(Cont Table 4)

B. Current Smoking

Current Smoking (−)	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	6211	131781	557	1.03	0.94-1.12	103	1.20	0.98-1.46	223	1.16	1.01-1.33
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	1157	24452	83	1.19	0.89-1.58	14	1.08	0.54-2.15	28	1.05	0.65-1.70
4.14-4.65	1426	30769	112	1.00		20	1.00		43	1.00	
4.66-5.17	1365	28898	111	0.99	0.76-1.29	15	0.73	0.37-1.44	43	1.05	0.68-1.61
5.18-5.68	1123	23764	118	1.10	0.84-1.43	25	1.29	0.70-2.35	51	1.32	0.87-2.01
5.69-6.20	613	13036	68	1.01	0.74-1.37	12	1.04	0.50-2.15	28	1.16	0.71-1.89
6.21-6.71	331	6913	31	0.85	0.56-1.27	5	0.72	0.27-1.96	12	0.93	0.48-1.79
6.72-	196	3950	34	1.76	1.19-2.62	12	3.49	1.66-7.33	18	2.68	1.52-4.74

C. Body Mass Index

Body Mass Index <25	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	7258	151366	700	1.11	1.03-1.21	135	1.33	1.12-1.59	278	1.22	1.08-1.39
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	1559	31987	127	1.00	0.79-1.27	20	0.80	0.45-1.42	45	1.00	0.67-1.50
4.14-4.65	1825	38630	156	1.00		30	1.00		55	1.00	
4.66-5.17	1640	34307	147	1.07	0.85-1.35	24	0.90	0.53-1.56	64	1.39	0.96-2.00
5.18-5.68	1160	24216	126	1.22	0.96-1.56	22	1.13	0.64-2.00	51	1.50	1.01-2.23
5.69-6.20	615	12899	77	1.19	0.90-1.57	19	1.49	0.82-2.70	32	1.47	0.94-2.30
6.21-6.71	293	6036	35	1.08	0.74-1.58	9	1.46	0.68-3.16	16	1.51	0.85-2.68
6.72-	166	3293	32	1.97	1.33-2.92	11	3.40	1.64-7.06	15	2.84	1.57-5.13
Body Mass Index >=25	No of Persons	Person-years	CVD			CHD			Cardiac Death		
			No deaths	HR	95%CI	No deaths	HR	95%CI	No deaths	HR	95%CI
1SD increment of serum total cholesterol	1951	41656	184	0.95	0.81-1.11	37	1.32	0.95-1.84	70	1.17	0.92-1.51
Category of baseline serum total cholesterol level (mmol/L)											
<4.14	240	5113	25	1.42	0.84-2.39	3	1.30	0.29-5.87	7	0.88	0.35-2.20
4.14-4.65	357	7631	33	1.00		4	1.00		14	1.00	
4.66-5.17	438	9379	34	0.88	0.54-1.43	4	0.86	0.21-3.49	8	0.47	0.20-1.14
5.18-5.68	413	8810	43	0.95	0.59-1.51	14	2.79	0.88-8.83	17	0.86	0.41-1.79
5.69-6.20	266	5714	24	0.78	0.46-1.34	5	1.17	0.30-4.57	10	0.70	0.30-1.63
6.21-6.71	149	3164	13	0.77	0.40-1.50	2	0.85	0.15-4.87	6	0.81	0.30-2.21
6.72-	88	1846	12	1.23	0.62-2.44	5	4.47	1.13-17.77	8	1.89	0.76-4.70

SD: standard deviation, HR: hazard ratio; 95% CI: 95% confidence interval, CVD: cardiovascular disease, CHD: coronary heart disease, HF: heart failure

The HRs were adjusted based on the following factors:

A. age, sex, the serum albumin level, body mass index, diabetes, smoking status and drinking status

B. age, sex, the serum albumin level, body mass index, hypertension, diabetes and drinking status

C. age, sex, the serum albumin level, body mass index, hypertension, diabetes, smoking status and drinking status

Table 5. PAF and excess deaths due to hypercholesterolemia for CVD, CHD and cardiac death

	CVD death	CHD death	Cardiac Death
JAS definition (TC >=5.69 mmol/L)			
HR (95%CI)	1.08 (0.92-1.28)	1.55 (1.10-2.19)	1.29 (1.00-1.66)
PAF	1.7%	10.6%	5.6%
Excess Death	14.6	18.2	19.5
ATPIII definition (TC >=6.21 mmol/L)			
HR (95%CI)	1.19 (0.95-1.48)	1.79 (1.16-2.74)	1.53 (1.11-2.12)
PAF	1.7%	6.9%	4.6%
Excess Death	14.6	11.9	15.6

HR: hazard ratio, 95%CI: 95% confidence interval, JAS: Japan Atherosclerosis Society, TC: total cholesterol, CVD: cardiovascular disease, CHD: coronary heart disease, PAF: population attributable fraction

to hypercholesterolemia was 14.6, 18.2 and 19.5 for CVD, CHD and cardiac death, respectively. The PAF of hypercholesterolemia determined based on the JAS definition (TC level ≥5.69 mmol/L) was 1.7%, 10.6% and 5.6% for CVD, CHD and cardiac death, respectively. Using the ATP Ⅲ definition (a TC level of ≥6.21 mmol/L), the PAFs for CVD, CHD and cardiac death were 1.7%, 6.9% and 4.6%, respectively. The number of excess deaths was also estimated to be 14.6 for CVD, 11.9 for CHD and 15.6 for cardiac death.

Discussion

In this 24-year Japanese cohort study, we found a 1-SD increment in the serum TC level to be positively associated with an increased risk of CVD, CHD and cardiac death. Similar results were also observed after classifying the participants by age (<65 or ≥65 years) and sex. Moreover, in the analyses of the seven TC level categories, the highest TC level (≥6.72 mmol/L) was found to be significantly associated with an increased risk of death due to CVD, especially CHD and cardiac death, and the PAFs of these diseases were moderately high. Furthermore, the relationship between the serum TC level and cardiovascular outcome was similar to that observed after classifying the subjects based on other risk factors, such as the prevalence of hypertension and current smoking. To the best of our knowledge, this is the first study to estimate the PAF for CVD death due to a high serum TC level in a Japanese population with a long follow-up period. Our results were based on data collected in 1980 and were not influenced by statins, as these drugs were not available in the market at the time of the survey.

In previous studies conducted in Western countries, the estimated PAF of CVD death due to hypercholesterolemia was found to be the highest among that for other risk factors[2-4]. However, the estimated PAFs in our study were lower than those for other risk factors, such as smoking (29%) and hypertension (8%), reported in previous studies assessing Japanese populations[6, 7]. Generally, the serum TC levels are lower in Asia countries, including Japan, than in Western countries[20, 21]. This observation may reflect the different impact of PAF of CVD death due to hypercholesterolemia. Furthermore, the reason for this difference appears to be due to differences in the prevalence of CHD and stroke in the Japanese population. Compared to that observed in Western countries, the prevalence of CHD is lower than that of stroke in Japan[20]. Moreover, as demonstrated in this study, hypercholesterolemia is not related to stroke mortality, although hypertension, smoking and diabetes are positively associated with stroke as well as CHD. Consequently, these factors resulted in lower PAFs due to hypercholesterolemia than those due to other risk factors in the present study.

As described above, neither a 1-SD increment nor high serum TC level were found to be associated with death due to stroke. This observation is consistent with the findings of other studies conducted in Japan[22-24]. In Japan, hypertension is the strongest risk factor for stroke; therefore, the influence of hypercholesterolemia is thought to be relatively weak[25]. Moreover, one subtype of stroke, atherothrombotic infarction, is positively associated with hypercholesterolemia, although its incidence among stroke cases is relatively low in Japan[26, 27]. This may be why hypercholesterolemia is not positively associated with stroke in Japan. The absence of a positive association between the cholesterol levels and stroke mortality has also been noted in studies in Western countries, especially

among elderly populations or patients with high blood pressure[28]. This is why the HRs and PAFs for total CVD death due to hypercholesterolemia were lower than those for CHD and/or cardiac death in the present study.

Moreover, we found a significant interaction between the serum TC level and smoking for CHD death, and the HR for smoking was higher than that for non-smoking in the subgroup analysis. These results are consistent with those reported by the Asia Pacific Cohort Studies Collaboration[29] and NIPPON DATA80[30] studies, although such findings have not been observed consistently[31]. Further research is therefore needed to confirm our findings.

As demonstrated in previous studies[8, 9], hypercholesterolemia is an important risk factor for CHD in Japan. However, the rate of fatalities from CHD among the Japanese population is lower than that observed in Western countries[32-34]. Heart failure is representative of end-stage CHD; therefore, HF may be registered as the cause of death in patients with CHD who survive a heart attack. Shiba *et al.* showed that the frequency of HF with an ischemic etiology is increasing in Japan[35]. Hence, we evaluated the endpoint "cardiac death," defined as death due to CHD or HF, and observed a similar result as that obtained for CHD only. Notably, the estimated PAF of CHD was higher than that of cardiac death, although this trend reversed when considering the estimated number of excess deaths.

As mentioned above, although the PAFs for smoking and hypertension estimated in other studies[6] are very high (29%), the rate of smoking is currently decreasing in Japan, and the prevalence of hypertension has not changed, even after adjusting for age[36, 37]. In contrast, the incidence of hypercholesterolemia is increasing[37]. As a result, the PAF of hypercholesterolemia may be higher in the future than that estimated in this study. Moreover, because the generation with hypercholesterolemia is reaching an age at which the risk for CVD increases, treating hypercholesterolemia is becoming increasingly important.

This study is associated with several limitations. First, we assessed risk factors, including the serum TC level, at baseline only; therefore, the relationship between the serum TC level and mortality may have been underestimated due to random errors in measuring the serum TC levels, known as the regression dilution effect[38]. Moreover, we did not have any information regarding changes in lifestyle or medications among the study subjects. For these reasons, it is necessary to apply the PAFs determined in this study to the present Japanese population with caution. Second, we were unable to obtain detailed information regarding the subtypes of stroke and heart failure because we could not view the subjects' death certificates. In Japan, the single underling cause of death is determined according to the ICD-9 code (until 1994) or ICD-10 code (from 1995 onwards) by a government officer based on a review of the death certificate. However, we believe this process may minimize the potential for information bias because the endpoints of NIPPON DATA were not defined by the researchers themselves. Third, we did not have access to data for other lipid parameters, such as high-density lipoprotein cholesterol, because the lipid profiles, with the exception of the TC level, were not generally evaluated in Japan during the baseline survey period.

Conclusion

In conclusion, in this long-term cohort study, hypercholesterolemia was shown to be significantly associated with an increased risk of death due to CVD, especially CHD and CHD plus HF, in both sexes among middle-aged and elderly community-dwelling Japanese individuals. The estimated PAFs of these diseases due to hypercholesterolemia were lower than those noted in Western countries and the values for other traditional risk factors. Nevertheless, we believe than managing hypercholesterolemia in Japanese is necessary in order to prevent the development of CVD in these populations.

Declaration

Dr. Sugiyama had full access to all of the data in this study and takes responsibility for the integrity of the data collection and the accuracy of the data analysis.

Acknowledgments

We would like to thank the members of the NIPPON DATA80/90 Research Group, who are listed in the Appendix in the online-only Data Supplement.

Notice of Grant Support

This study was supported by a Grant-in-aid from the Ministry of Health, Labour and Welfare under the auspices of the Japanese Association for Cerebro-Cardiovascular Disease Control, a Research Grant for Cardiovascular Diseases (7A-2) from the Ministry of Health, Labour and Welfare and a Health and Labour Sciences Research Grant, Japan (Comprehensive

Research on Aging and Health [H11-Chouju-046, H14-Chouju-003, H17-Chouju-012, H19-Chouju-Ippan-014] and Comprehensive Research on Life Style–Related Diseases Including Cardiovascular Diseases and Diabetes Mellitus [H22-Jyunkankitou-Seisyu-Sitei-017]).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

List of Contributors for NIPPON DATA80

Chairperson: Hirotsugu Ueshima (Department of Health Science, Shiga University of Medical Science, Otsu, Shiga).

