL-C (mg/dL)	95% CI		P value	LDL-C (mg/dL)	95% CI		P value
Unadjusted	− 3.16	− 5.52	− 0.79	0.009	− 1.64	− 4.30	+ 1.02	0.226
Model 1								
≥ 12 years	− 4.83	− 7.44	− 2.22	< 0.001	− 0.52	− 3.77	+ 2.73	0.754
< 12 years	+ 2.77	− 2.78	+ 8.31	0.328	− 2.57	− 6.88	+ 1.75	0.243
Model 2								
≥ 12 years	− 3.65	− 6.24	− 1.06	0.006	+ 0.85	− 2.55	+ 4.26	0.622
< 12 years	+ 4.40	− 1.03	+ 9.84	0.112	− 2.81	− 7.13	+ 1.51	0.202
Model 3								
≥ 12 years	− 3.66	− 6.25	− 1.07	0.006	+ 0.75	− 2.67	+ 4.17	0.668
< 12 years	+ 4.46	− 0.97	+ 9.90	0.107	− 2.82	− 7.14	+ 1.50	0.200

*1 SD difference for dietary cholesterol is 67.5 mg/1,000 kcal for men and 65.9 mg/1,000 kcal for women. Coefficients for dietary cholesterol were based on the use of indicator variables for education years. Tests were also performed to clarify whether the coefficients differed between the education years of ≥ 12 and < 12 years.

Model 1 = dietary cholesterol + education years (≥ 12 / < 12 years) + dietary cholesterol × education years

Model 2 = Model 1 + age, BMI, menopausal status in women (before/in the middle or after), number of cigarettes, alcohol intake, SFA, PUFA, MUFA, TFA, soluble fiber, and site

Model 3 = Model 2 + employment status ("employed in a company"/ "the others")

P values for the interaction term between dietary cholesterol and education years (dietary cholesterol × education years) are as follows: $P = 0.015$ for men and $P = 0.457$ for women in Model 1, $P = 0.007$ for men and $P = 0.162$ for women in Model 2, and $P = 0.007$ for men and $P = 0.174$ for women in Model 3.

ical health check-ups conducted in the companies would enlarge the health disparities in which employed and more educated people had more opportunities to have healthier lifestyles than nonemployed and less educated people. Many epidemiological studies indicated that the education level has inverse relationships with blood pressure, death from coronary heart diseases and CVDs[34-38]. These data are presumably explained as the examples of reverse causality or temporal confounding[10, 11], i.e, educated people go to hospitals, take care about their lifestyles and reduce their food intake in response to high blood pressure or other risk factors.

Data from 1970s indicate that serum cholesterol and dietary cholesterol/egg consumption were both markedly higher among the employed in urban areas than among inhabitants in rural areas[39]. However, the nationwide health promotion program, including health screening and education[40], has resulted in health disparities. The health effectiveness of individual education programs has been confirmed in many studies[36, 41-44]. Employed people in a company, particularly in large-scale companies, have more opportunities to undergo medical health check-ups and obtain health information than the others[45]. Nonemployed men in the present study may have less access to health care services and fewer opportunities and incentives to change their lifestyles. This is a cross-sectional study; it has not been established whether the findings in the nonemployed are related to limited healthcare access and employment-sponsored intervention programs. Shekelle et al. reported that the association between dietary and serum cholesterol is positive for men with levels of serum cholesterol > 250 mg/dL and inverse for men with levels of ≥ 250 mg/dL[11]. It is consistent with the present study that men with higher serum LDL-C had a significantly inverse association compared with men with lower serum LDL-C. Archer et al. suggested that higher education level is associated with higher prevalence of dietary cholesterol restriction[46]. The novelty of this study is its confirmation that the direction of the relationship between dietary cholesterol and serum LDL-C is largely attributed to the employment status and education years in Japanese men. In this situation, more number of men that are employed and educated modified their diets rather than men that are nonemployed and less educated as a whole. Since there were no associations between dietary cholesterol intake and employment status/education years, not all employed and educated men but employed and educated men with higher serum LDL-C would be inclined to restrict their dietary cholesterol intake. The results of the present study might indicate that lifestyle modifications have the potential to change or reverse the apparently positive relationship between dietary cholesterol and serum cholesterol, particularly in observational studies.

Previous INTERMAP/INTERLIPID studies and a Japanese study have reported that dietary cholesterol intake is markedly higher in Japan than in the US[9, 39], and the main food group supplying dietary cholesterol in Japan is eggs (49%), whereas it is meat in the UK[47]. A previous Japanese study has reported that lower egg consumption is significantly associated with a higher prevalence of dietary cholesterol restriction among people with higher serum cholesterol[10]. The findings in the present study suggest that employed and more educated men reduce their dietary cholesterol intake by restricting their egg intake in Japan.

