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.

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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.

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High-density Lipoprotein Subclasses and Risk of Stroke and its Subtypes in Japanese Population

The Circulatory Risk in Communities Study

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Background and Purpose—High-density lipoprotein (HDL) cholesterol is an established protective factor for ischemic stroke. However, the contribution of HDL subclasses to stroke risk and its subtypes is uncertain.

Methods—A prospective nested case–control study of 40- to 85-year-old Japanese was undertaken using frozen serum samples collected from 5280 men and 7524 women. They participated in cardiovascular risk surveys from 1985 to 1999 (1 community) and 1989 to 1998 (2 communities) under Circulatory Risk in Communities Study. HDL cholesterol subclasses were classified by high-performance liquid chromatography into 3 subgroups: S-HDL (very small or small HDL), M-HDL (medium HDL), and L-HDL (large or very large HDL) cholesterol. One control subject per case was matched by sex, age, community, serum storage year, and fasting status.

Results—In 2005, we identified 241 strokes (155 ischemic and 86 hemorrhagic). S-HDL and M-HDL cholesterol levels were inversely associated with total stroke risk, ischemic stroke, specifically lacunar infarction, and hemorrhagic stroke. After adjustment for cardiovascular risk factors, these associations remained statistically significant. Multivariable conditional odds ratios (95% confidence interval) for 1 SD (0.12 mmol/L) increment of S-HDL cholesterol levels were 0.34 (0.23–0.52) for total stroke, 0.38 (0.23–0.63) for ischemic stroke, 0.33 (0.18–0.61) for lacunar infarction, 0.30 (0.14–0.65) for hemorrhagic stroke, and 0.30 (0.12–0.77) for intraparenchymal hemorrhage. The respective multivariable odds ratios for 1SD (0.10 mmol/L) increment of M-HDL cholesterol levels were 0.56 (0.41–0.75), 0.63 (0.45–0.88), 0.59 (0.40–0.87), 0.41 (0.21–0.80), and 0.38 (0.16–0.90). No associations were found between L-HDL cholesterol levels and risk of total stroke and its subtypes.

Conclusions—Small- to medium-sized HDL, not large HDL, cholesterol levels were inversely associated with total stroke risk. **(*Stroke*. 2013;44:327-333.)**

Key Words: high-density lipoprotein cholesterol ■ Japanese ■ nested case–control study ■ particle size ■ stroke

High-density lipoprotein (HDL) particles are heterogeneous in structure, having differential effect on their antiatherogenic properties.[1] Small, dense HDL particles display higher cholesterol efflux capacity,[2] potent protection for low-density lipoprotein (LDL) oxidation[3,4], and possess stronger anti-inflammatory properties than large HDL particles.[5]

Lipoprotein subclasses were quantified by gradient gel electrophoresis and nuclear magnetic resonance methods, the findings on the associations of HDL subclasses and cardiovascular disease have been inconsistent. Case–control studies using the gradient gel electrophoresis method reported that small HDL particles were inversely associated with the progression of coronary atherosclerosis[6] and risk of coronary heart disease,[7,8] and other studies showed opposite trends with prevalence of carotid atherosclerosis[9] and ischemic stroke risk.[10] In addition, studies using nuclear magnetic resonance observed that only large HDL particles, not small HDL or medium HDL particles, were inversely associated with risk of cardiovascular disease,[11] whereas another study with the nuclear magnetic resonance method showed that larger HDL particles were inversely associated, and smaller HDL particles

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were positively associated with the prevalence of coronary artery disease.[12]

High-performance liquid chromatography (HPLC) with gel permeation columns is an alternative method for classifying and quantifying lipoproteins according to particle sizes.[13] This method can provide cholesterol levels of major lipoproteins and their subclasses using a small amount of serum or plasma and measure simultaneously cholesterol levels in each lipoprotein fraction and lipoprotein particle size distribution. The HPLC defines 5 HDL subclasses based on HDL particle diameter size, which is similar to gradient gel electrophoresis and nuclear magnetic resonance methods.[13,14] Advantages of the HPLC method include its direct cholesterol determination in HDL and HDL subclasses within 16 minutes by using a small amount of plasma (<10 μL).[13,15]

In the present study, a prospective nested case–control study of men and women was conducted in 3 Japanese communities of the Circulatory Risk in Communities Study (CIRCS) using stored serum samples. We applied the HPLC method to assess HDL subclasses and to seek their associations with risk of stroke and its subtypes.

