Time-Related Changes in Relationships Between the Keys Score, Dietary Lipids, and Serum Total Cholesterol in Japan — NIPPON DATA80/90/2010 —

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Background: In recent years, individuals with high serum total cholesterol (TC) have been more likely to modify their diet by reducing their intake of dietary lipids. The aim of this study was to investigate time-related changes in the relationships between the Keys score, dietary lipids, and TC between 1980 and 2010 in representative Japanese populations.

Methods and Results: We used 3 cross-sectional cohort studies conducted among Japanese representative populations in 1980, 1990, and 2010 (NIPPON DATA80/90/2010) for the analyses. We analyzed data for 10,365, 7,714, and 2,657 participants, respectively, in each dataset. The relationships between TC and the Keys score (calculated by dietary lipids, cholesterol, and energy intake)/dietary lipids were examined. In men, a 1-unit higher Keys score correlated with 0.92 mg/dL and 0.64 mg/dL TC in 1980 and 1990, respectively, but not in 2010. In women, a 1-unit higher Keys score correlated with 0.70 mg/dL and 0.74 mg/dL TC in 1980 and 1990, respectively, but with 0.33 mg/dL in 2010. In men and women, dietary cholesterol, saturated fatty acid (SFA), and trans fatty acid (TFA) levels were positively associated with TC concentrations in 1980 and 1990, but not in 2010.

Conclusions: In these representative Japanese populations, a higher Keys score as well as dietary cholesterol, SFA, and TFA levels correlated with higher TC concentrations in 1980/1990, but the relationships were weaker or not observed in 2010.

Key Words: Cohort study; Dietary cholesterol; Dietary lipids; Keys score; Serum total cholesterol

Japan achieved a remarkable breakthrough in the improvement of hypercholesterolemia in the late 20th century: the introduction of statin drugs in 1989;¹ the dissemination of dietary modifications and restrictions on dietary cholesterol and lipids in the diet through the growth of mass media and in the clinical setting since the late 1960s;^{2,3} the enactment of the Industrial Safety and Health Law that mandated annual health check-ups for employees in 1972; and the enactment of the Law for the Welfare of the Elderly in 1983.⁴ Under the health check-up system, detection of hypercholesterolemia and subsequent lifestyle modifications became available, particularly for middle-aged and elderly individuals.

In the context of these developments, we hypothesized that individuals with higher serum total cholesterol (TC) levels in recent years would be more likely to modify their diets (reduce dietary lipids and cholesterol intake) and take lipid-lowering medications than those with lower TC

concentrations; if a sufficient number of individuals do this, dietary lipids will not or will be inversely related to TC. Although previous studies reported that individuals with hypercholesterolemia modify their diets and restrict their intake of dietary lipids,^{5,6} time-related changes in the relationship between dietary lipids and TC against this background have not yet been examined.

Therefore, we examined changes in the relationships between the Keys score (dietary lipids) and TC between 1980 and 2010 in representative Japanese populations using NIPPON DATA80/90/2010.

Methods

Study Design and Participants

A pooled dataset of baseline data for analysis was obtained from the National Integrated Project for the Prospective Observation of Non-communicable Diseases and its Trends

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in the Aged conducted in 1980, 1990, and 2010 (NIPPON DATA80/90/2010). The methods of these studies are described in detail elsewhere.^{7,8}

NIPPON DATA80/90 was the combined data of participants in the National Survey on Circulatory Disorders (NSCD) and the dietary estimates from the National Nutrition Survey conducted in 1980 and 1990, respectively. Participants were those persons surveyed for the 3rd and 4th NSCD, conducted in 1980 and 1990. They were residents of 300 survey districts throughout Japan, randomly selected for the National Surveys by the Ministry of Health, Labour and Welfare of Japan. The participants for NIPPON DATA2010 were those who agreed to participate in the baseline survey and signed the consent form among participants in 300 districts throughout Japan, randomly selected for the NHNS by the Ministry of Health, Labour and Welfare of Japan in 2010. Surveys were conducted in a local health center, and consisted of physical examination, blood tests, and a self-administered questionnaire in which participants were asked about their habits of alcohol drinking, smoking, treatment for hypercholesterolemia (1990 and 2010), and history of cardiovascular disease (CVD). In NIPPON DATA90/2010, participants were asked about "...controlling your diet (type of food, meal size, amount of fat, or balance between meat and fish". If they answered "yes" to at least one of the above in the questionnaire, they were considered to be controlling their diet. Height in stocking feet and weight in light clothing were measured. Body mass index (BMI) was calculated as weight (in kg) divided by the square of height (in m).