Among women, no significant associations were observed after adjusting for physiological factors including age, menopause, employment status, and education years in this highly controlled study. This might suggest that dietary cholesterol intake is not a largely influential factor on serum LDL-C in women before menopause[48]. However, several factors need to be considered. First, experimental studies that originally reported positive relationships between dietary lipids and serum cholesterol involved men[6, 49]. Second, physiological characteristics such as menopause and age may have greater influences on serum LDL-C than environmental factors in women[50-53]. Third, the definition of an employed woman included those with an employed husband in this study, which means some women were not employed themselves. This may change the proportion of participation by these women in health check-ups. However, most women in this study underwent health check-ups and obtained health information at local health centers because the four sites were active communities to attend healthcare services in Japan. A previous study also reported that attendance rates for health check-ups were higher among women than men[54]. Therefore, these complex conditions for women may have resulted in the obscured effect of employment status and education in the present study.

The strength of this study was that all data acquired were attained with high-quality control for nutritional, physical, and laboratory examinations[9, 16, 17]. The limitations of the present study include the lack of individual information regarding participation in health check-ups; different characteristics (factory workers and residents were mixed) among the four sites; education, employment, and some covariates in the questionnaire were self-reported; and small number of nonemployed individuals. However, the disparity in participation in health check-ups among employment status has been reported in other study[45], and the findings of the analysis without adjustments for site were consistent with the main results. Although a large number of men with the other job were from Aito town, the direction of coefficient values among employment statuses had the same tendency as the main result (Employed had a negative and nonemployed had a positive direction), and a small number of subjects in one site might weaken the statistical power for detecting *P* value.

Conclusion

Higher intake of dietary cholesterol correlated with higher concentrations of serum LDL-C in male participants that were less educated and nonemployed (includes self-employed, homemakers, farmers, fishermen, and retired employees), whereas the inverse was observed in those that were more educated and employed in a company. Further studies are needed to determine whether the effects observed in the nonemployed and less educated are related because of their limited access to health care and employment-sponsored intervention programs designed to encourage positive behaviors.

Acknowledgments

We thank Dr. Hisatomi Arima, Department of Preventive Medicine and Public Health, Faculty of Medicine, Fukuoka University and Dr. Akira Fujiyoshi, Department of Public Health, Shiga University of Medical Science for the critical revision to the manuscript, and Dr. Robert Douglas Abbott, Center for Epidemiologic Research in Asia, Shiga University of Medical Science and Dr. Sachiko Tanaka, Department of Public Health, Shiga University of Medical Science for their statistical expertise and advice, neither of whom were compensated for their contributions. Prof. Paul Elliott is the director of MRC-PHE Centre for Environment and Health and acknowledges support from the Medical Research Council and Public Health England (MR/L01341X/1). He is a Professor at UK Dementia Research Institute (DRI), UK DRI at Imperial College London. UK DRI is funded by the Medical Research Council, Alzheimer's Society and Alzheimer's Research, UK. The INTERMAP/INTERLIPID study has been accomplished through the fine work of the staff at local, national, and international centers; a partial listing of colleagues is given in the References[9, 16] of this manuscript.

Notice of Grant Support

This work was supported partly by a grant-in-aid for Scientific Research (A); the Ministry of Education, Science, Sports, and Culture of Japan (No. 090357003); and the Suntory Company. The INTERMAP study was supported by the National, Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA (Grant 2-RO1-HL50490), as well as national and local agencies in the four countries.

Conflicts of Interest

None declared. The study funders/sponsors had no role in the design or conduct of the study: collection, management, analysis, or interpretation of data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Author Contributions

Yukiko Okami and Hirotsugu Ueshima designed the research; Hirotsugu Ueshima, Nagako Okuda, Hideaki Nakagawa, Kiyomi Sakata, Shigeyuki Saitoh, Akira Okayama, Katsushi Yoshita, Sohel R. Choudhury, Queenie Chan, Paul Elliott, and Jeremiah Stamler organized the research and provided essential materials; Yasuyuki Nakamura, Nagako Okuda, Akira Okayama, Jeremiah Stamler, and Katsuyuki Miura critically revised the manuscript for intellectual content; Yukiko Okami and Hirotsugu Ueshima analyzed data; Yukiko Okami and Hirotsugu Ueshima wrote the manuscript; Yukiko Okami had the primary responsibility for the final content. All authors have read and approved the final manuscript.