Co-Chairperson: Akira Okayama (The First Institute for Health Promotion and Health Care, Japan Anti-Tuberculosis Association, Tokyo) for the NIPPON DATA80, Tomonori Okamura (Department of Preventive Medicine and Public Health, Keio University, Tokyo) for the NIPPON DATA90.

Research members: Shigeyuki Saitoh (Department of 2nd Internal Medicine, Sapporo Medical University, Sapporo, Hokkaido), Kiyomi Sakata (Department of Hygiene and Preventive Medicine, Iwate Medical University, Morioka, Iwate), Atsushi Hozawa (Preventive Medicine and Epidemiology, Tohoku Medical Megabank Organization, Tohoku University, Sendai, Miyagi), Takehito Hayakawa (Department of Hygiene and Preventive Medicine, Fukushima Medical University, Fukushima), Yosikazu Nakamura (Department of Public Health, Jichi Medical University, Shimotsuke, Tochigi), Yasuhiro Matsumura(Faculty of Healthcare, Kiryu University, Midori City, Gunma), Nobuo Nishi, Nagako Okuda (Project for the National Health and Nutrition Survey, National Institute of Health and Nutrition, Tokyo), Toru Izumi (Faculty of Medicine, Kitasato University, Sagamihara, Kanagawa), Toshiyuki Ojima (Department of Community Health and Preventive Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka), Koji Tamakoshi (Department of Public Health and Health Information Dynamics, Nagoya University Graduate School of Medicine, Nagoya, Aichi), Hideaki Nakagawa (Department of Epidemiology and Public Health, Kanazawa Medical University, Kanazawa, Ishikawa), Katsuyuki Miura, Takayoshi Ohkubo, Yoshikuni Kita (Department of Health Science, Shiga University of Medical Science, Otsu, Shiga), Aya Kadota (Department of School Nursing and Health Education, Osaka Kyoiku University, Kashiwara), Yasuyuki Nakamura (Cardiovascular Epidemiology, Kyoto Women's University, Kyoto), Yoshihiro Miyamoto (Department of Preventive Cardiology, National Cerebral and Cardiovascular Center), Katsushi Yoshita (Osaka City University Graduate School of human life science, Osaka), Kazunori Kodama, Fumiyoshi Kasagi (Radiation Effects Research Foundation, Hiroshima), and Yutaka Kiyohara (Department of Environmental Medicine, Kyushu University, Fukuoka).

References

1) http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/suikei11/ (in Japanese)
2) Emberson JR, Whincup PH, Morris RW, Walker M: Reassessing the contribution of serum total cholesterol, blood pressure and cigarette smoking to the aetiology of coronary heart disease: impact of regression dilution bias. Eur Heart J, 2003; 24: 1719-1726
3) Singh-Manoux A, Nabi H, Shipley M, Guéguen A, Sabia S, Dugravot A, Marmot M, Kivimaki M: The role of conventional risk factors in explaining social inequalities in coronary heart disease: the relative and absolute approaches to risk. Epidemiology, 2008; 19: 599-605
4) Grau M, Subirana I, Elosua R, Fitó M, Covas MI, Sala J, Masiá R, Ramos R, Solanas P, Cordon F, Nieto FJ, Marrugat J; REGICOR Investigators: Why should population attributable fractions be periodically recalculated? An example from cardiovascular risk estimation in southern Europe. Prev Med, 2010; 51: 78-84
5) Woodward M, Martiniuk A, Lee CM, Lam TH, Vanderhoorn S, Ueshima H, Fang X, Kim HC, Rodgers A, Patel A, Jamrozik K, Huxley R; Asia Pacific Cohort Studies Collaboration: Elevated total cholesterol: its prevalence and population attributable fraction for mortality from coronary heart disease and ischaemic stroke in the Asia-Pacific region. Eur J Cardiovasc Prev Rehabil, 2008; 15: 397-401
6) Hozawa A, Okamura T, Murakami Y, Kadowaki T, Nakamura K, Hayakawa T, Kita Y, Nakamura Y, Abbott RD, Okayama A, Ueshima H; NIPPON DATA80 Research Group: Joint impact of smoking and hypertension on cardiovascular disease and all-cause mortality in Japan: NIPPON DATA80, a 19-year follow-up. Hypertens Res, 2007; 30: 1169-1175
7) Ikeda A, Iso H, Yamagishi K, Inoue M, Tsugane S: Blood pressure and the risk of stroke, cardiovascular disease, and all-cause mortality among Japanese: the JPHC Study.Am J Hypertens, 2009; 22: 273-280
8) Okamura T, Tanaka H, Miyamatsu N, Hayakawa T, Kadowaki T, Kita Y, Nakamura Y, Okayama A, Ueshima H; NIPPON DATA80 Research Group: The relationship between serum total cholesterol and all-cause or cause-specific mortality in a 17.3-year study of a Japanese cohort. Atherosclerosis, 2007; 190: 216-223
9) Okamura T, Kadowaki T, Hayakawa T, Kita Y, Okayama A, Ueshima H; Nippon Data80 Research Group: What

cause of mortality can we predict by cholesterol screening in the Japanese general population? J Intern Med, 2003; 253: 169-180
10) Okamura T, Hayakawa T, Kadowaki T, Kita Y, Okayama A, Elliott P, Ueshima H; NIPPONDATA80 Research Group: Resting heart rate and cause-specific death in a 16.5-year cohort study of the Japanese general population. Am Heart J, 2004; 147: 1024-1032
11) Ueshima H, Choudhury SR, Okayama A, Hayakawa T, Kita Y, Kadowaki T, Okamura T, Minowa M, Iimura O: Cigarette smoking as a risk factor for stroke death in Japan: NIPPON DATA80. Stroke, 2004; 35: 1836-1841
12) Okamura T, Hayakawa T, Kadowaki T, Kita Y, Okayama A, Elliott P, Ueshima H; NIPPON DATA80 research group: A combination of serum low albumin and above-average cholesterol level was associated with excess mortality. J Clin Epidemiol, 2004; 57: 1188-1195
13) Takashima N, Ohkubo T, Miura K, Okamura T, Murakami Y, Fujiyoshi A, Nagasawa SY, Kadota A, Kita Y, Miyagawa N, Hisamatsu T, Hayakawa T, Okayama A, Ueshima H; NIPPON DATA80 Research Group: Long-term risk of BP values above normal for cardiovascular mortality: a 24-year observation of Japanese aged 30 to 92 years. J Hypertens, 2012; 30: 2299-2306
14) Miyamatsu N1, Kadowaki T, Okamura T, Hayakawa T, Kita Y, Okayama A, Nakamura Y, Oki I, Ueshima H: Different effects of blood pressure on mortality from stroke subtypes depending on BMI levels: a 19-year cohort study in the Japanese general population--NIPPON DATA80. J Hum Hypertens, 2005; 19: 285-291
15) Nakamura Y, Ueshima H, Okamura T, Kadowaki T, Hayakawa T, Kita Y, Tamaki S, Okayama A; NIPPON DATA80 Research Group: Association between fish consumption and all-cause and cause-specific mortality in Japan: NIPPON DATA80, 1980-99. Am J Med, 2005; 118: 239-245
16) Nakamura M, Sato S, Shimamoto T: Current status of CDC lipid standardization and international needs for standardization in epidemiological studies and clinical trials in Japan. J Atheroscler Thromb, 2004; 11: discussion 36-37
17) Kadowaki S, Okamura T, Hozawa A, Kadowaki T, Kadota A, Murakami Y, Nakamura K, Saitoh S, Nakamura Y, Hayakawa T, Kita Y, Okayama A, Ueshima H; NIPPON DATA Research Group: Relationship of elevated casual blood glucose level with coronary heart disease, cardiovascular disease and all-cause mortality in a representative sample of the Japanese population. NIPPON DATA80. Diabetologia, 2008; 51: 575-582
18) Rockhill B, Newman B, Weinberg C: Use and misuse of population attributable fractions. Am J Public Health, 1998; 88: 15-19
19) 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; Japan Atherosclerosis Society: 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, 2013; 20: 517-523
20) Ueshima H, Sekikawa A, Miura K, Turin TC, Takashima N, Kita Y, Watanabe M, Kadota A, Okuda N, Kadowaki T, Nakamura Y, Okamura T: Cardiovascular disease and risk factors in Asia: a selected review. Circulation, 2008; 118: 2702-2709
21) Zhang X, Patel A, Horibe H, Wu Z, Barzi F, Rodgers A, MacMahon S, Woodward M; Asia Pacific Cohort Studies Collaboration: Cholesterol, coronary heart disease, and stroke in the Asia Pacific region. Int J Epidemiol, 2003; 32: 563-572
22) Cui R, Iso H, Toyoshima H, Date C, Yamamoto A, Kikuchi S, Kondo T, Watanabe Y, Koizumi A, Inaba Y, Tamakoshi A; JACC Study Group: Serum total cholesterol levels and risk of mortality from stroke and coronary heart disease in Japanese: the JACC study. Atherosclerosis, 2007; 194: 415-420
23) Nago N, Ishikawa S, Goto T, Kayaba K: Low cholesterol is associated with mortality from stroke, heart disease, and cancer: the Jichi Medical School Cohort Study. J Epidemiol, 2011; 21: 67-74
24) Tanabe N, Iso H, Okada K, Nakamura Y, Harada A, Ohashi Y, Ando T, Ueshima H; Japan Arteriosclerosis Longitudinal Study Group: Serum total and non-high-density lipoprotein cholesterol and the risk prediction of cardiovascular events - theJALS-ECC -. Circ J, 2010; 74: 1346-1356
25) Iso H: Lifestyle and cardiovascular disease in Japan. J Atheroscler Thromb, 2011; 18: 83-88
26) Imamura T, Doi Y, Arima H, Yonemoto K, Hata J, Kubo M, Tanizaki Y, Ibayashi S, Iida M, Kiyohara Y: LDL cholesterol and the development of stroke subtypes and coronary heart disease in a general Japanese population: the Hisayama study. Stroke, 2009; 40: 382-388
27) Cui R, Iso H, Yamagishi K, Saito I, Kokubo Y, Inoue M, Tsugane S; JPHC Study Group: High serum total cholesterol levels is a risk factor of ischemic stroke for general Japanese population: the JPHC study. Atherosclerosis, 2012; 221: 565-569
28) Prospective Studies Collaboration, Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, Collins R: Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet, 2007; 370: 1829-1839
29) Nakamura K, Barzi F, Huxley R, Lam TH, Suh I, Woo J, Kim HC, Feigin VL, Gu D, Woodward M; Asia Pacific Cohort Studies Collaboration: Does cigarette smoking exacerbate the effect of total cholesterol and high-density lipoprotein cholesterol on the risk of cardiovascular diseases? Heart, 2009; 95: 909-916
30) Hozawa A, Okamura T, Kadowaki T, Murakami Y, Nakamura K, Hayakawa T, Kita Y, Nakamura Y, Okayama A; Hirotsugu Ueshima for NIPPON DATA80 Research group.: Is weak association between cigarette smoking and cardiovascular disease mortality observed in Japan explained by low total cholesterol? NIPPON DATA80. Int J Epidemiol, 2007; 36: 1060-1067
31) Nakamura K1, Nakagawa H, Sakurai M, Murakami Y, Irie F, Fujiyoshi A, Okamura T, Miura K, Ueshima H;