The total numbers of community dwellers aged 30 years (20 years for NIPPON DATA2010) and older who were eligible and invited to participate were 10,546 (4,639 men and 5,907 women) in 1980, 8,383 (3,504 men and 4,879 women) in 1990, and 2,891 (1,236 men and 1,655 women) in 2010. People with an implausible energy intake (<500 or >5,000 kcal/day) or missing data for age, BMI, drinking, smoking, TC, and Keys score (dietary lipids), or who were under 30 years old (for NIPPON DATA2010) were excluded from the analysis, leaving 10,365 (4,558 men and 5,807 women) for NIPPON DATA80, 7,714 (3,220 men and 4,494 women) for NIPPON DATA90, and 2,657 (1,130 men and 1,527 women) for NIPPON DATA2010 (**Figure S1**). The Institutional Review Board of Shiga University of Medical Science (No. 22-29, 2010) approved this study.

Dietary Assessment

Dietary intakes for NIPPON DATA80/90 were assessed for each household using a 3-day semi-weighing dietary record method. Trained dietitians reviewed dietary records with the participants and edited them as necessary. Survey data were processed centrally to calculate total energy intake and the intake of nutrients and food groups. In this study, the nutrient intake of each household member was estimated by proportional distribution method, dividing the household intake data of the National Nutrition Survey in 1980/1990 proportionally with average intakes, as categorized by sex and age groups. In addition, other nutrients known to have effects on CVD risk factors, such as trans fatty acids (TFA), were calculated based on the INTERMAP Food Table and INTERMAP Japan dietary survey data.⁷ The detailed procedure and results of the validation for these estimations have been reported elsewhere.^{7,9} In NIPPON DATA2010, 1-day dietary surveys

with semi-weighing household dietary records and information on the food distribution ratio among family members were conducted by trained dietary interviewers.⁸

Dietary intakes of cholesterol (mg/1,000kcal), saturated fatty acids (SFA) (%kcal), polyunsaturated fatty acids (PUFA) (%kcal), monounsaturated fatty acids (MUFA) (%kcal), TFA (%kcal), and dietary fiber (g/1,000 kcal) were used in the analysis. The Keys score, which has been widely used as a reflector of TC in previous studies,^{10–12} was calculated using the following equation:¹³ φ (unit) = $2.7 \times S - 1.35 \times P + 1.5 \times C^{1/2}$, where S is SFA (%kcal), P is PUFA (%kcal), and C is dietary cholesterol (mg/1,000 kcal). Keys et al developed this equation to predict the average change in the serum cholesterol level by dietary change. One score increment in the Keys score is expected to correspond to an increase in serum cholesterol by 1 mg/dL. The predicted change in serum cholesterol from the Keys equation is highly correlated with the observed change in serum cholesterol ($r=0.92$).¹³

Serum Lipid Measurements

Non-fasting blood samples were obtained at each baseline for NIPPON DATA80/90/2010. Serum was separated and centrifuged soon after blood coagulation. Samples were shipped to one laboratory for blood chemistry measurements. TC was measured enzymatically and standardized by the Center for Disease Control and Prevention/National Heart, Lung, and Blood Institute (CDC-NHLBI) Lipids Standardization Program and US Cholesterol Reference Method Laboratory Network (CRMLN).^{14,15} Serum high-density lipoprotein (HDL) cholesterol was measured in 1990 and 2010, but not in 1980.

Statistical Analysis

In the description of the characteristics of participants for variables related to TC (mg/dL), means and standard deviations (SD) or percentages were calculated separated by sex and datasets, with an analysis of variance (ANOVA) test or chi-squared test for comparisons among the 3 datasets (NIPPON DATA 80/90/2010). The relationship between the Keys score and TC (<200, 200–239, >239) was compared among the 3 datasets with the ANOVA test. A multiple linear regression analysis was used to examine the relationship between TC as a dependent variable and the Keys score as an independent variable and required covariates, including age, BMI, smoking status, drinking status, and dietary fiber intake. Dummy variables were the smoking status (non-smoker, ex-smoker, current smoker) and drinking status (non-drinker, ex-drinker, current drinker). Model 1 was adjusted for age, while Model 2 was further adjusted for BMI, smoking status, drinking status, and dietary fiber. We then calculated the difference in TC (mg/dL) per 1-unit increase in the Keys score. The same analyses were conducted among the participants excluded for being treated for hypercholesterolemia and controlling their diet (1990 and 2010) or with a history of CVD. As a subgroup analysis, we stratified those controlling their diet in Model 2 in order to assess the effects on the relationship between the Keys score and TC. Stratification by age at 50 or 55 years old or by age group (30–39, 40–59, 60–79, and 80–95 years old) was also performed in order to observe the effects of treatment, diet, and differences between age groups. Another multiple regression analysis was conducted between TC as a dependent variable and 1 SD of an increase in dietary cholesterol (mg/1,000 kcal), SFA (%kcal),