References

1) Qureshi AI1 SF, Ahmed S, Nasar A, Divani AA, Kirmani JF.: Regular egg consumption does not increase the risk of stroke and cardiovascular diseases. Med Sci Monit, 2007; 13(1): CR1-8
2) Berger S, Raman G, Vishwanathan R, Jacques PF, Johnson EJ: Dietary cholesterol and cardiovascular disease: a systematic review and meta-analysis. Am J Clin Nutr, 2015; 102: 276-294
3) Rong Y, Chen L, Zhu T, Song Y, Yu M, Shan Z, Sands A, Hu FB, Liu L: Egg consumption and risk of coronary heart disease and stroke: dose-response meta-analysis of prospective cohort studies. BMJ, 2013; 346: e8539
4) Connor WE, Hodges RE, Bleiler RE: The serum lipids in men receiving high cholesterol and cholesterol-free diets. J Clin Invest, 1961; 40: 894-901
5) Keys A, Parlin RW: Serum cholesterol response to changes in dietary lipids. Am J Clin Nutr, 1966; 19: 175-181
6) Hegsted DM, McGandy RB, Myers ML, Stare FJ: Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr, 1965; 17: 281-295
7) Keys A: Serum cholesterol response to dietary cholesterol. Am J Clin Nutr, 1984; 40: 351-359
8) McNamara DJ: Cholesterol intake and plasma cholesterol: an update. J Am Coll Nutr, 1997; 16: 530-534
9) Ueshima H, Okayama A, Saitoh S, Nakagawa H, Rodriguez B, Sakata K, Okuda N, Choudhury SR, Curb JD: Differences in cardiovascular disease risk factors between Japanese in Japan and Japanese-Americans in Hawaii: the INTERLIPID study. J Hum Hypertens, 2003; 17: 631-639
10) Nakamura Y, Iso H, Kita Y, Ueshima H, Okada K, Konishi M, Inoue M, Tsugane S: Egg consumption, serum total cholesterol concentrations and coronary heart disease incidence: Japan Public Health Center-based prospective study. Br J Nutr, 2006; 96: 921-928
11) Shekelle RB, Stamler J, Paul O, Shryock AM, Liu S, Lepper M: Dietary lipids and serum cholesterol level: change in diet confounds the cross-sectional association. Am J Epidemiol, 1982; 115: 506-514
12) Robinson JG, Stone NJ: The 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular disease risk: a new paradigm supported by more evidence. Eur Heart J, 2015; 36: 2110-2118
13) Okamura T, Sugiyama D, Tanaka T, Dohi S: Worksite wellness for the primary and secondary prevention of cardiovascular disease in Japan: the current delivery system and future directions. Prog Cardiovasc Dis, 2014; 56: 515-521
14) Okuda N, Ueshima H, Okayama A, Saitoh S, Nakagawa H, Rodriguez BL, Sakata K, Choudhury SR, Curb JD, Stamler J: Relation of long chain n-3 polyunsaturated fatty acid intake to serum high density lipoprotein cholesterol among Japanese men in Japan and Japanese-American men in Hawaii: the INTERLIPID study. Atherosclerosis, 2005; 178: 371-379
15) Nakamura Y, Ueshima H, Okuda N, Higashiyama A, Kita Y, Kadowaki T, Okamura T, Murakami Y, Okayama A, Choudhury SR, Rodriguez B, Curb JD, Stamler J: Relation of dietary and other lifestyle traits to difference in serum adiponectin concentration of Japanese in Japan and Hawaii: the INTERLIPID Study. Am J Clin Nutr, 2008; 88: 424-430
16) Stamler J, Elliott P, Dennis B, Dyer AR, Kesteloot H, Liu K, Ueshima H, Zhou BF: INTERMAP: background, aims, design, methods, and descriptive statistics (nondietary). J Hum Hypertens, 2003; 17: 591-608
17) Dennis B, Stamler J, Buzzard M, Conway R, Elliott P, Moag-Stahlberg A, Okayama A, Okuda N, Robertson C, Robinson F, Schakel S, Stevens M, Van Heel N, Zhao L, Zhou BF: INTERMAP: the dietary data--process and quality control. J Hum Hypertens, 2003; 17: 609-622
18) Albano JD, Ward E, Jemal A, Anderson R, Cokkinides VE, Murray T, Henley J, Liff J, Thun MJ: Cancer mortality in the United States by education level and race. Journal of the National Cancer Institute, 2007; 99: 1384-1394