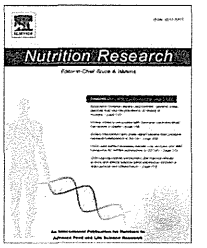
EPOCH-JAPAN Research Group: EPOCH-JAPAN Research Group. Influence of smoking combined with another risk factor on the risk of mortality from coronary heart disease and stroke: pooled analysis of 10 Japanese cohort studies. Cerebrovasc Dis, 2012; 33: 480-491
32) Kitamura A, Iso H, Iida M, Naito Y, Sato S, Jacobs DR, Nakamura M, Shimamoto T, Komachi Y: Trends in the incidence of coronary heart disease and stroke and the prevalence of cardiovascular risk factors among Japanese men from 1963 to 1994. Am J Med, 2002; 112: 104-109
33) Funabashi N, Shima M, Adachi M, Watanabe S, Masuda Y: Analysis of the treatment of acute myocardial infarction using ambulance records in Japanese cities. Jpn Circ J, 1999; 63: 170-176
34) Tunstall-Pedoe H, Kuulasmaa K, Mähönen M, Tolonen H, Ruokokoski E, Amouyel P: Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. Lancet, 1999; 353: 1547-1557
35) Shiba N, Nochioka K, Miura M, Kohno H, Shimokawa H; CHART-2 Investigators: Trend of westernization of etiology and clinical characteristics of heart failure patients in Japan-first report from the CHART-2 study. Circ J, 2011; 75: 823-833
36) http://www.mhlw.go.jp/stf/houdou/2r98520000020qbb.html (in Japanese)
37) http://www.mhlw.go.jp/stf/houdou/2r9852000001r5gc.html (in Japanese)
38) MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A, Stamler J: Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. Lancet, 1990; 335: 765-774

NUTRITION RESEARCH 35 (2015) 14–22

Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com



The reasonable reliability of a self-administered food frequency questionnaire for an urban, Japanese, middle-aged population: the Suita study☆,☆☆

Koutatsu Maruyama[a,b,*], *Yoshihiro Kokubo*[b], *Tamami Yamanaka*[c], *Makoto Watanabe*[b], *Hiroyasu Iso*[c], *Tomonori Okamura*[d], *Yoshihiro Miyamoto*[b]

[a] *Department of Basic Medical Research and Education, Ehime University Graduate School of Medicine, Shitsukawa, Toon, Ehime 791-0295, Japan*
[b] *Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, 5-7-1, Fujishiro-dai, Suita, Osaka 565-8565, Japan*
[c] *Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Yamadaoka, 2-2, Suita-shi, Osaka 565-0871, Japan*
[d] *Department of Preventive Medicine and Public Health, Keio University, 35 Shinanomachi Shinjuku, Tokyo, 160-8582, Japan*

ARTICLE INFO

Article history:
Received 22 July 2014
Revised 20 October 2014
Accepted 22 October 2014

Keywords:
Japanese
Urban area
FFQ
Validity
Repeatability
Cohort study
CVD

ABSTRACT

Because few studies have developed food frequency questionnaires (FFQ) and examined their reliability for Japanese urban populations, FFQ developed for urban Japanese populations may show reasonable reliability for estimating intakes of nutrients and food groups. Therefore, the objective of this study was to examine the reliability of an FFQ developed for a prospective cohort study in a Japanese urban area. A total of 29 men and 29 women aged 47 to 78 years were selected from participants in the Suita study from February 1997 to February 1998. Seven-consecutive-day dietary records (DR) was collected in each season (28-day DR). The FFQ were administered 3 times in total in each season, except in autumn. We calculated Spearman correlation coefficients to assess the validation of the first and third FFQ compared with 28-day DR and to assess the repeatability for 3-, 6-, and 9-month intervals. Reasonable validity of each FFQ compared with 28-day DR were observed for energy intake and for 27 nutrients, and 11 food groups were selected. Median (range) Spearman rank correlation coefficients for energy-adjusted nutrient and food group intakes of the first FFQ were 0.52 (0.14-0.88) and 0.53 (0.24-0.74), and those of the third FFQ were 0.51 (0.07-0.84) and 0.57 (0.16-0.75), respectively. The repeatability of each interval was relatively good; median (range) Spearman correlation coefficients of nutrients for 3-, 6-, and 9-month intervals were 0.67 (0.40-0.85), 0.63 (0.25-0.93), and 0.62 (0.31-0.87), respectively; those for food groups were 0.58 (0.42-0.76), 0.56 (0.24-0.80), and 0.65 (0.30-0.76), respectively. In conclusion, this FFQ is useful for evaluating the associations of nutrient and food intakes with cardiovascular diseases and their risk factors in Japanese urban populations.

Abbreviations: DR, dietary records; FFQ, food frequency questionnaires.

☆ Conflict of interest: None.

☆☆ Author contributions: K.M. participated in the study analysis, interpretation of data, and drafting of the manuscript and provided statistical expertise. Y.K. participated in the study concept and design, acquisition of data and interpretation of data, and critical revision of the manuscript. Y.M., T.O., M.W., and H.I. participated in acquisition and interpretation of data and critical revision of the manuscript. T.Y. participated in the interpretation of data.

* *Corresponding author.* Department of Basic Medical Research and Education, Ehime University Graduate School of Medicine, Shitsukawa, Toon, Ehime 791-0295, Japan. Tel.: +81 89 960 5283; fax: +81 89 960 5284.

E-mail addresses: maruyama.kotatsu.mu@ehime-u.ac.jp, m_koutatsu_@hotmail.com (K. Maruyama).

http://dx.doi.org/10.1016/j.nutres.2014.10.012
0271-5317/

1. Introduction

The food frequency questionnaire (FFQ) is one of the most commonly used assessment tools for diet. A large number of FFQ have been developed to adapt characteristics of study populations for most epidemiological studies.

Compared with the Western diet, the Japanese diet is characterized by higher intakes of salt, fish, rice, and soy foods and lower intakes of dietary fats and dairy products (eg, calcium-rich food) [1–4]. Furthermore, high-carbohydrate and high-salt intakes are associated with increased risk of all-cause mortality and lifestyle-related diseases [5–7], although intakes of those nutrients have decreased during the last 3 decades [8]. Most FFQ for Japanese have relatively high validity for estimating those nutrients [9].

On the other hand, there are also region-specific dietary habits in Japan. The National Health and Nutrition Survey in Japan [8] showed that the largest metropolitan areas tended to have higher intakes of saturated fatty acids, bread, and meats while tending to have lower intakes of n-3 unsaturated fatty acids, vitamin D, rice, and fish compared with other areas.

Those differences between areas may affect differences in lifestyle-related disease incidents between both areas. The secular trend of incidence of coronary heart disease was higher in urban than in rural areas; urban areas have experienced an increase in incidence, whereas rural areas have experienced a decrease [10]. In addition, the incidence of stroke was approximately twice as high as that of coronary heart disease in urban areas, whereas it was 2 to 4 times that in rural areas [10–13].

Several studies in Japan have examined the reliability of the FFQ among Japanese rural populations [9]. However, few FFQ have been developed for Japanese urban populations [14–17]. Furthermore, most other validation studies in Japan do not include urban populations or mixed areas [9] despite that food distribution and urbanization include large metropolitan areas and rural areas in Japan [8]. Therefore, FFQ developed for rural areas could not estimate the nutrient and food intakes for urban populations correctly.

The hypothesis of this study was that the FFQ developed for urban Japanese populations have reasonable reliability for estimating intakes of energy, nutrient, and food groups. Because few FFQ were developed and their reliability for urban Japanese populations was confirmed, the objective of this study was to examine the validity and repeatability of a self-administered FFQ developed for a middle-aged population from prospective cohort studies in Japanese urban areas.

2. Methods and materials

2.1. Study design and subjects

The subjects were recruited from participants of the Suita study. The Suita study is a prospective cohort study conducted in a Japanese urban area, the details of which can be found elsewhere [11,12,18–21]. In brief, this cohort, which is supported by the National Cerebral and Cardiovascular Center, has approximately 6500 men and women aged 30 to 79 years who live in Suita City, an urban area located on the north side of Osaka Prefecture (its population was approximately 345 000 in 1990). This cohort was established in 1989 to focus on protection for cardiovascular diseases.

All study participants underwent regular health checkups between September 1989 and March 1994. Participants continued to visit the National Cerebral and Cardiovascular Center every 2 years since then for regular health checkups. At baseline and at regular health checkup surveys, the participants were evaluated in terms of potential risk factors, including anthropometric indices, blood pressure, blood sample test results, and electrocardiogram results, and their lifestyles were assessed. After the baseline survey, the participants were followed up and cardiovascular events were assessed (ie, stroke and coronary heart disease).

To validate the FFQ for the Suita study, 31 married couples (31 men and 31 women) aged 47 to 78 years were selected from participants of this study. They also participated in the validation study for FFQ of the Japan Public Health Center–Based Prospective Study Cohort II [22,23]. We excluded 2 men and 2 women from the analysis because they did not completely answer the 28-day dietary records (DR); thus, we included 58 men and women for this analysis. Figure shows the timeline of this study. Seven-consecutive-day DR were administered to each subject in each of the 4 seasons. The FFQ was also collected in each season, except in autumn (November 1997). This study was approved by the Institutional review board of the National Cerebral and Cardiovascular Center, and informed consent was obtained from each participant.

2.2. Dietary records

Because the participants also participated in the validation study for the FFQ of the Japan Public Health Center–Based Prospective Study Cohort II, information in the DR for this study was the same. The data collection procedure for the DR

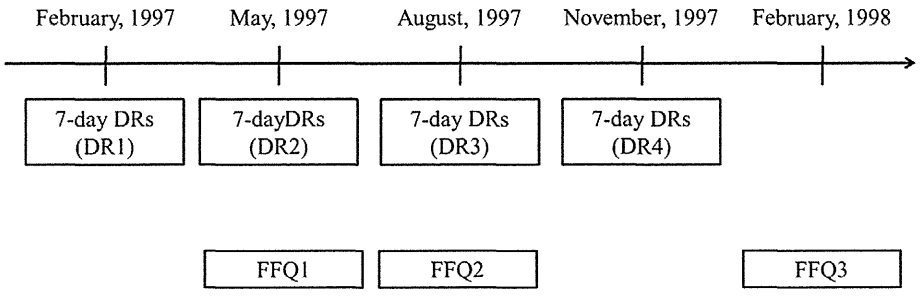


Figure – Timeline of the study for reliability of the self-administered FFQ. The 7-day DR and FFQ were conducted at almost the same time in each season, except for the lack of the FFQ in November 1997.

methodology is described in detail elsewhere [22,23]. In brief, 7-consecutive-day DR were collected in each of the 4 seasons, which were then used as 28-day DR for 1 year. The subjects received the booklet for recording, and local dietitians instructed them how to record. We asked the subjects to record all foods in detail, including seasonings and beverages, method of preparation, and names of dishes, and to measure the weight or size of each food. Local dietitians collected and then checked their DR by the end of each season. We averaged all food intakes in each 28-day period and then considered them as the individual's habitual food intake. Daily energy and major nutrients intakes were calculated by using the *Standard Tables of Food Composition in Japan (Fifth Revised and Enlarged Edition)*[24]. Daily food intakes were also calculated, and all foods were categorized into 18 food groups based on standard tables.

2.3. *Food frequency questionnaire*

The developed self-administered FFQ consisted of a number of questions and included items under 50 types of food, 6 alcohol beverages, 8 dishes, 10 salty food and dishes, as well as 7 questions concerning the fat content of milk and types of meat, fish, and oil consumed (see Supplementary data). Three questions assessed dietary habits (eg, seasoning and noodle soup) to estimate salt intake. We asked for the weekly or monthly frequency of consumption of 49 foods (excluding salty food and dishes) and 6 alcohol beverages, whereas for vegetable consumption, we asked for the daily frequency in an open-ended style.

The frequency of dry fish was grouped into 5 categories: "rarely," "once or twice per week," "every other day," "once per day," and "twice per day." Almost all food and alcohol beverages, excluding rice, bread, and beer, were shown as a standard portion size in the FFQ. Portion sizes of beer were categorized into "large (633 mL)," "medium (500 mL)," "small (350 mL)," and "glass (200 mL)."

In addition, we asked for the frequencies of principal foods at each mealtime (breakfast, lunch, dinner, or snack), including rice, bread, noodles, sweet buns, and cereal, because habitual intake of principal food at each mealtime may reflect their dietary habit. It may also be useful to estimate cereal and carbohydrate intakes more correctly. Portion sizes of rice were grouped into 4 categories: "large (200 g)," "medium (standard; 150 g)," "small (110 g)," and "other (open-ended)." Bread portions were categorized into "quarter of a loaf," "one-fifth of a loaf," "one-sixth of a loaf," and "one-eighth of a loaf."