Table 1. Participant Characteristics (NIPPON DATA80/90/2010, 1980–2010, Japan)

	1980	1990	2010	P value
Men	(n=4,558)	(n=3,220)	(n=1,130)	
Age (years)	49.9±13.4	53.3±13.6	61.5±13.9	<0.001
BMI (kg/m ²)	22.5±2.9	23.0±3.0	24.0±3.2	<0.001
Smoking status, n (%)				<0.001
Non-smoker	830 (18.2)	657 (20.4)	308 (27.3)	
Ex-smoker	847 (18.6)	779 (24.2)	501 (44.3)	
Current smoker	2,881 (63.2)	1,784 (55.4)	321 (28.4)	
Drinking status, n (%)				<0.001
Non-drinker	2,127 (46.7)	1,126 (35.0)	396 (35.0)	
Ex-drinker	263 (5.8)	226 (7.0)	36 (3.2)	
Current drinker	2,168 (47.6)	1,868 (58.0)	698 (61.8)	
Dietary energy (kcal/day)	2,399±482	2,323±457	2,145±557	<0.001
Dietary fat (%kcal)	20.0±5.2	22.3±4.5	23.2±6.7	<0.001
Dietary cholesterol (mg/1,000 kcal)	164.6±55.2	183.1±53.0	155.7±83.0	<0.001
SFA (%kcal)	5.65±1.47	5.90±1.35	6.04±2.32	<0.001
PUFA (%kcal)	5.27±1.39	5.58±1.30	5.31±1.87	<0.001
MUFA (%kcal)	7.44±1.95	7.90±1.76	7.84±2.79	<0.001
TFA (%kcal)	0.32±0.19	0.33±0.17	–	0.019*
Total fiber (g/1,000 kcal)	7.7±1.8	7.0±1.9	7.8±3.0	<0.001
Keys score (unit)**	27.1±5.7	28.5±5.1	27.1±8.5	<0.001
Serum total cholesterol (mg/dL)***	186.4±32.8	198.6±36.8	202.2±34.1	<0.001
Non-HDL-cholesterol (mg/dL)	–	148.3±38.7	144.9±34.3	<0.001*
Being treated for hypercholesterolemia, n (%)	–	216 (6.7)	163 (14.4)	<0.001
Controlling their diet, n (%)	–	1,891 (58.7)	776 (68.7)	<0.001
CVD history, n (%)	144 (3.2)	170 (5.3)	132 (11.7)	<0.001
Women	(n=5,807)	(n=4,494)	(n=1,527)	
Age (years)	50.0±13.5	52.4±13.9	59.3±14.7	<0.001
BMI (kg/m ²)	22.8±3.4	22.9±3.3	22.8±3.5	0.619
Smoking status, n (%)				<0.001
Non-smoker	5,097 (87.8)	3,955 (88.0)	1,291 (84.5)	
Ex-smoker	139 (2.4)	117 (2.6)	135 (8.8)	
Current smoker	571 (9.8)	422 (9.4)	101 (6.6)	
Drinking status, n (%)				<0.001
Non-drinker	5,538 (95.4)	4,149 (92.3)	1,158 (75.8)	
Ex-drinker	98 (1.7)	48 (1.1)	21 (1.4)	
Current drinker	171 (2.9)	297 (6.6)	348 (22.8)	
Dietary energy (kcal/day)	1,929±390	1,865±365	1,753±431	<0.001
Dietary fat (%kcal)	21.7±5.7	24.4±5.0	25.7±7.1	<0.001
Dietary cholesterol (mg/1,000 kcal)	178.6±59.0	199.8±59.0	170.6±90.1	<0.001
SFA (%kcal)	6.18±1.66	6.48±1.54	6.89±2.57	<0.001
PUFA (%kcal)	5.76±1.52	6.08±1.40	5.73±2.03	<0.001
MUFA (%kcal)	8.12±2.16	8.66±1.97	8.57±3.00	<0.001
TFA (%kcal)	0.35±0.22	0.37±0.19	–	0.005*
Fiber (g/1,000 kcal)	9.2±2.1	8.4±2.3	9.2±3.5	<0.001
Keys score (unit)**	28.7±6.1	30.3±5.7	29.7±9.3	<0.001
Serum total cholesterol (mg/dL)***	191.2±34.0	206.9±38.8	210.0±35.7	<0.001
Non-HDL-cholesterol (mg/dL)	–	150.0±40.2	142.4±36.7	<0.001*
Being treated for hypercholesterolemia, n (%)	–	352 (7.8)	271 (17.8)	<0.001
Controlling their diet, n (%)	–	3,079 (68.5)	1,170 (76.6)	<0.001
CVD history, n (%)	130 (2.2)	170 (3.8)	80 (5.2)	<0.001