19) Satizabal CL, Beiser AS, Chouraki V, Chene G, Dufouil C, Seshadri S: Incidence of Dementia over Three Decades in the Framingham Heart Study. N Engl J Med, 2016; 374: 523-532
20) Hak AE, Witteman JC, Hugens W, Keyzer JJ, Pop VJ, Uitterlinden AG, Pols HA: The increase in cholesterol with menopause is associated with the apolipoprotein E genotype. A population-based longitudinal study. Atherosclerosis, 2004; 175: 169-176
21) Eberle E, Doering A, Keil U: Weight change and change of total cholesterol and high-density-lipoprotein cholesterol. Results of the MONICA Augsburg cohort study. Ann Epidemiol, 1991; 1: 487-492
22) Hjermann I, Helgeland A, Holme I, Lung-Larsen G, Leren P: The intercorrelation of serum cholesterol, cigarette smoking and body weight. The Oslo Study. Acta Med Scand, 1976; 200: 479-485
23) Schuitemaker GE, Dinant GJ, van der Pol GA, van Wersch JW: Relationship between smoking habits and low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, and triglycerides in a hypercholesterolemic adult cohort, in relation to gender and age. Clin Exp Med, 2002; 2: 83-88
24) Craig WY, Palomaki GE, Haddow JE: Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. BMJ, 1989; 298: 784-788
25) Nakamura Y, Okuda N, Turin TC, Fujiyoshi A, Okamura T, Hayakawa T, Yoshita K, Miura K, Ueshima H: Fatty acids intakes and serum lipid profiles: NIPPON DATA90 and the national nutrition monitoring. J Epidemiol, 2010; 20 Suppl 3: S544-548
26) Ascherio A, Katan MB, Zock PL, Stampfer MJ, Willett WC: Trans fatty acids and coronary heart disease. N Engl J Med, 1999; 340: 1994-1998
27) Nakanishi N, Yoshida H, Nakamura K, Kawashimo, Tatara K: Influence of alcohol intake on risk for increased low-density lipoprotein cholesterol in middle-aged Japanese men. Alcohol Clin Exp Res, 2001; 25: 1046-1050
28) Hein HO, Suadicani P, Gyntelberg F: Alcohol consumption, serum low density lipoprotein cholesterol concentration, and risk of ischaemic heart disease: six year follow up in the Copenhagen male study. BMJ, 1996; 312: 736-741
29) Choudhury SR, Ueshima H, Kita Y, Kobayashi KM, Okayama A, Yamakawa M, Hirao Y, Ishikawa M, Miyoshi Y: Alcohol intake and serum lipids in a Japanese population. Int J Epidemiol, 1994; 23: 940-947
30) Brown L, Rosner B, Willett WW, Sacks FM: Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr, 1999; 69: 30-42
31) Mensink RP, Katan MB: Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb, 1992; 12: 911-919
32) Mensink RP, Zock PL, Kester AD, Katan MB: Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr, 2003; 77: 1146-1155
33) Kuroda K: Changes in health-maintaining and -promoting behavior of the Japanese seen through secondary analysis of 'Hoken eisei-kisochosa' and 'Kokuminseikatsu-kisochosa' by the Ministry of Health and Social Welfare: Healthism in post-war Japan. Journal of the Socio-Cultural Research Institute, Ryukoku University: society and culture, 2004; 6: 307-324
34) Dyer AR, Stamler J, Shekelle RB, Schoenberger J: The relationship of education to blood pressure: findings on 40,000 employed Chicagoans. Circulation, 1976; 54: 987-992
35) Stamler R, Shipley M, Elliott P, Dyer A, Sans S, Stamler J: Higher blood pressure in adults with less education. Some explanations from INTERSALT. Hypertension, 1992; 19: 237-241
36) Stamler J, Elliott P, Appel L, Chan Q, Buzzard M, Dennis B, Dyer AR, Elmer P, Greenland P, Jones D, Kesteloot H, Kuller L, Labarthe D, Liu K, Moag-Stahlberg A, Nichaman M, Okayama A, Okuda N, Robertson C, Rodriguez B, Stevens M, Ueshima H, Horn LV, Zhou B: Higher blood pressure in middle-aged American adults with less education-role of multiple dietary factors: the INTERMAP study. J Hum Hypertens, 2003; 17: 655-775
37) Liu K, Cedres LB, Stamler J, Dyer A, Stamler R, Nanas S, Berkson DM, Paul O, Lepper M, Lindberg HA, Marquardt J, Stevens E, Schoenberger JA, Shekelle RB, Collette P, Shekelle S, Garside D: Relationship of education to major risk factors and death from coronary heart disease, cardiovascular diseases and all causes, Findings of three Chicago epidemiologic studies. Circulation, 1982; 66: 1308-1314
38) Clay CM, Dyer AR, Liu K, Stamler J, Shekelle RB, Berkson DM, Paul O, Schoenberger JA, Lepper M, Lindberg HA and et al.: Education, smoking and non-cardiovascular mortality: findings in three Chicago epidemiological studies. Int J Epidemiol, 1988; 17: 341-347
39) Ueshima H, Iida M, Shimamoto T, Konishi M, Tanigaki M, Doi M, Nakanishi N, Takayama Y, Ozawa H, Komachi Y: Dietary intake and serum total cholesterol level: their relationship to different lifestyles in several Japanese populations. Circulation, 1982; 66: 519-526
40) Takeuchi K: The relationship between undergoing a basic health checkup based on the Health and Medical Services Law for the Aged and medical expenditure covered under the National Health Insurance in Japan. Nihon Eiseigaku Zasshi, 2002; 56: 673-681
41) Nakamura M, Masui S, Oshima A, Okayama A, Ueshima H: Effects of stage-matched repeated individual counseling on smoking cessation: A randomized controlled trial for the high-risk strategy by lifestyle modification (HISLIM) study. Environ Health Prev Med, 2004; 9: 152-160
42) Schneider RJ, Barengo N, Haapala I, Tavella M: Association between dietary habits, education, serum triglycerides and blood cholesterol among women of Cabildo, Buenos Aires. Medicina (B Aires), 2006; 66: 517-525
43) Ikeda A, Iso H, Toyoshima H, Fujino Y, Mizoue T, Yoshimura T, Inaba Y, Tamakoshi A: The relationships between interest for and participation in health screening and risk of mortality: the Japan Collaborative Cohort Study. Prev Med, 2005; 41: 767-771
44) Tada H, Kawashiri MA, Nohara A, Inazu A, Kobayashi J, Yasuda K, Mabuchi H, Yamagishi M, Hayashi K: Lipid Management in a Japanese Community:Attainment Rate of Target Set by the Japan Atherosclerosis Society Guide-