The amount of each food intake was calculated by multiplying the standard or selected portion size by the frequency (per day). For milk, meat, fish, and oil, answers to 7 questions that asked the fat content of those foods assessed the weight of the food components. Salt intake was also weighted by answers to 3 questions related to salt intake (seasoning and noodle soup). The estimated daily energy and major nutrients intakes in the FFQ were calculated by using the *Standard Tables of Food Composition in Japan (Fifth Revised and Enlarged Edition)*[24]. Daily food intakes were also all calculated, and all foods were categorized on the basis of the standard tables.

2.4. *Selected foods and nutrients*

Intakes of 11 food groups were categorized on the basis of the standard tables, and energy and 27 nutrients were calculated. We also estimated the intake of animal and plant protein, animal and plant fat, calcium from milk and dairy products, and caffeine because those nutrients could be estimated by using the standard tables. Those nutrients have been shown to be associated with risk of cardiovascular diseases and their risk factors [25–27].

2.5. *Statistical analyses*

All nutrient and food group intakes in men and women were adjusted for energy intake by residual methods [28] using linear regression analysis after logarithmic transformation. We calculated the means and SDs for energy, for each nutrient, and for food groups of 28-day DR and respective FFQ (FFQ1, FFQ2, and FFQ3) and presented them as means ± SD. To assess the validation of the FFQ compared with 28-day DR, we calculated the Spearman correlation coefficients of 28-day DR with FFQ1 and FFQ3. We also divided the subjects into tertiles according to intakes for energy, energy-adjusted nutrients, and food groups for 28-day DR, FFQ1, and FFQ3, respectively, and then calculated the percentages of the same and extreme categories for energy, energy-adjusted nutrient, and food group intakes. To assess the repeatability, we calculated Spearman correlation coefficients among respective FFQ at 3-, 6-, and 9-month intervals. SAS Statistical Package version 9.4 (Statistical Analysis System Inc, Cary, NC, USA) was used for analyses.

3. Results

3.1. *Validation of FFQ*

Table 1 shows the means and correlations of nutrient intakes between the 28-day DR and the FFQ. Most of the mean crude intakes of energy and nutrients calculated by the FFQ were systematically lower than those calculated by the 28-day DR, excluding sodium, calcium, polyunsaturated fatty acids, vitamin D, and ethanol.

Spearman correlation coefficients between the FFQ and the 28-day DR were calculated to examine the validity of the FFQ. Median and range values of Spearman correlation coefficients for crude intakes of energy and nutrients estimated by FFQ1 were 0.47 and 0.07 (soluble fiber) and 0.89 (ethanol). After adjustment for energy intake, intakes for nutrients were 0.52 and 0.14 (caffeine) and 0.88 (ethanol), and crude intakes of those estimated by FFQ3 were 0.44 and 0.19 (polyunsaturated fatty acids) and 0.84 (ethanol). After adjustment for energy intake, those for intakes of nutrients were 0.51 and 0.07 (caffeine) and 0.84 (ethanol) (Table 1).

Percentages of joint classifications of energy and energy-adjusted nutrient intakes by tertiles between 28-day DR and FFQ are shown in Table 2. Similar trends in Spearman correlation coefficients were observed. Ethanol, carbohydrate, and calcium intakes have relatively high percentage of the same category and low percentage of the extreme category. In contrast, caffeine, α-tocopherol, and n-3 polyunsaturated

Table 1 – Nutrient intakes for 28-day DR with FFQ1 and FFQ3

	Nutrient intakes			Spearman correlation coefficients			
				DR vs FFQ1		DR vs FFQ3	
	28-d DR	FFQ1	FFQ3	Crude	Energy adjusted	Crude	Energy adjusted
Energy (kcal)	2043 ± 323	1907 ± 329	1847 ± 300	0.58		0.45	–
Total protein (g)	78.6 ± 12.4	76.5 ± 17.9	76.6 ± 15.3	0.53	0.48	0.47	0.54
Plant protein (g)	37.0 ± 6.3	35.4 ± 6.6	35.7 ± 8.1	0.60	0.65	0.53	0.71
Animal protein (g)	41.6 ± 9.3	41.1 ± 13.7	40.9 ± 11.9	0.41	0.41	0.43	0.43
Total fat (g)	54.7 ± 10.3	53.3 ± 15.9	52.1 ± 12.8	0.56	0.57	0.44	0.66
Plant fat (g)	26.9 ± 6.4	28.7 ± 9.7	27.7 ± 8.5	0.46	0.42	0.22	0.38
Animal fat (g)	27.7 ± 7.3	24.5 ± 9.0	24.3 ± 7.3	0.54	0.63	0.62	0.75
Carbohydrate (g)	286.7 ± 49.7	252.8 ± 51.0	242.8 ± 43.8	0.71	0.76	0.59	0.80
Energy from protein (%energy)	15.5 ± 1.6	16.1 ± 2.8	16.7 ± 2.6	–	0.61	–	0.61
Energy from fat (%energy)	24.2 ± 3.5	25.0 ± 5.7	25.4 ± 4.8	–	0.61	–	0.65
Energy from carbohydrate (%energy)	56.2 ± 5.5	53.4 ± 8.3	52.9 ± 7.1	–	0.65	–	0.75
Sodium (mg)	3793 ± 763	4258 ± 1240	3863 ± 996	0.32	0.48	0.39	0.43
Potassium (mg)	3065 ± 485	2454 ± 527	2501 ± 510	0.46	0.47	0.49	0.53
Calcium (mg)	664 ± 189	724 ± 257	719 ± 261	0.68	0.69	0.48	0.54
Calcium from milk and dairy products (mg)	230 ± 161	267 ± 181	262 ± 181	0.85	0.74	0.75	0.69
Magnesium (mg)	320 ± 51	300 ± 72	304 ± 73	0.55	0.59	0.51	0.60
Retinol (μg)	320 ± 286	352 ± 340	316 ± 224	0.46	0.50	0.40	0.46
β-Carotene (μg)	3866 ± 1369	2940 ± 1027	3005 ± 1061	0.47	0.58	0.29	0.29
Retinol equivalent (μg)	689 ± 315	643 ± 370	617 ± 265	0.53	0.55	0.33	0.36
Vitamin D (μg)	8.59 ± 3.03	11.48 ± 6.51	10.66 ± 5.19	0.28	0.23	0.28	0.20
α-Tocopherol (mg)	8.15 ± 1.46	6.92 ± 1.68	6.81 ± 1.47	0.36	0.25	0.24	0.16
Vitamin K (μg)	251 ± 77	227 ± 66	241 ± 90	0.47	0.54	0.40	0.43
Vitamin B_6 (mg)	1.49 ± 0.27	1.32 ± 0.32	1.32 ± 0.28	0.36	0.43	0.44	0.54
Vitamin B_{12} (μg)	9.91 ± 5.30	8.80 ± 4.04	8.21 ± 3.16	0.26	0.23	0.27	0.36
Folic acid (μg)	457 ± 94	312 ± 86	314 ± 81	0.51	0.58	0.31	0.35
Vitamin C (mg)	150.3 ± 34.2	94.8 ± 34.4	101.2 ± 25.2	0.61	0.59	0.46	0.47
Saturated fatty acids (g)	15.7 ± 3.8	15.2 ± 5.1	14.7 ± 4.2	0.73	0.74	0.69	0.83
Monounsaturated fatty acids (g)	18.2 ± 3.9	17.2 ± 5.4	16.7 ± 4.3	0.51	0.47	0.44	0.66
Polyunsaturated fatty acids (g)	13.0 ± 2.4	13.5 ± 4.3	13.6 ± 3.7	0.37	0.31	0.19	0.25
n-3 polyunsaturated fatty acids (g)	2.5 ± 0.5	2.6 ± 1.0	2.6 ± 0.8	0.20	0.15	0.33	0.22
n-6 polyunsaturated fatty acids (g)	10.4 ± 2.1	10.9 ± 3.6	11.0 ± 3.3	0.44	0.35	0.22	0.34
Cholesterol (mg)	331 ± 98	298 ± 124	299 ± 101	0.61	0.60	0.52	0.64
Dietary fiber (g)	16.9 ± 2.9	12.5 ± 2.8	2.8 ± 0.7	0.40	0.46	0.45	0.50
Soluble fiber (g)	3.8 ± 0.8	2.8 ± 0.6	9.5 ± 2.1	0.07	0.20	0.36	0.33
Insoluble fiber (g)	12.3 ± 2.1	9.5 ± 2.2	12.4 ± 2.8	0.45	0.49	0.49	0.55
Caffeine (g)	0.19 ± 0.07	0.02 ± 0.02	0.02 ± 0.04	0.16	0.14	0.25	0.07
Ethanol (g)	10.0 ± 15.4	12.6 ± 21.7	11.4 ± 18.6	0.89	0.88	0.84	0.84
Median				0.47	0.52	0.44	0.51

Nutrient intakes for 28-day DR with FFQ1 and FFQ3 are means ± SD.
Spearman correlation coefficients ≥0.27 ($P < .05$) and ≥0.36 ($P < .01$).

fatty acids, which have lower Spearman correlation coefficients, have relatively low percentage of the same category and high percentage of the extreme category.

We also show in Table 3 the mean and Spearman correlation coefficients of food group intakes between the 28-day DR and FFQ. Similar differences for mean intakes linked with nutrients were observed. Intakes for most of the food groups, excluding pulses (including soy), nuts and seeds, fruits, milk and dairy products, and confectioneries, as calculated by each FFQ, were systematically higher than values calculated by the 28-day DR.

Median and range values of Spearman correlation coefficients between FFQ1 and 28-day DR for intakes of food groups were 0.57 and 0.17 (vegetables) and 0.74 (cereals). After adjustment for energy intake, these were 0.53 and 0.24 (meats) and 0.75 (cereals). Those between FFQ3 and 28-day DR were 0.57 and 0.19 (algae, nuts, and seeds) and 0.74 (cereals). After adjustment for energy intake, these were 0.57 and 0.16 (algae) and 0.78 (cereals).

Percentages of joint classifications of energy-adjusted food group intakes by tertiles between 28-day DR and FFQ were also calculated (Table 4). They showed trends similar to those of Spearman correlation coefficients. Cereal intake comprises a relatively high percentage of the same category and a lower percentage of the extreme category. In contrast, nuts, seeds, and algae have a relatively low percentage of the same category and a high percentage of the extreme category.

3.2. *Repeatability of FFQ*

In this analysis, we administered the FFQ in 3 seasons (ie, at 3-, 6-, and 9-month intervals) to address repeatability. Mean values of

Table 2 – Joint classification of energy and energy-adjusted nutrient intakes by tertiles of 28-day DR with FFQ1 and FFQ

	DR vs FFQ1		DR vs FFQ3	
	Same category	Extreme category	Same category	Extreme category
Energy (kcal)	56.9	8.6	51.7	6.9
Total protein (g)	53.4	12.1	55.2	10.3
Plant protein (g)	51.7	3.4	58.6	0.0
Animal protein (g)	44.8	10.3	43.1	12.1
Total fat (g)	53.4	12.1	55.2	3.4
Plant fat (g)	50.0	19.0	50.0	12.1
Animal fat (g)	63.8	5.2	63.8	5.2
Carbohydrate (g)	63.8	1.7	72.4	3.4
Energy from protein (%energy)	56.9	8.6	50.0	8.6
Energy from fat (%energy)	50.0	8.6	53.4	5.2
Energy from carbohydrate (%energy)	56.9	8.6	53.4	1.7
Sodium (mg)	55.2	13.8	53.4	8.6
Potassium (mg)	48.3	10.3	56.9	8.6
Calcium (mg)	58.6	3.4	39.7	8.6
Calcium from milk and dairy products (mg)	67.2	5.2	58.6	6.9
Magnesium (mg)	51.7	6.9	58.6	6.9
Retinol (μg)	48.3	10.3	51.7	10.3
β-Carotene (μg)	53.4	1.7	44.8	10.3
Retinol equivalent (μg)	43.1	5.2	41.4	6.9
Vitamin D (μg)	46.6	15.5	43.1	15.5
α-Tocopherol (mg)	31.0	13.8	39.7	19.0
Vitamin K (μg)	51.7	3.4	43.1	5.2
Vitamin B_6 (mg)	50.0	12.1	55.2	6.9
Vitamin B_{12} (μg)	44.8	24.1	39.7	15.5
Folic acid (μg)	65.5	6.9	48.3	10.3
Vitamin C (mg)	55.2	3.4	44.8	10.3
Saturated fatty acids (g)	60.3	1.7	72.4	0.0
Monounsaturated fatty acids (g)	44.8	10.3	55.2	3.4
Polyunsaturated fatty acids (g)	43.1	15.5	44.8	13.8
n-3 polyunsaturated fatty acids (g)	37.9	17.2	34.5	17.2
n-6 polyunsaturated fatty acids (g)	39.7	15.5	43.1	15.5
Cholesterol (mg)	41.4	3.4	46.6	1.7
Dietary fiber (g)	46.6	8.6	62.1	10.3
Soluble fiber (g)	39.7	12.1	41.4	13.8
Insoluble fiber (g)	44.8	6.9	62.1	10.3
Caffeine (g)	34.5	17.2	31.0	17.2
Ethanol (g)	75.9	0.0	65.5	0.0

All values are percentages.