Values are mean±SD, or n (%) (as indicated). P values were obtained using ANOVA or the chi-squared test. *Unpaired t-test. **Keys score=2.7×S–1.35×P+1.5×√C. S, saturated fatty acids (%kcal); P, polyunsaturated fatty acids (%kcal); C, dietary cholesterol (mg/1,000 kcal). ***SI conversion factors: In order to convert serum total cholesterol to mmol/L, multiply values by 0.0259. BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, trans fatty acids.

Table 2. Relationships Between Serum Total Cholesterol (mg/dL) and the Keys Score* Among All Participants and the Participants Excluded for Being Treated for Hypercholesterolemia or Controlling Their Diet (NIPPON DATA80/90/2010, 1980–2010, Japan)

	Men			Women		
	1980	1990	2010	1980	1990	2010
Unadjusted	(n=4,558)	(n=3,220)	(n=1,130)	(n=5,807)	(n=4,494)	(n=1,527)
β^{**}	1.00	0.74	0.00	0.06	−0.08	0.18
(95% CI)	(0.84, 1.17)	(0.49, 0.99)	(−0.23, 0.24)	(−0.09, 0.20)	(−0.28, 0.12)	(−0.01, 0.37)
P value	<0.001	<0.001	0.981	0.446	0.417	0.065
Model 1						
β^{**}	0.97	0.67	−0.06	0.65	0.64	0.33
(95% CI)	(0.80, 1.14)	(0.41, 0.92)	(−0.29, 0.17)	(0.51, 0.79)	(0.44, 0.84)	(0.14, 0.52)
P value	<0.001	<0.001	0.624	<0.001	<0.001	<0.001
Model 2						
β^{**}	0.92	0.64	−0.07	0.70	0.74	0.33
(95% CI)	(0.75, 1.09)	(0.39, 0.89)	(−0.30, 0.17)	(0.55, 0.84)	(0.54, 0.94)	(0.13, 0.53)
P value	<0.001	<0.001	0.564	<0.001	<0.001	0.001
Excluded participants being treated for hypercholesterolemia						
Model 2		(n=3,004)	(n=967)		(n=4,142)	(n=1,256)
β^{**}	—	0.60	−0.05	—	0.73	0.34
(95% CI)	—	(0.35, 0.85)	(−0.30, 0.20)	—	(0.53, 0.92)	(0.12, 0.56)
P value	—	<0.001	0.684	—	<0.001	0.003
Excluded participants being treated for hypercholesterolemia or controlling their diet						
Model 2		(n=1,280)	(n=320)		(n=1,381)	(n=332)
β^{**}	—	0.62	−0.18	—	0.80	0.11
(95% CI)	—	(0.24, 0.99)	(−0.62, 0.26)	—	(0.47, 1.13)	(−0.26, 0.48)
P value	—	0.001	0.414	—	<0.001	0.561

Model 1 was adjusted for age, and Model 2 was further adjusted for BMI, smoking status (non-smoker, ex-smoker, and current smoker), drinking status (non-drinker, ex-drinker, and current drinker), and dietary fiber (g/1,000 kcal). *Keys score=2.7×S−1.35×P+1.5×√C. S, saturated fatty acids (%kcal); P, polyunsaturated fatty acids (%kcal); C, dietary cholesterol (mg/1,000 kcal). ** β is shown as a natural coefficient of the Keys score. BMI, body mass index; CI, confidence interval.

PUFA (%kcal), MUFA (%kcal), TFA (%kcal), and dietary fiber (g/1,000 kcal), adjusted for age, BMI, smoking status, and drinking status. We calculated the standardized coefficient values of the Keys score, age, BMI, dietary fiber, smoking status, and drinking status in order to clarify the effective size of each independent variable on TC. In addition, non-HDL-cholesterol was compared between 1990 and 2010 and the associations between the Keys score and non-HDL-cholesterol were assessed with linear regression models. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA); two-tailed $P < 0.05$ was considered to be significant.