lines for the Prevention of Atherosclerotic Cardiovascular Diseases 2012. J Atheroscler Thromb, 2017; 24: 338-345
45) Funahashi H: Attributes of non-participants aged 40-59 years in specific health check-ups. Nihon Koshu Eisei Zasshi, 2013; 60: 119-127
46) Archer SL, Hilner JE, Dyer AR, Greenlund KJ, Colangelo LA, Kiefe CI, Liu K: Association of education with dietary intake among young adults in the bi-ethnic Coronary Artery Risk Development in Young Adults (CARDIA) cohort. Public Health Nutr, 2003; 6: 689-695
47) Sakurai M, Stamler J, Miura K, Brown IJ, Nakagawa H, Elliott P, Ueshima H, Chan Q, Tzoulaki I, Dyer AR, Okayama A, Zhao L: Relationship of dietary cholesterol to blood pressure: the INTERMAP study. J Hypertens, 2011; 29: 222-228
48) Akahoshi M, Soda M, Nakashima E, Shimaoka K, Seto S, Yano K: Effects of menopause on trends of serum cholesterol, blood pressure, and body mass index. Circulation, 1996; 94: 61-66
49) Keys A, Anderson JT, Grande F: Prediction of serum-cholesterol responses of man to changes in fats in the diet. Lancet, 1957; 273: 959-966
50) Stevenson JC, Crook D, Godsland IF: Influence of age and menopause on serum lipids and lipoproteins in healthy women. Atherosclerosis, 1993; 98: 83-90
51) Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR: Menopause and risk factors for coronary heart disease. New England Journal of Medicine, 1989; 321: 641-646
52) Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi S, Egusa G, Hiro T, Hirobe K, Iida M, Kihara S, Kinoshita M, Maruyama C, Ohta T, Okamura T, Yamashita S, Yokode M, Yokote K: Women. Executive Summary of the Japan Atherosclerosis Society (JAS) Guidelines for the Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases in Japan-2012 Version. J Atheroscler Thromb, 2014; 21: 291-295
53) Current state of and recent trends in serum lipid levels in the general Japanese population. Research Committee on Serum Lipid Level Survey 1990 in Japan. J Atheroscler Thromb, 1996; 2: 122-132
54) Thorogood M, Coulter A, Jones L, Yudkin P, Muir J, Mant D: Factors affecting response to an invitation to attend for a health check. J Epidemiol Community Health, 1993; 47: 224-228