Table 3 – Intakes for food groups for 28-day DR with FFQ1 and FFQ3

	Food group intakes			DR vs FFQ1		DR vs FFQ3	
	28-d DR	FFQ1	FFQ3	Crude	Energy adjusted	Crude	Energy adjusted
Cereals (g)	453.1 ± 127.3	415.5 ± 119.8	406.9 ± 116.7	0.74	0.75	0.74	0.78
Pulses (g)	70.9 ± 27.8	130.0 ± 52.3	156.3 ± 8.4	0.52	0.54	0.65	0.67
Nuts and seeds (g)	4.3 ± 3.6	5.5 ± 8.9	5.0 ± 8.1	0.42	0.44	0.19	0.26
Vegetables (g)	289.6 ± 66.4	214.0 ± 8.7	217.5 ± 84.3	0.17	0.34	0.35	0.44
Fruits (g)	154.0 ± 74.8	158.1 ± 98.3	174.5 ± 67.4	0.70	0.71	0.57	0.57
Algae (g)	8.6 ± 5.9	2.1 ± 1.2	2.1 ± 1.4	0.57	0.49	0.19	0.16
Fishes and shellfishes (g)	97.4 ± 30.8	92.7 ± 46.3	89.8 ± 39.6	0.31	0.30	0.39	0.31
Meats (g)	60.0 ± 20.1	45.4 ± 19.9	49.1 ± 18.0	0.23	0.24	0.33	0.41
Eggs (g)	34.4 ± 15.8	26.4 ± 17.1	28.3 ± 14.8	0.62	0.53	0.66	0.68
Milk and dairy products (g)	172.8 ± 120.9	227.0 ± 146.4	216.4 ± 146.5	0.76	0.67	0.72	0.66
Confectioneries (g)	35.9 ± 24.4	46.2 ± 32.8	37.8 ± 27.2	0.70	0.66	0.63	0.66
Median				0.57	0.53	0.57	0.57

Food group intakes for 28-day DR with FFQ1 and FFQ3 are means ± SD.
Spearman correlation coefficients ≥0.27 ($P < .05$) and ≥0.36 ($P < .01$).

Table 4 – Joint classification of energy-adjusted food group intakes by tertiles of 28-day DR with FFQ1 and FFQ3

	DR vs FFQ1		DR vs FFQ3	
	Same category	Extreme category	Same category	Extreme category
Cereals (g)	70.7	1.7	74.1	1.7
Pulses (g)	53.4	8.6	63.8	5.2
Nuts and seeds (g)	41.4	10.3	34.5	13.8
Vegetables (g)	37.9	6.9	43.1	8.6
Fruits (g)	63.8	5.2	55.2	3.4
Algae (g)	51.7	6.9	32.8	12.1
Fishes and shellfishes (g)	44.8	17.2	48.3	17.2
Meats (g)	37.9	13.8	46.6	15.5
Eggs (g)	48.3	6.9	60.3	1.7
Milk and dairy products (g)	60.3	8.6	60.3	5.2
Confectioneries (g)	55.2	3.4	56.9	5.2

All values are percentages.

energy and crude- and energy-adjusted nutrient and food group intakes between each FFQ showed slight differences (Tables 5 and 6). Spearman correlation coefficients among 3 FFQ had mostly intermediate to high values and were statistically significant. Median (range) values of Spearman correlation coefficients for crude intakes of energy and nutrients for 3-, 6-, and 9-month intervals were 0.69 (0.43-0.83), 0.61 (0.29-0.92), and 0.64 (0.33-0.88), respectively. These correlations were not altered after adjustment for energy intake. Those for intakes of nutrients for 3-, 6-, and 9-month intervals were 0.67 (0.40-0.85), 0.63 (0.25-0.93), and 0.62 (0.31-0.87), respectively (Table 3). Table 4 shows the median (range) values of Spearman correlation coefficients for crude intakes of food groups. Those for intakes of food groups for 3-, 6-, and 9-month intervals were 0.66 (0.41-0.77), 0.64 (0.35-0.81), and 0.66 (0.36-0.81), respectively. After adjustment for energy intake, those for intakes of food groups for 3-, 6-, and 9-month intervals were 0.58 (0.42-0.76), 0.56 (0.24-0.80), and 0.65 (0.30-0.76), respectively.

4. Discussion

In this study, we examined the reliability of a self-administered FFQ developed for a Japanese, urban, middle-aged population. We observed reasonable validity compared with 28-day DR and a relatively high repeatability for 3 intervals for energy, for 27 nutrients, and for 11 food groups selected. This finding agrees with our research hypothesis.

On the basis of previous validation studies conducted in a Japanese urban area, Tokudome et al [16] determined the Spearman correlation coefficients for energy and for intakes of 22 selected nutrients among 202 middle-aged men and women. Participants live in Aichi Prefecture in the third metropolitan area in Japan. The Spearman correlation coefficient for energy intake was 0.36 in men and 0.37 in women. The medians (ranges) of Spearman correlation coefficients for energy-adjusted nutrients were 0.35 (0.13-0.76) in men and 0.34 (0.11-0.47) in women. Another validation study conducted among 78 middle-aged female dietitians in the same prefecture also observed reasonable validity of the semiquantitative FFQ; Spearman correlation coefficients for energy intake were 0.42 in men and 0.42 in women. Medians (ranges) of Spearman correlation coefficients for 35 energy-adjusted nutrients and 14 foods were 0.45 (0.22-0.71) and 0.42 (0.31-0.68), respectively [15].

Another study that examined FFQ validation with 144 urban, middle-aged, cancer screenees showed that the median (range) deattenuated correlation coefficients of 45 nutrients were 0.57 (0.23-0.89) for men and 0.47 (0.08-0.94) for women. The respective Spearman correlation coefficients for 43 food groups were 0.51 (0.10-0.98) for men and 0.51 (−0.36 to 0.88) for women [14]. Furthermore, a review of 21 FFQ developed for the Japanese population, including rural area populations, indicated that correlation coefficients between DR and FFQ for nutrient intake ranged from 0.31 to 0.56 [9].

We also observed a relatively high repeatability for the 3 intervals for almost all nutrients and food groups. A previous study among 844 men and 1074 women who participated in an annual health checkup in Aichi Prefecture examined the repeatability of a short FFQ for a 1-year interval [15,17]. The study showed that the median (range) Spearman correlation coefficients between the time points for energy, 24 nutrients, and 15 food groups were 0.66 (0.55-0.74) in men and 0.77 (0.69-0.84) in women. In addition, a review of 21 Japanese FFQ indicated that correlations of twice administration of the same FFQ at intervals of 9 to 12 months ranged from 0.50 to 0.72 [9].

Major large-cohort studies in Japan also developed FFQ and examined their validity and repeatability among rural residents. Median correlation coefficients to assess validity for energy and for major nutrients ranged from 0.32 to 0.43. Those to assess repeatability ranged from 0.24 to 0.50, those to assess validity for food intakes ranged from 0.28 to 0.42, and those to assess repeatability ranged from 0.34 to 0.48 [29–31]. The Prospective Urban and Rural Epidemiological study examined the reliability of an FFQ among both urban and rural areas in participating countries. They observed that the median correlation coefficients to assess validity for energy and major nutrients ranged from 0.45 to 0.56 in urban areas and from 0.08 to 0.52 in rural areas; those to assess 1-year repeatability ranged from 0.43 to 0.46 in urban areas and from 0.42 to 0.44 in rural areas [32–34]. In addition, this FFQ asked in detail about principal foods, including cereals, and about salt-intake-related dietary habits. Validations of carbohydrate and sodium intakes were relatively high compared with those for the other FFQ for Japanese populations (median correlation coefficients of carbohydrate and sodium intakes for 19 studies were 0.50 and 0.33, respectively) [9] and for urban populations in other countries [32–34]. It is a feature of this FFQ. Furthermore, we observed similar validities between FFQ1 and FFQ3 compared with the 28-day DR, excluding intakes for several nutrients and food groups, for example, β-carotene, vitamin C, and algae. However, these nutrient and food group intakes may be affected by seasonal variability [35], or may have small opportunity for each meal. Therefore, we did not observe educational bias in this study, and the reliability of this FFQ was reasonable compared with previous findings.