Results

Participant characteristics in NIPPON DATA80/90/2010 are shown in **Table 1**. Among men and women, age, current drinkers, dietary fat intake, SFA, TFA, TC, participants being treated for hypercholesterolemia, controlling their diet, and with a CVD history significantly increased, and current smokers and total energy intake decreased between 1980 and 2010. Non-HDL-cholesterol decreased from 1990 to 2010 in both men and women. This was also observed in analyses where participants under treatment for hypercholesterolemia were excluded. Among men, BMI also increased between 1980 and 2010. Dietary intakes of cholesterol and PUFA and the Keys score remained at the same level over time in men and women. The relationship between the Keys score and TC is shown in **Table S1**. In

men, positive correlations were observed in 1980 and 1990 and became negative in 2010. No correlations were found in any dataset for women.

Table 2 shows the results of the linear regression analysis with TC as a dependent variable and the Keys score as an independent variable. In men, a 1-unit higher Keys score was associated with significantly higher TC in 1980, but the positive association was lower in 1990 and absent in 2010. In women, no associations were observed between the Keys score and TC in unadjusted models in any of the datasets. This was consistent with the findings shown in **Table S1**. However, positive correlations between the Keys score and TC were observed in all datasets after adjustment for age, although the coefficient value was lower and non-significant in 2010 (**Table 2**). **Figure S2** shows scatter plots of TC and the Keys score stratified by sex. The attenuation of the relationship between the Keys score and TC from 1980/1990 to 2010 was similarly observed after adjustment for other covariates and among excluded male and female participants being treated for hypercholesterolemia or controlling their diet. Similar results were observed among male and female participants without a history of CVD (data not shown). The association between the Keys score and non-HDL-cholesterol (mg/dL) was also attenuated from 1990 to 2010 (data not shown). **Table S2** shows the standardized coefficient values of all independent variables with TC in Model 2. In men, the most associated factor with TC was BMI and second was the Keys score, and both coefficients decreased in 2010. Age was negatively

Table 3. Relationships Between Serum Total Cholesterol (mg/dL) and the Keys Score* Among All Participants, Stratified by Age Group (30–39, 40–59, 60–79, and 80–95 Years Old) (NIPPON DATA80/90/2010, 1980–2010, Japan)

Keys score	Men			Women		
	1980	1990	2010	1980	1990	2010
30–39 years old	(n=1,216)	(n=605)	(n=108)	(n=1,580)	(n=976)	(n=230)
β^{**}	0.62	0.54	0.48	0.82	1.17	0.16
(95% CI)	(0.33, 0.91)	(−0.01, 1.09)	(−0.48, 1.44)	(0.57, 1.08)	(0.79, 1.55)	(−0.25, 0.58)
P value	<0.001	0.052	0.324	<0.001	<0.001	0.441
40–59 years old	(n=2,203)	(n=1,505)	(n=310)	(n=2,773)	(n=2,063)	(n=456)
β^{**}	1.02	0.49	−0.51	0.74	0.69	0.43
(95% CI)	(0.76, 1.28)	(0.10, 0.87)	(−0.94, −0.08)	(0.54, 0.94)	(0.39, 0.98)	(0.09, 0.77)
P value	<0.001	0.013	0.020	<0.001	<0.001	0.014
60–79 years old	(n=1,075)	(n=1,026)	(n=626)	(n=1,350)	(n=1,319)	(n=734)
β^{**}	1.20	0.79	0.04	0.65	0.58	0.21
(95% CI)	(0.86, 1.53)	(0.36, 1.23)	(−0.27, 0.35)	(0.33, 0.97)	(0.21, 0.96)	(−0.08, 0.50)
P value	<0.001	<0.001	0.794	<0.001	0.002	0.162
80–95 years old	(n=64)	(n=84)	(n=86)	(n=104)	(n=136)	(n=107)
β^{**}	0.90	1.29	0.52	−0.25	1.67	0.62
(95% CI)	(−0.52, 2.31)	(−0.02, 2.59)	(−0.16, 1.20)	(−1.62, 1.12)	(0.39, 2.94)	(−0.03, 1.27)
P value	0.211	0.054	0.130	0.714	0.011	0.063

All models were adjusted for age, BMI, smoking status (non-smoker, ex-smoker, and current smoker), drinking status (non-drinker, ex-drinker, and current drinker), and dietary fiber (g/1,000 kcal). *Keys score=2.7×S−1.35×P+1.5×C. S, saturated fatty acids (%kcal); P, polyunsaturated fatty acids (%kcal); C, dietary cholesterol (mg/1,000 kcal). ** β is shown as a natural coefficient of the Keys score. BMI, body mass index; CI, confidence interval.