Methods

Surveyed Populations

The present study was an ancillary study of the CIRCS.[16] CIRCS is a dynamic cohort of Japanese men and women aged ≥30 years in 5 communities across Japan, overseen by a research team from the Osaka Medical Center for Health Science and Promotion, Osaka University and the University of Tsukuba. The surveyed populations comprised 13 314 men and women aged 40 to 85 years, who participated in cardiovascular risk surveys between 1985 and 2000 in a mideastern rural community (Kyowa; participants and census population for 40–85 years; n=6829 and n=8557, respectively) and between 1989 and 1998 in northeastern rural community (Ikawa; n=2570 and n=2981, respectively) and a southwest rural community (Noichi; n=3915 and n=7169, respectively). The participation rate in cardiovascular risk surveys among men and women aged 40 to 85 years was 80% in Kyowa, 86% in Ikawa, 55% in Noichi, and 71% for the total population. A 1.0- to 2.0-mL serum sample obtained from each participant was stored at −80°C for 1 to 20 years (median, 10.5 years). Participants with a history of stroke or coronary heart disease (n=510) were excluded from the analyses. The participants were followed up to determine the incidence of stroke occurring by the end of 2005. The Ethics Committee of Osaka University, The University of Tsukuba and the Osaka Medical Center for Health Science and Promotion approved this study.

Surveillance of Stroke and Classification of Stroke Subtypes

Susceptible cases of stroke were ascertained from national insurance claims, ambulance records, death certificates (cases with stroke as the underlying cause of death [International Classification of Diseases, 9th revision: 430–438] were selected), reports by local physicians, and reports by public health nurses and volunteers. To confirm the diagnosis of stroke, we called, visited, or invited the susceptible subjects to participate in annual cardiovascular risk surveys to obtain clinical histories. In addition, physicians obtained medical histories and reviewed medical records, including computed tomograpy/magnetic resonance imaging from local clinics and hospitals. In the case of deaths, histories were obtained from families, and medical records were reviewed.

The diagnosis of stroke was made according to the criteria of the National Survey of Stroke,[17] which requires a constellation of neurological deficits of sudden or rapid onset lasting ≥24 hours or until death. Strokes were classified as intraparenchymal hemorrhage, subarachnoid hemorrhage, or ischemic stroke (lacunar infarction, large-artery occlusive infarction, and embolic infarction) by computed tomograpy/magnetic resonance imaging using standardized criteria.[18] Strokes with negative findings on imaging studies and unclassified strokes were excluded. For each new case of stroke, 1 control subject was selected randomly from the participants with no incident stroke, matched for sex, age (±2 years), community, year of serum storage, and fasting status at serum collection (<8 and ≥8 hours).

Determination of HDL Particle Size

Nonfasting venous blood was collected in 7- to 10-mL plain tubes and allowed to stand for 30 minutes for serum separation. The serum samples were aliquoted immediately and placed on dry ice at survey sites and then stored at −80°C.

Serum lipoprotein analyses were performed by HPLC with gel permeation columns (LipoSEARCH; Skylight-Biotec, Inc., Akita, Japan).[15] By this method, HDL was classified by particle size into 5 subgroups: 13.5 to 15.0 nm (very large HDL), 12.1 nm (large HDL), 10.9 nm (medium HDL), 9.8 nm (small HDL), and 7.6 to 8.8 nm (very small HDL).[13] To simplify data analysis, we grouped these HDL subclasses as follows: S-HDL (very small or small HDL), M-HDL (medium HDL), and L-HDL (large or very large HDL).

Statistical Analysis

The odds ratios and 95% confidence intervals for total stroke and stroke subtype were estimated according to quartiles and 1SD increment of total HDL, S-HDL, M-HDL, and L-HDL cholesterol levels with conditional logistic regression models. Adjustment was made for hypertension status (normal, borderline, and hypertension), body mass index (kg/m^2), current alcohol intake (g/d), cigarette smoking status (never, ex-smoker, and current), cholesterol-lowering medication (yes/no), log-transformed triglycerides levels (mmol/L), and serum glucose category (normal, impaired glucose tolerance, and diabetes mellitus). SAS version 9.1.3 was used for the statistical analyses (2-tailed).