Table 5 – Nutrient intakes among the 3 FFQ

	Nutrient intakes			Spearman correlation coefficients					
	FFQ1	FFQ2	FFQ3	FFQ1 vs FFQ2		FFQ1 vs FFQ3		FFQ2 vs FFQ3	
				Crude	Energy adjusted	Crude	Energy adjusted	Crude	Energy adjusted
Energy (kcal)	1897 ± 328	1870 ± 322	1847 ± 300	0.76	–	0.53	–	0.49	–
Total protein (g)	74.8 ± 17.5	78.0 ± 16.9	76.6 ± 15.3	0.72	0.65	0.69	0.71	0.68	0.71
Plant protein (g)	35.4 ± 6.6	35.7 ± 7.9	35.7 ± 8.1	0.73	0.72	0.66	0.78	0.64	0.71
Animal protein (g)	39.5 ± 13.3	42.4 ± 12.6	40.9 ± 11.9	0.70	0.61	0.68	0.62	0.79	0.70
Total fat (g)	53.0 ± 15.9	55.1 ± 14.0	52.1 ± 12.8	0.69	0.69	0.64	0.71	0.65	0.61
Plant fat (g)	28.7 ± 9.7	28.3 ± 9.2	27.7 ± 8.5	0.66	0.69	0.63	0.63	0.51	0.52
Animal fat (g)	24.2 ± 8.9	26.7 ± 8.0	24.3 ± 7.3	0.69	0.60	0.71	0.77	0.72	0.72
Carbohydrate (g)	252.8 ± 51.0	240.3 ± 52.5	242.8 ± 43.8	0.79	0.74	0.61	0.75	0.58	0.83
Energy from protein (%energy)	15.8 ± 2.7	16.8 ± 2.6	16.7 ± 2.6	–	0.70	–	0.75	–	0.77
Energy from fat (%energy)	25.0 ± 5.8	26.6 ± 5.0	25.4 ± 4.8	–	0.71	–	0.74	–	0.74
Energy from carbohydrate (%energy)	53.7 ± 8.3	51.4 ± 7.0	52.9 ± 7.1	–	0.67	–	0.68	–	0.79
Sodium (mg)	4223 ± 1236	4130 ± 1114	3863 ± 996	0.43	0.40	0.40	0.32	0.34	0.36
Potassium (mg)	2431 ± 520	2495 ± 548	2501 ± 510	0.76	0.67	0.63	0.57	0.56	0.56
Calcium (mg)	721 ± 256	717 ± 242	719 ± 261	0.80	0.78	0.68	0.67	0.64	0.69
Calcium from milk and dairy products (mg)	267 ± 181	272 ± 168	262 ± 181	0.80	0.77	0.82	0.74	0.72	0.76
Magnesium (mg)	298 ± 72	307 ± 74	304 ± 73	0.80	0.71	0.68	0.70	0.65	0.61
Retinol (μg)	352 ± 340	396 ± 312	316 ± 224	0.77	0.75	0.69	0.75	0.68	0.66
β-Carotene (μg)	2940 ± 1027	2862 ± 1349	3005 ± 1061	0.59	0.54	0.29	0.25	0.33	0.33
Retinol equivalent (μg)	643 ± 370	682 ± 335	617 ± 265	0.68	0.73	0.60	0.66	0.57	0.58
Vitamin D (μg)	11.40 ± 6.48	12.42 ± 7.86	10.66 ± 5.19	0.64	0.57	0.60	0.58	0.73	0.57
α-Tocopherol (mg)	6.74 ± 1.63	6.80 ± 1.77	6.81 ± 1.47	0.60	0.61	0.65	0.59	0.58	0.57
Vitamin K (μg)	227 ± 66	231 ± 86	241 ± 90	0.69	0.70	0.59	0.56	0.71	0.70
Vitamin B_6 (mg)	1.31 ± 0.32	1.38 ± 0.32	1.32 ± 0.28	0.77	0.66	0.67	0.71	0.69	0.71
Vitamin B_{12} (μg)	8.67 ± 4.01	9.13 ± 3.26	8.21 ± 3.16	0.59	0.57	0.57	0.63	0.67	0.69
Folic acid (μg)	312 ± 85	319 ± 92	314 ± 81	0.67	0.69	0.53	0.47	0.45	0.43
Vitamin C (mg)	94.7 ± 34.4	95.7 ± 36.8	101.2 ± 25.2	0.64	0.61	0.38	0.38	0.35	0.39
Saturated fatty acids (g)	15.2 ± 5.1	15.7 ± 4.3	14.7 ± 4.2	0.71	0.73	0.71	0.83	0.66	0.76
Monounsaturated fatty acids (g)	17.2 ± 5.4	18.0 ± 4.8	16.7 ± 4.3	0.70	0.70	0.63	0.72	0.66	0.64
Polyunsaturated fatty acids (g)	13.5 ± 4.3	14.0 ± 4.1	13.6 ± 3.7	0.65	0.53	0.58	0.41	0.63	0.39
n-3 polyunsaturated fatty acids (g)	2.5 ± 1.0	2.7 ± 1.1	2.6 ± 0.8	0.62	0.51	0.57	0.42	0.70	0.45
n-6 polyunsaturated fatty acids (g)	10.9 ± 3.6	11.2 ± 3.3	11.0 ± 3.3	0.66	0.63	0.57	0.51	0.57	0.50
Cholesterol (mg)	291 ± 123	309 ± 108	299 ± 101	0.78	0.78	0.68	0.79	0.83	0.83
Dietary fiber (g)	12.5 ± 2.8	12.4 ± 3.5	12.4 ± 2.8	0.70	0.66	0.50	0.41	0.57	0.62
Soluble fiber (g)	2.8 ± 0.6	2.8 ± 0.9	2.8 ± 0.7	0.64	0.64	0.44	0.39	0.52	0.58
Insoluble fiber (g)	9.5 ± 2.2	9.4 ± 2.6	9.5 ± 2.1	0.68	0.64	0.50	0.44	0.60	0.62
Caffeine (g)	0.02 ± 0.02	0.02 ± 0.03	0.02 ± 0.04	0.65	0.41	0.61	0.42	0.46	0.31
Ethanol (g)	12.6 ± 21.7	11.4 ± 17.2	11.4 ± 18.6	0.83	0.85	0.92	0.93	0.88	0.87
Median				0.69	0.67	0.61	0.63	0.64	0.62

Nutrient intakes among the 3 FFQ are means ± SD.
Spearman correlation coefficients ≥0.27 ($P < .05$) and ≥0.36 ($P < .01$).

This present study was not designed to examine the repeatability for a 1-year interval. Most recent studies examined such repeatability because seasonal variation may modify the repeatability of an FFQ [28]. Furthermore, seasonal variations of dietary habits of Japanese were observed [35]. However, food preservation and distribution techniques in Japan have been developed since around 1985 [36,37]. Differences in availability and affordability of most foods could be small in all seasons. In addition, the repeatability of the FFQ for 3-, 6-, and 9-month intervals in the present study may be better compared with those of previous studies in Japan. Therefore, we presumed that the repeatability of this FFQ for 1 year could also be considered as reasonable.

This study also has limitations. The sample size was not sufficient for separate evaluation of the validity and repeatability for men and women. In addition, the validation measurements (Pearson or Spearman correlation coefficients) for men generally tended to be lower than those for women. The other limitation was that all of the participants of this study were not randomly selected; thus, there might be selection bias such as volunteer bias in our results. On the other hand, they completed the 28-day DR, and dietary information from the 28-day DR is one of the criterion standards [28]. Therefore, the effect of selection bias on the reliability of this FFQ may be weak.

In conclusion, this FFQ developed for a Japanese urban cohort to estimate habitual nutritional intakes has reasonable

Table 6 – Intakes for food groups among the 3 FFQ

	Food group intakes			Spearman correlation coefficients					
	FFQ1	FFQ2	FFQ3	FFQ1 vs FFQ2		FFQ1 vs FFQ3		FFQ2 vs FFQ3	
				Crude intake	Energy adjusted	Crude intake	Energy adjusted	Crude intake	Energy adjusted
Cereals (g)	415.5 ± 19.8	396.6 ± 132.9	406.9 ± 116.7	0.70	0.76	0.80	0.80	0.71	0.75
Pulses (g)	130.0 ± 52.3	149.5 ± 68.4	156.3 ± 78.4	0.44	0.42	0.42	0.38	0.65	0.61
Nuts and seeds (g)	5.5 ± 8.9	4.8 ± 6.6	5.0 ± 8.1	0.76	0.65	0.74	0.56	0.66	0.43
Vegetables (g)	214.0 ± 78.7	207.2 ± 105.6	217.5 ± 84.3	0.58	0.51	0.35	0.24	0.36	0.30
Fruits (g)	158.1 ± 98.3	163.0 ± 99.2	174.5 ± 67.4	0.64	0.51	0.46	0.48	0.63	0.67
Algae (g)	2.1 ± 1.2	2.2 ± 1.2	2.1 ± 1.4	0.61	0.58	0.58	0.49	0.45	0.40
Fishes and shellfishes (g)	84.7 ± 43.5	89.9 ± 45.1	81.9 ± 36.7	0.66	0.53	0.64	0.58	0.81	0.65
Meats (g)	45.4 ± 19.9	54.0 ± 18.7	49.1 ± 18.0	0.41	0.51	0.37	0.48	0.57	0.58
Eggs (g)	26.4 ± 17.1	28.6 ± 16.8	28.3 ± 14.8	0.77	0.73	0.66	0.72	0.77	0.76
Milk and dairy products (g)	227.0 ± 146.4	226.8 ± 131.4	216.4 ± 146.5	0.75	0.73	0.81	0.70	0.73	0.74
Confectioneries (g)	46.2 ± 32.8	40.5 ± 32.4	37.8 ± 27.2	0.74	0.67	0.70	0.71	0.74	0.73
Median				0.66	0.58	0.64	0.56	0.66	0.65

reliability. Therefore, this FFQ should be useful for evaluating the associations of nutritional intakes with cardiovascular diseases and their risk factors for Japanese urban populations.

Competing interests

The authors declare that they have no competing interests.

Acknowledgment

We thank the members of the Suita Medical Foundation and the Suita City Health Center. We also thank researchers and medical staff in the Department of Preventive Cardiology, National Cardiovascular Center, for performing medical examinations and follow-up. We also thank Satuki-Junyukai, the society members of the Suita study. This study was supported by Grants-in-Aid for Cancer Research and the Third-Term Comprehensive Ten-Year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare of Japan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nutres.2014.10.012.

REFERENCES

[1] Tzoulaki I, Patel CJ, Okamura T, Chan Q, Brown IJ, Miura K, et al. A nutrient-wide association study on blood pressure. Circulation 2012;126:2456–64.
[2] Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, et al. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. Circulation 2006;113: 195–202.
[3] Hu EA, Pan A, Malik V, Sun Q. White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review. BMJ 2012;344:e1454. http://dx.doi.org/10.1136/bmj.e1454.
[4] Yamori Y. Food factors for atherosclerosis prevention: Asian perspective derived from analyses of worldwide dietary biomarkers. Exp Clin Cardiol 2006;11:94–8.
[5] Noto H, Goto A, Tsujimoto T, Noda M. Low-carbohydrate diets and all-cause mortality: a systematic review and meta-analysis of observational studies. PLoS One 2013;8(1):e55030.
[6] Hu T, Bazzano LA. The low-carbohydrate diet and cardiovascular risk factors: evidence from epidemiologic studies. Nutr Metab Cardiovasc Dis 2014;24:337–43.
[7] Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. BMJ 2013;346:f1326.
[8] Kenkyukai Kenko Eiyo Joho. The National Health and Nutrition Survey in Japan, 2010. Daiichi-shuppan: Tokyo, Japan; 2013 [in Japanese].
[9] Wakai K. A review of food frequency questionnaires developed and validated in Japan. J Epidemiol 2009;19:1–11.
[10] Kitamura A, Sato S, Kiyama M, Imano H, Iso H, Okada T, et al. Trends in the incidence of coronary heart disease and stroke and their risk factors in Japan, 1964 to 2003: the Akita-Osaka study. J Am Coll Cardiol 2008;52:71–9.
[11] Kokubo Y, Kamide K, Okamura T, Watanabe M, Higashiyama A, Kawanishi K, et al. Impact of high-normal blood pressure on the risk of cardiovascular disease in a Japanese urban cohort. The Suita study. Hypertension 2008;52:652–9.
[12] Kokubo Y, Okamura T, Yoshimasa Y, Miyamoto Y, Kawanishi K, Kotani Y, et al. Impact of metabolic syndrome components on incidence of cardiovascular disease in a general urban Japanese population: the Suita study. Hypertens Res 2008;31: 2027–35.
[13] Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi S, et al. Absolute risk of cardiovascular disease and lipid management targets. J Atheroscler Thromb 2013;20:689–97.
[14] Takachi R, Ishihara J, Iwasaki M, Hosoi S, Ishii Y, Sasazuki S, et al. Validity of a self-administered food frequency questionnaire for middle-aged urban cancer screenees: comparison with 4-day weighed dietary records. J Epidemiol 2011;21:447–58.
[15] Tokudome S, Imaeda N, Tokudome Y, Fujiwara N, Nagaya T, Sato J, et al. Relative validity of a semi-quantitative food frequency questionnaire versus 28 day weighed diet records in Japanese female dietitians. Eur J Clin Nutr 2001;55:735–42.
[16] Tokudome Y, Goto C, Imaeda N, Hasegawa T, Kato R, Hirose K, et al. Relative validity of a short food frequency