Table 4. Relationships Between Serum Total Cholesterol (mg/dL) and Dietary Cholesterol, SFA, PUFA, MUFA, TFA, and Dietary Fiber by 1 Standard Deviation* Among All Participants (NIPPON DATA80/90/2010, 1980–2010, Japan)

	Men			Women		
	1980 (n=4,558)	1990 (n=3,220)	2010 (n=1,130)	1980 (n=5,807)	1990 (n=4,494)	2010 (n=1,527)
Dietary cholesterol						
β	1.73	1.04	−0.70	1.03	1.70	0.32
(95% CI)	(0.50, 2.95)	(−0.59, 2.67)	(−2.24, 0.83)	(0.01, 2.06)	(0.42, 2.99)	(−0.96, 1.60)
P value	0.006	0.211	0.369	0.048	0.010	0.625
SFA						
β	3.70	2.51	−0.44	1.63	3.43	0.16
(95% CI)	(1.25, 6.18)	(−0.74, 5.77)	(−2.78, 1.89)	(−0.43, 3.70)	(0.89, 5.98)	(−1.64, 1.95)
P value	0.003	0.130	0.710	0.120	0.008	0.862
PUFA						
β	−1.36	0.07	−0.12	−0.46	0.37	−1.78
(95% CI)	(−3.50, 0.78)	(−2.69, 2.83)	(−2.22, 1.98)	(−2.23, 1.30)	(−1.89, 2.63)	(−3.49, −0.07)
P value	0.214	0.960	0.912	0.606	0.751	0.042
MUFA						
β	1.80	0.45	0.94	2.21	0.21	3.15
(95% CI)	(−1.53, 5.12)	(−3.91, 4.81)	(−2.07, 3.94)	(−0.55, 4.97)	(−3.28, 3.69)	(0.78, 5.52)
P value	0.289	0.839	0.541	0.116	0.908	0.009
TFA						
β	1.50	2.11	—	2.07	1.16	—
(95% CI)	(0.15, 2.85)	(0.13, 4.09)		(0.97, 3.16)	(−0.39, 2.72)	
P value	0.030	0.037	—	<0.001	0.143	—
Dietary fiber						
β	−1.10	−2.33	−0.79	−0.26	0.76	−0.01
(95% CI)	(−2.45, 0.26)	(−4.01, −0.65)	(−2.49, 0.92)	(−1.28, 0.76)	(−0.47, 1.99)	(−1.32, 1.30)
P value	0.112	0.007	0.366	0.619	0.228	0.985

Dietary cholesterol, SFA, PUFA, MUFA, TFA, and dietary fiber were included in the same model as independent variables and adjusted for age, BMI, smoking status (non-smoker, ex-smoker, and current smoker), and drinking status (non-drinker, ex-drinker, and current drinker). *1 standard deviation differences for dietary cholesterol, SFA, PUFA, MUFA, TFA, and dietary fiber are shown in Table 1. Abbreviations as in Tables 1,2.

associated with TC in men. In women, age was the most strongly associated with TC, BMI was the second, and Keys score was third in all datasets. Furthermore, all coefficient values decreased in 2010, which was similar to the results obtained for men.

Table 3 shows the results of the linear regression analysis with TC and the Keys score stratified by age groups (30–39, 40–59, 60–79, and 80–95 years old) (Model 2). When data were observed horizontally, the positive association between the Keys score and TC abated from 1980/1990 to 2010 in all age groups, except for 80–95-year-old women. In 40–59-year-old men, the positive correlation observed in 1980 became weaker in 1990 and negative in 2010. Women showed smaller changes in coefficient values than men during the time periods examined. The attenuation of relationships between 1980/1990 and 2010 was observed in all stratified groups at 50 or 55 years old ($<50/\geq 50$ or $<55/\geq 55$) in both men and women (data not shown). When data were observed vertically, the coefficient values in men slightly increased from the younger to older generation in 1980/1990, but not in 2010. In women, coefficient values slightly decreased from the younger to older generation in 1980/1990, but not in 2010.

Table 4 shows the results of the multiple linear regression analysis with 6 dietary factors as independent variables and TC as a dependent variable. One SD in the dietary intakes of cholesterol, SFA, PUFA, MUFA, TFA, and dietary fiber was included in the same model as independent variables and adjusted for age, BMI, smoking status, and drinking status. When results were observed horizontally, the positive correlation observed for dietary cholesterol and SFA with TC in 1980 was attenuated in 1990 and became negative in 2010 in men. In women, the positive correlation between dietary cholesterol and TC in 1980/1990 became negative in 2010. TFA positively correlated with TC in men and women. When data were observed vertically, SFA or MUFA had the largest coefficient values in all datasets. The coefficient value of dietary cholesterol on TC was nearly one-half of that of SFA in 1980/1990 for both men and women.

Similar results were obtained in both men and women when excluding participants with a history of CVD, being treated for hypercholesterolemia, and controlling their diet (data not shown).

Discussion

In men and women, a higher Keys score, dietary cholesterol, and SFA correlated with higher TC concentrations in 1980/1990; however, this relationship was attenuated in 2010. In men, the positive correlation in 1980 was attenuated in 1990 and became negative in 2010. These changes were similar among participants not being treated for hypercholesterolemia or with a history of CVD.

The marked decreases observed in coefficient values for the Keys score, dietary cholesterol, and SFA between 1980/1990 and 2010 suggested frequent restriction of dietary lipid intake among healthy individuals in recent years. Health education on restricting the amount of dietary cholesterol and fat intake in the clinical setting started in the 1960s.^{2,3,16} Because of this, and rapid economic growth in Japan, health and diet information was circulated through the mass media. A national survey reported that 37.7% of individuals in the general population in 1980 were controlling their diet.¹⁷ The present study indicated that

50–70% of individuals in 1990 made a conscious effort to control their diet and this increased to 60–80% in 2010. Previous studies reported that “healthism” or “health fads” spread widely throughout the nation from the mid-1970s.^{17,18} The combined result showed that the proportion of the general population consciously or subconsciously considering their health or diet markedly increased from around the 1980s. Because no correlations were observed between the Keys score and TC in the present study, even among participants not controlling their diet, we assumed that relatively large numbers of participants may unconsciously modify their diet or just did not recognize their attention to their diet. This question needs to be addressed in future studies.

A new nationwide health promotion program started in 2008. This employment-sponsored program, including health screening and subsequent healthcare advice, has improved health awareness in Japan.¹⁹ The effectiveness of individual education programs for modifying diets and lowering the risk of CVD has been confirmed in a number of studies.^{12,20–22} A previous study reported that less educated individuals have higher Keys scores, sodium intake, and blood pressure than more educated individuals because education increases healthy behaviors and lifestyle improvements, specifically in the working generation aged 40–59 years old.^{12,23} The present results support the interpretation that individuals with higher TC abstained from eating dietary lipids significantly more than those with lower TC among men aged 40–59 years old in 2010. Furthermore, the present study adds further information that this paradoxical logic has been frequently observed in recent years, with TC concentrations reflecting dietary lipid intake. Otherwise, participants who knew their TC level was high might have modified their diet intentionally when the survey was conducted. In either case, this phenomenon in which exposure and outcomes are more likely to be opposite (reverse causality or temporal confounding) has already been reported,^{5,6} indicating that individuals with high TC modify their diets and abstain from eating SFA and dietary cholesterol, specifically from eggs, in Japan. Because the biological effect of dietary lipids on TC is likely to be consistent over time, the time-related changes observed in these 3 cross-sectional epidemiological cohorts appear to be attributed to this reverse causality. However, the present study cannot confirm this. Results stratified by age group indicated that the aging population during the period examined did not confound the relationships because younger and older age groups showed attenuation between 1980/1990 and 2010. However, whether time-related changes are related to other historical backgrounds or confounding factors, such as the characteristics of each dataset, warrants consideration. Although the composition of serum cholesterol might have changed, attenuation in the relationship of the Keys score and non-HDL-cholesterol was also observed in a similar fashion.

Keys et al reported that SFA intake strongly correlated with CHD death rates in the seven countries study.²⁴ The Keys equation, in which the subduplicate power of dietary cholesterol multiplied by 1.5, is the most accurate number for predicating TC based on 39 experimental studies.²⁵ Other experimental studies confirmed that a 100mg/day change in dietary cholesterol reduced serum TC concentrations by 2.5mg/dL on average.²⁶ Because Japanese individuals eat as much as 350–450mg/day of dietary

cholesterol (equivalent of 2 eggs),²⁷ the effects of dietary cholesterol on TC are not negligible considering the population strategy in Japan. Following the abolishment of the upper limit of the recommended dietary cholesterol in the USA in 2015,²⁸ the USDA added that this abolishment did not suggest that dietary cholesterol was no longer important to consider because the healthy eating pattern in the dietary guidelines limited dietary cholesterol to less than 300 mg/day.²⁹