questionnaire for assessing nutrient intake versus three-day weighed diet records in middle-aged Japanese. J Epidemiol 2005;15:135–45.
[17] Imaeda N, Goto C, Tokudome Y, Hirose K, Tajima K, Tokudome S. Reproducibility of a short food frequency questionnaire for Japanese general population. J Epidemiol 2007;17:100–7.
[18] Higashiyama A, Wakabayashi I, Ono Y, Watanabe M, Kokubo Y, Okayama A, et al. Association with serum gamma-glutamyltransferase levels and alcohol consumption on stroke and coronary artery disease: the Suita study. Stroke 2011;42:1764–7.
[19] Okamura T, Kokubo Y, Watanabe M, Higashiyama A, Ono Y, Nishimura K, et al. A revised definition of the metabolic syndrome predicts coronary artery disease and ischemic stroke after adjusting for low density lipoprotein cholesterol in a 13-year cohort study of Japanese: the Suita study. Atherosclerosis 2011;217:201–6.
[20] Watanabe M, Okamura T, Kokubo Y, Higashiyama A, Okayama A. Elevated serum creatine kinase predicts first-ever myocardial infarction: a 12-year population-based cohort study in Japan, the Suita study. Int J Epidemiol 2009;38:1571–9.
[21] Kokubo Y, Okamura T, Watanabe M, Higashiyama A, Ono Y, Miyamoto Y, et al. The combined impact of blood pressure category and glucose abnormality on the incidence of cardiovascular diseases in a Japanese urban cohort: the Suita study. Hypertens Res 2010;33:1238–43.
[22] Ishihara J, Sobue T, Yamamoto S, Yoshimi I, Sasaki S, Kobayashi M, et al. Validity and reproducibility of a self-administered food frequency questionnaire in the JPHC Study Cohort II: study design, participant profile and results in comparison with Cohort I. J Epidemiol 2003;13(1 Suppl):S134–47.
[23] Tsugane S, Sasaki S, Kobayashi M, Tsubono Y, Akabane M, JPHC. Validity and reproducibility of the self-administered food frequency questionnaire in the JPHC Study Cohort I: study design, conduct and participant profiles. J Epidemiol 2003;13(1 Suppl):S2–S12.
[24] Science and Technology Agency. Standard Tables of Food Composition in Japan Fifth Revised and Enlarged Edition. Tokyo: Printing Bureau, Ministry of Finance; 2005 [in Japanese].
[25] Mineharu Y, Koizumi A, Wada Y, Iso H, Watanabe Y, Date C, et al. Coffee, green tea, black tea and oolong tea consumption and risk of mortality from cardiovascular disease in Japanese men and women. J Epidemiol Community Health 2011;65:230–40.
[26] Umesawa M, Iso H, Ishihara J, Saito I, Kokubo Y, Inoue M, et al. Dietary calcium intake and risks of stroke, its subtypes, and coronary heart disease in Japanese: the JPHC Study Cohort I. Stroke 2008;39:2449–56.
[27] Umesawa M, Sato S, Imano H, Kitamura A, Shimamoto T, Yamagishi K, et al. Relations between protein intake and blood pressure in Japanese men and women: the Circulatory Risk in Communities Study (CIRCS). Am J Clin Nutr 2009;90:377–84.
[28] Willet WC. Nutritional epidemiology. 2nd ed. New York, NY: Oxford University Press; 1998.
[29] Date C, Fukui M, Yamamoto A, Wakai K, Ozeki A, Motohashi Y, et al. Reproducibility and validity of a self-administered food frequency questionnaire used in the JACC study. J Epidemiol 2005;15(Suppl 1):S9–S23.
[30] Ogawa K, Tsubono Y, Nishino Y, Watanabe Y, Ohkubo T, Watanabe T, et al. Validation of a food-frequency questionnaire for cohort studies in rural Japan. Public Health Nutr 2003;6: 147–57.
[31] Tsubono Y, Kobayashi M, Sasaki S, Tsugane S, JPHC. Validity and reproducibility of a self-administered food frequency questionnaire used in the baseline survey of the JPHC Study Cohort I. J Epidemiol 2003;13(1 Suppl):S125–33.
[32] Dehghan M, Ilow R, Zatonska K, Szuba A, Zhang X, Mente A, et al. Development, reproducibility and validity of the food frequency questionnaire in the Poland arm of the Prospective Urban and Rural Epidemiological (PURE) study. J Hum Nutr Diet 2012;25:225–32.
[33] Dehghan M, López Jaramillo P, Dueñas R, Anaya LL, Garcia RG, Zhang X, et al. Development and validation of a quantitative food frequency questionnaire among rural- and urban-dwelling adults in Colombia. J Nutr Educ Behav 2012;44:609–13.
[34] Dehghan M, del Cerro S, Zhang X, Cuneo JM, Linetzky B, Diaz R, et al. Validation of a semi-quantitative Food Frequency Questionnaire for Argentinean adults. PLoS One 2012;7(5): e37958.
[35] Sasaki S, Takahashi T, Iitoi Y, Iwase Y, Kobayashi M, Ishihara J, et al. Food and nutrient intakes assessed with dietary records for the validation study of a self-administered food frequency questionnaire in JPHC Study Cohort I. J Epidemiol 2003;13(1 Suppl):S23–50.
[36] White paper on economics. Economic Planning Agency of Japan; 1987 [in Japanese].
[37] White paper on the National Lifestyle. Economic Planning Agency of Japan; 1987 [in Japanese].

Original Article

The Relationship between Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Ligands Containing Apolipoprotein B and the Cardio-Ankle Vascular Index in Healthy Community Inhabitants: The KOBE Study

Daisuke Sugiyama[1,2], Aya Higashiyama[2,3,4], Ichiro Wakabayashi[3], Yoshimi Kubota[2,3], Yoshiko Adachi[3], Akiko Hayashibe[3], Kuniko Kawamura[2], Kazuyo Kuwabara[1], Kunihiro Nishimura[2,4], Aya Kadota[2,5], Yoko Nishida[2], Takumi Hirata[2], Hironori Imano[2,6], Naomi Miyamatsu[2,7], Yoshihiro Miyamoto[2,4], Tatsuya Sawamura[8] and Tomonori Okamura[1,2]

[1]Department of Preventive Medicine and Public Health, School of Medicine, Keio University, Tokyo, Japan
[2]Foundation for Biomedical Research and Innovation, Kobe, Japan
[3]Department of Environmental and Preventive Medicine, Hyogo College of Medicine, Hyogo, Japan
[4]Department of Preventive Medicine and Epidemiologic Informatics, National Cerebral and Cardiovascular Center, Osaka, Japan
[5]Center for Epidemiologic Research in Asia/Department of Public Health, Shiga University of Medical Science, Shiga, Japan
[6]Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan
[7]Department of Clinical Nursing, Shiga University of Medical Science, Shiga, Japan
[8]Department of Vascular Physiology, National Cerebral and Cardiovascular Center, Osaka, Japan

Aims: Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 ligands containing apolipoprotein B (LAB) and lectin-like oxidized LDL receptor-1 (LOX-1) are known as LOX-1-related modified LDL indicators. These indicators play an important role in the early phase atherosclerosis, but the relationship between these indicators and subclinical atherosclerosis, as represented by the cardio-ankle vascular index (CAVI), has not been assessed. We herein investigated the association of LOX-1-related modified LDL indicators and the CAVI in healthy, Japanese urban community inhabitants who were considered to be at low risk for cardiovascular disease (CVD).

Methods: The participants were 515 healthy Japanese (310 men and 205 women) without a history of CVD, cancer or the use of medication for hypertension, diabetes or dyslipidaemia. To estimate the association between LOX-1-related modified LDL indicators (LAB, soluble form of LOX-1 (sLOX-1)) and the CAVI, we performed multivariable linear regression analyses with possible confounders such as the serum LDL cholesterol level.

Results: The plasma LAB showed a positive association with the CAVI in men (standardized coefficient: 0.11, $p=0.04$). This relationship was not observed in women. On the other hand, no clear association was observed between the CAVI and the plasma sLOX-1 level in either sex.

Conclusions: The plasma LAB levels may represent a useful marker for detecting potential atherosclerosis in healthy individuals considered to be at low risk for atherosclerosis and CVD. Further studies are needed to confirm the present findings.

J Atheroscler Thromb, 2015; 22: 499-508.

Key words: Lectin-like oxidized low-density lipoprotein receptor-1, LOX-1 ligand containing ApoB, Cardio-ankle vascular index, Community-based study

Introduction

Endothelial dysfunction is currently considered to be an early phase in the development of atherosclerosis; the oxidative modification of low-density lipoprotein (LDL) is considered to play a key role in endothelial dysfunction[1]. Lectin-like oxidative LDL receptor-1 (LOX-1) is the receptor for oxidative and/

or modified LDL in endothelial cells[2], and both LOX-1 and its ligands containing apolipoprotein B (LAB) are involved in endothelial dysfunction[3, 4]. Because a specific ELISA assay for LAB using a monoclonal anti-ApoB antibody can recognize ApoB-48 and ApoB-100, as well as recombinant LOX-1[5], this assay can measure the biological activity of ApoB-containing lipoprotein based on its binding to LOX-1. This parameter reflects the atherogenicity of whole modified LDL better than one specific antigenic determinant of oxidized LDL[6, 7]. Therefore, this ELISA assay is thought to be suitable for evaluating the biologic activity of atherogenic lipoproteins. In fact, recent studies have shown that the soluble form of LOX-1 (sLOX-1) and LAB both are possible biomarkers of not only the risk of cardiovascular disease (CVD), but also subclinical atherosclerotic disease[8-11].

On the other hand, since pathological studies have shown that atherosclerosis of the aorta preceded effects in other organs[12, 13], the cardio-ankle vascular index (CAVI) is considered to be a useful screening tool for subclinical atherosclerosis[14, 15]. The CAVI is also a novel arterial stiffness parameter[16, 17] that is associated with CVD[18-20]. In a recent clinical report, the serum LAB was associated with an increased intima-media thickness (IMT) in Caucasian men in the US, but not in Japanese men[11]. Since the absolute risk for CVD in Japanese men is lower than that in men in the US[21], a more sensitive measurement, such as the CAVI, may be more suitable for detecting potential atherosclerosis in Japanese people.

Accordingly, we conducted this cross-sectional study to investigate the association between LOX-1-related modified LDL indicators (LAB, sLOX-1) and the CAVI in Japanese urban community inhabitants considered to be healthy and at lower risk for CVD than the general population.

Methods

Study Participants

This study is based on data from the baseline survey of the Kobe Orthopedic and Biomedical Epidemiological study (the KOBE study). The KOBE study is a population-based cohort study; one of its endpoints is the incidence of lifestyle-related diseases such as hypertension, diabetes mellitus and dyslipidaemia. All study participants were volunteers, who resided in Kobe City (one of the major cities in the Kansai area, which is the second largest urban area in Japan). The subjects ranged in age from 40-74 years old. The KOBE participants had to meet the following criteria: 1) no current medication use for hypertension, diabetes mellitus or dyslipidaemia; and 2) no history of CVD or cancer. The details of the KOBE study were reported elsewhere[22]. Informed consent was obtained from each participant in writing. As part of the baseline survey of this cohort study, the CAVI was assessed in 549 individuals from July 2010 to December 2011. Of these, 17 participants were excluded; 16 had incomplete data and one had a high triglyceride level (≥400 mg/dL). Seventeen additional participants were excluded because they had a history of using medication for hypertension, diabetes mellitus or dyslipidaemia after the recruiting process. The remaining 515 individuals (310 men and 205 women) were included in this study. The present study was approved by the Ethics Committee at the Institute of Biomedical Research and Innovation.

Data Collection and Standardization

The study participants were asked to respond to questionnaires about lifestyle-related factors, such as the use of medications, smoking (current smoker or not) and alcohol consumption (current drinker or not). The body mass index (BMI) was calculated as the weight (kg) divided by the height squared (m^2). After a five minute rest period, the blood pressure was measured twice in each participant using an automatic sphygmomanometer (BP-103i II; Nihon Colin, Tokyo, Japan), and the mean value for each participant was recorded.

Blood samples were obtained from all participants after they had fasted for at least 10 h, and blood samples were tested in the commissioned clinical laboratory centre (SRL Inc., Tokyo, Japan). The haemoglobin A1c (HbA1c) level was measured by the latex coagulating method. Enzymatic methods were used to measure the serum total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride levels. The LDL cholesterol (LDL-C) level was calculated by Friedewald's formula. The serum high sensitivity C-reactive protein (hs-CRP, mg/L) level was measured by a BN II nephelometer (Dade Behring, Deerfield, IL, USA).