However, recent large-scale observational studies and meta-analyses have demonstrated that a high intake of dietary cholesterol or SFA was related to lower total mortality and CVD or non-CVD mortality.^{30–32} Because humans are animals that can think and act for themselves, it is very natural that participants with high TC or other CVD risk factors would restrict their food intake or select healthy foods according to health information or their doctor's guidance. A previous study conducted in Japan in 1990 reported that SFA intake and the Keys score correlated with TC.³³ Therefore, a shift in the social background related to the reverse direction between dietary lipids and TC may have occurred between 1990 and 2010. If this hypothesis is accurate, negative relationships between dietary cholesterol and SFA with CVD risk in observational studies need to be interpreted very carefully.

Regarding other factors, BMI was identified as a more important determinant of TC concentration irrespective of sex. Similar to the Keys score, the coefficient values in BMI decreased between 1980/1990 and 2010, which is consistent with previous findings showing that the relationship between being overweight and a high TC concentration gradually weakened between 1980 and 2010.³⁴ Reverse causality may have occurred in BMI, similar to the Keys score; in recent years individuals with higher TC have attempted to decrease their weight more than those with lower TC. In women, age was the most strongly associated factor with TC, which may be related to changes associated with menopause.³⁵ The reason why TC gradually increased between 1980 and 2010 in men and women may be the aging of the population and increases in BMI.

In terms of dietary fatty acids not included in the calculation of the Keys score, TFA positively correlated with TC in men and women. MUFA also positively correlated with TC. Another study reported that the effects of TFA on the ratio of low-density lipoprotein (LDL) cholesterol to HDL-cholesterol were significantly stronger than those of SFA.³⁶ However, the effects of MUFA on TC remain controversial.^{37,38} Our results suggest that not only dietary cholesterol, SFA, and PUFA, but also TFA and MUFA need to be considered as influential on TC in future studies. Accordingly, the Keys equation would need to be developed according to the dietary and lifestyle factors identified/assessed after the Keys equation was established. In other words, the addition of BMI, TFA and MUFA into the Keys equation might be possible, and the prediction of LDL-cholesterol instead of TC would be more beneficial according to the new scientific findings to date.

Study Strengths and Limitations

The present study had several strengths. Our study participants were from the NHNS, which was conducted in 300 randomly selected districts across Japan in 1980, 1990, and 2010. Therefore, our data were representative of Japanese in each era. Additionally, to the best of our knowledge, this is the first study to demonstrate time-related changes in the

relationships between dietary lipids and TC in Japanese representative populations. Our study also had some limitations. First, the methods of dietary surveys differed between 1980/1990 and 2010: 1-day household surveys in 1980/1990 and 3-day individual surveys in 2010. Although both were dietary record methods, the difference between 1980/1990 and 2010 possibly had an effect on the results in 2010. Second, because it was an observational study, it was difficult to completely control unknown confounding factors. Third, the response rate of the NHNS has been decreasing, especially for blood tests,^{8,39} and the small sample size of 2010 has been affected by this. Thus, the generalizability of the dataset in 2010 might be limited compared with the other datasets. Fourth, information on the treatment of hypercholesterolemia was unobtainable for the datasets in 1980. However, we assume that there were few people under treatment for hypercholesterolemia at that time because statins only became a major treatment in Japan from 1989.¹

Conclusions

In men and women, a higher Keys score and increased intake of dietary lipids, particularly dietary cholesterol and SFA, correlated with higher TC concentrations in 1980; however, this relationship was attenuated in 1990. A similar relationship was completely absent or further weakened in the dataset of 2010; an inverse correlation was observed in 40–59-year-old men. These results may possibly be related to the recent increase in awareness of hypercholesterolemia and knowledge of dietary improvement in Japan.

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Supplementary Files

Supplementary File 1

Table S1. Relationships between the Keys score and serum

total cholesterol (mg/dL) among all participants, NIPPON DATA80/90/2010, 1980–2010, Japan

Table S2. Relationships between serum total cholesterol (mg/dL) and the Keys score, age, BMI, dietary fiber, smoking status, and drinking status by 1 standard deviation among all participants, NIPPON DATA80/90/2010, 1980–2010, Japan

Figure S1. Participant flow chart.

Figure S2. Scatter plots of serum total cholesterol (mg/dL) and the Keys score stratified by gender, NIPPON DATA80/90/2010, 1980–2010, Japan.

Please find supplementary file(s);
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