The plasma LAB levels were measured using an enzyme-linked immunosorbent assay (ELISA)[5]. The intra- and inter-assay coefficients of variance (CV) for LAB were 2.2% and 13.1%, respectively (n=26). The

Address for correspondence: Daisuke Sugiyama, Department of Preventive Medicine and Public Health, School of Medicine, Keio University 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
E-mail: dsugiyama@z8.keio.jp
Received: June 9, 2014
Accepted for publication: September 28, 2014

Table 1. The characteristics of the study participants

	Men	Women
Number of participants	310	205
Age (years)	61 ± 9	63 ± 8
BMI (kg/m^2)	23 ± 3	21 ± 2
SBP (mmHg)	123 ± 17	117 ± 18
DBP (mmHg)	78 ± 10	71 ± 10
HbA1c (%)	5.2 ± 0.5	5.2 ± 0.3
TC (mg/dL)	204 ± 28	226 ± 32
HDL-C (mg/dL)	61 ± 14	72 ± 15
TG (mg/dL)	88 (63, 120)	74 (56, 96)
LDL-C (mg/dL)	122 ± 27	137 ± 29
HR (beat/min)	58 ± 9	61 ± 8
Current smoker (%)	10	1.5
Current drinker (%)	76.8	33.2
hs-CRP (mg/L)	0.29 (0.14, 0.54)	0.24 (0.10, 0.55)
LAB (μg/L)	22700 (18090, 30470)	24660 (18090, 32240)
sLOX-1 (ng/L)	113 (93, 141)	116 (100, 140)
CAVI	8.0 ± 0.9	7.9 ± 0.9

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, TC: total cholesterol, HDL-C; high-density lipoprotein cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein cholesterol, HR: heart rate, hs-CRP: high sensitivity C-reactive protein, LAB: lectin-like oxidized LDL receptor-1 (LOX-1) ligand containing apolipoprotein B, sLOX-1: soluble form of LOX-1, CAVI: cardio-ankle vascular index
The values were expressed as the means ± standard deviation (SD) or medians (interquartile ranges) for continuous variables, and as percentages for categorical variables.

plasma sLOX-1 levels were measured by a sandwich ELISA using two types of monoclonal antibodies against the extracellular domain of LOX-1[10]. The blood collection tubes for LAB and sLOX-1 were identical to those used for blood glucose; the tubes contained sodium fluoride, heparin sodium and ethylenediaminetetraacetic acid (EDTA-2Na). The intra- and inter-assay CVs for sLOX-1 were 2.4% and 7.8%, respectively (n = 26).

The CAVI was measured using a VaSera CAVI instrument (Fukuda Denshi Co. Ltd., Tokyo, Japan). After a five minute rest, the CAVI was assessed, and the heart rate was measured simultaneously. The CAVI was calculated using the formula below:

$$CAVI = a\ \{(2\rho/\Delta P) \times \ln (Ps/Pd)\ PWV^2\} + b$$

where Ps is the systolic blood pressure, Pd is the diastolic blood pressure, PWV is the pulse wave velocity, ΔP is Ps - Pd, ρ is the blood density and a and b are constants. The VaSera is equipped with both measurement and calculation systems, which allowed for automatic calculation of the CAVI.

Statistical Analysis

Sex-specific analyses were performed. The basic characteristics were expressed as the means ± standard deviation (SD) or medians (and interquartile ranges) for continuous variables, and as percentages for categorical variables. The correlation between the LAB and sLOX-1 (both log-transformed) was estimated by Pearson's product-moment correlation in each sex. The sex-specific CAVI was also compared by tertiles of the LAB or sLOX-1 using an analysis of covariance after adjusting for age, BMI, systolic blood pressure, heart rate, HbA1c, current smoking status and current alcohol consumption (drinker or not).

Univariable linear regression analyses were performed to determine the relationships between the CAVI and the variables used in multivariable models (LAB, sLOX-1, age, BMI, systolic blood pressure, heart rate, HbA1c, current smoker or not, current alcohol drinker or not, LDL-C, hs-CRP and HDL-C). The LAB, sLOX-1 and hs-CRP were log-transformed in all of the regression analyses. Multiple adjustments were also performed with linear regression models to estimate the association between LAB and sLOX-1 and the CAVI.

Model 1 included the age, BMI, systolic blood pressure, heart rate, HbA1c, current smoking status and current alcohol consumption. Model 2 included

Table 2. The results of the univariable linear regression analyses for the relationship between the CAVI and the variables used in the multivariable models

	Men			Women		
	Coefficient	95%CI	*p* value	Coefficient	95%CI	*p* value
Ln LAB	0.525	0.260 to 0.790	<0.001	0.112	−0.159 to 0.383	0.415
Ln sLOX-1	0.212	−0.070 to 0.493	0.140	−0.133	−0.397 to 0.131	0.322
Age	0.061	0.052 to 0.071	<0.001	0.067	0.054 to 0.079	<0.001
BMI	−0.023	−0.062 to 0.017	0.258	−0.048	−0.098 to 0.001	0.056
SBP	0.015	0.009 to 0.021	<0.001	0.017	0.010 to 0.024	<0.001
HR	0.014	0.003 to 0.026	0.017	0.017	0.002 to 0.032	0.024
HbA1c	0.420	0.228 to 0.612	<0.001	0.556	0.182 to 0.930	0.004
Current smoker	0.235	−0.586 to 0.115	0.188	−0.845	−1.861 to 0.171	0.103
Current drinker	−0.170	−0.419 to 0.079	0.180	−0.114	−0.375 to 0.146	0.388
LDL-C	0.001	−0.003 to 0.005	0.616	0.003	−0.002 to 0.007	0.209
Ln hs-CRP	0.186	0.090 to 0.281	<0.001	0.168	0.072 to 0.263	0.001
HDL-C	−0.008	−0.015 to 0.000	0.037	−0.009	−0.017 to −0.001	0.026

CAVI: cardio-ankle vascular index, 95%CI; 95% confidence interval, LAB; lectin-like oxidized LDL receptor 1(LOX-1) ligand containing apolipoprotein B, sLOX-1; soluble form of LOX-1, BMI: body mass index, SBP: systolic blood pressure, HR: heart rate, LDL-C: low density lipoprotein cholesterol, hs-CRP: high sensitivity C-reactive protein, HDL-C; high density lipoprotein cholesterol

the variables in Model 1, plus the serum LDL-C levels. In Model 3, the hs-CRP was added to the variables in Model 2. Model 4 included all of the variables in Model 3, as well as the HDL-C. The adjusted coefficient of determination (adjusted R-squared) was also calculated for each model.

To evaluate the collinearity between variables, especially the LDL-C and LAB or sLOX-1 in regression models, we estimated the variance inflation factor (VIF) in each Model. If the estimated VIF for one variable is over 10, there is strong possibility of the existence of collinieariy[23]. All statistical analyses were performed using the R version 3.0.1 software program (R Foundation for Statistical Computing, Vienna, Austria). The significance level was set at $p<0.05$, and all statistical tests were two-tailed.

Results

Table 1 shows the characteristics of study participants. The mean age was 61 ± 9 years in men and 63 ± 8 years in women. The median LAB (μg/L) was 22,700 (18,090, 30,470) in men and 24,660 (18,090, 32,240) in women. The median sLOX-1 (ng/L) was 113 (93, 141) in men and 116 (100, 140) in women. Neither the LAB nor sLOX-1 was significantly different between the genders. The correlations between the LAB and sLOX-1 were not significant in either gender ($r=0.04$, $p=0.47$ in men and $r=0.05$, $p=0.51$ in women). The mean blood pressure and HbA1c did not meet the criteria for hypertension or diabetes. The mean LDL-C levels in women were higher than those in men, but did not reach the abnormal range. The current smoking rate was 10.0% in men and 1.5% in women, and the current drinking rate was 76.8% in men and 33.2% in women. The mean CAVI was 8.0 ± 0.9. The CAVI values did not differ significantly between men and women (8.0 ± 0.9 and 7.9 ± 0.9, respectively).

The results of the univariable linear regression analyses for the relationship between the CAVI and the variables used in the multivariable models were presented in **Table 2**. There was a significant positive association between the LAB and CAVI in men, but not in women. On the other hand, there were no associations between the sLOX-1 (log-transformed) and CAVI in either men or women.

The associations between the LAB (log-transformed) and CAVI in the multivariable linear regression models are presented in **Table 3**. After stratification by sex, statistically significant positive associations were shown in all three models for men. The coefficients or standardized coefficients in all models for men were not very different. In contrast, no associations were observed in any of the models for women. The plasma sLOX-1 (log-transformed) was also not associated with the CAVI in any model of the sex-specific analyses (**Table 4**). The serum LDL-C levels were not significantly associated with the CAVI in any of the results for model 2. Even if hs-CRP was added as an independent variable in the multivariable linear regression models, the results remained unchanged

Table 3. The results of the multivariable linear regression analyses for the relationship between the LAB and the CAVI

		Coefficient	95%CI	Standardized Coefficient	*p* value	VIF
Men						
Model 1	Ln LAB	0.232	0.008 to 0.456	0.096	0.042	1.120
			Adjusted R-squared: 0.389			
Model 2	Ln LAB	0.255	0.005 to 0.506	0.106	0.046	1.398
	LDL-C	−0.001	−0.004 to 0.003	−0.021	0.685	1.325
			Adjusted R-squared: 0.387			
Model 3	Ln LAB	0.264	0.015 to 0.513	0.109	0.038	1.399
	LDL-C	0.000	−0.004 to 0.002	−0.029	0.572	1.333
	Ln hs-CRP	0.087	0.007 to 0.168	0.100	0.033	1.121
			Adjusted R-squared: 0.395			
Model 4	Ln LAB	0.247	−0.004 to 0.498	0.102	0.053	1.420
	LDL-C	−0.001	−0.005 to 0.002	−0.031	0.550	1.334
	Ln hs-CRP	0.081	0.000 to 0.162	0.093	0.051	1.146
	HDL-C	−0.004	−0.011 to 0.003	−0.055	0.290	1.378
			Adjusted R-squared: 0.395			
Women						
Model 1	Ln LAB	0.038	−0.177 to 0.253	0.019	0.727	1.092
			Adjusted R-squared: 0.424			
Model 2	Ln LAB	0.008	−0.235 to 0.251	0.004	0.949	1.392
	LDL-C	0.001	−0.003 to 0.005	0.034	0.598	1.487
			Adjusted R-squared: 0.422			
Model 3	Ln LAB	−0.001	−0.242 to 0.240	−0.001	0.993	1.393
	LDL-C	0.001	−0.003 to 0.005	0.042	0.512	1.491
	Ln hs-CRP	0.092	0.011 to 0.172	0.129	0.026	1.184
			Adjusted R-squared: 0.434			
Model 4	Ln LAB	−0.081	−0.326 to 0.164	−0.041	0.516	1.488
	LDL-C	0.002	−0.002 to 0.006	0.052	0.416	1.496
	Ln hs-CRP	0.091	0.012 to 0.170	0.129	0.024	1.184
	HDL-C	−0.008	−0.015 to −0.002	−0.142	0.011	1.153
			Adjusted R-squared: 0.449			

95%CI: 95% confidence interval, LAB: lectin-like oxidized LDL receptor-1 ligand containing apolipoprotein B, CAVI: cardio-ankle vascular index, LDL-C: low-density lipoprotein cholesterol, hs-CRP: high sensitivity C-reactive protein, HDL-C; high density lipoprotein cholesterol
Variables for Model 1: age, body mass index, systolic blood pressure, heart rate, HbA1c, current smoker or not, current alcohol drinker or not and LAB (log-transformed).
Variables for Model 2: Model 1 + LDL-C
Variables for Model 3: Model 2 + hs-CRP
Variables for Model 4: Model 3 + HDL-C

(Model 3 in **Table 3** and **Table 4**). Moreover, these results were also similar when the serum HDL-C levels were added to both linear regressions (Model 4 in **Tables 3** and **4**).

The multivariable adjusted CAVI levels tended to be higher according to LAB tertile in men, but this trend was not significant ($p=0.15$, ANCOVA, **Fig. 1**). The estimated VIFs indicated that there was little evidence for the existence of collinearity. The estimated VIFs for LAB, sLOX-1, LDL-C, hs-CRP and HDL-C are shown in **Tables 3** and **4**. In addition, the VIFs for the other variables were not over 1.5 (data not shown).