Results

Age-adjusted baseline characteristics of the controls according to quartiles of HDL subclasses are shown in Table 1. Body mass index was inversely associated with total HDL cholesterol and L-HDL, and the prevalence of current smokers was lower with the higher quartiles of L-HDL cholesterol levels. Total HDL cholesterol levels were positively associated with S-HDL, M-HDL, and L-HDL cholesterol levels, whereas triglycerides were inversely associated with total HDL cholesterol, S-HDL, M-HDL, and L-HDL cholesterol levels. Mean blood pressure, mean ethanol intake, and prevalence of hypertensive and glucose abnormality did not vary according to total HDL cholesterol, S-HDL, M-HDL, and L-HDL cholesterol levels. The prevalence of diabetes mellitus was lower with the higher quartiles of total HDL and L-HDL cholesterol levels.

During the follow-up period, we identified 241 incident strokes comprising 155 ischemic strokes (116 lacunar infarctions, 35 large-artery occlusive infarctions, and 11 embolic infarctions) and 86 hemorrhagic strokes (64 intraparenchymal hemorrhages and 22 subarachnoid hemorrhages).

Table 2 shows odd ratios and 95% confidence intervals for total stroke and stroke subtypes according to the quartiles and 1SD increment of total HDL, S-HDL, M-HDL, and L-HDL cholesterol levels. We did not show the results for large-artery occlusive infarction, embolic infarctions, and subarachnoid hemorrhage because of small incidence numbers. Total HDL cholesterol levels were inversely associated

Table 1. Age-adjusted Baseline Characteristics of Control Subjects According to Quartiles of High-density Lipoprotein Cholesterol Levels by High-density Lipoprotein Subclass

	Total HDL cholesterol, mg/dL					L-HDL, mg/dL					M-HDL, mg/dL					S-HDL, mg/dL				
	13.1–36.0	36.5–44.9	45.0–53.7	54.0–87.8	*P* for trend	5.1–12.1	12.1–17.1	17.1–24.2	24.3–51.6	*P* for trend	1.9–9.1	9.2–11.6	11.9–14.4	14.4–21.5	*P* for trend	3.9–11.7	11.7–14.6	14.7–17.9	17.9–39.6	*P* for trend
No. of controls	51	58	67	65		65	53	66	57		52	54	66	69		45	57	68	71	
Age, y	66	67	65	66	0.56	66	67	65	65	0.56	67	68	65	64	0.03	67	65	66	66	0.87
Men, %	55	52	47	51	0.74	57	50	44	54	0.58	47	51	57	48	0.78	40	56	56	49	0.61
Systolic BP, mm Hg	135	137	134	132	0.25	135	138	133	132	0.21	132	135	138	133	0.54	134	136	134	134	0.83
Diastolic BP, mm Hg	79	79	78	77	0.34	80	78	76	79	0.36	78	77	80	77	0.84	77	80	79	78	0.80
Hypertension, %	39	42	34	37	0.71	41	44	34	32	0.33	37	37	36	41	0.64	35	39	35	41	0.91
Body mass index, kg/m^2	23.9	23.8	23.0	22.8	0.03	24.2	24.0	23.3	21.6	<0.001	22.8	22.9	23.7	23.6	0.07	23.0	23.1	22.9	24.0	0.12
Ethanol intake, g/d	13.2	11.7	11.3	14.7	0.73	16.1	9.7	10.9	13.9	0.64	10.7	11.3	10.7	17.4	0.10	9.6	13.1	11.0	16.1	0.15
Current smokers, %	38	30	19	23	0.07	48	20	24	12	0.001	28	25	27	26	0.94	6	18	16	18	0.57
Cholesterol-lowering medication, %	4	3	5	0	0.23	5	6	2	0	0.96	2	2	5	3	0.78	2	2	4	3	0.85
Total HDL cholesterol, mmol/L	0.74	1.05	1.26	1.61	<0.001	0.90	1.05	1.32	1.51	<0.001	0.84	1.08	1.24	1.51	<0.001	0.85	1.15	1.25	1.38	<0.001
L-HDL, mmol/L	0.26	0.36	0.53	0.71	<0.002	0.23	0.37	0.52	0.81	<0.002	0.39	0.45	0.49	0.56	<0.002	0.43	0.52	0.50	0.44	0.91
M-HDL, mmol/L	0.20	0.30	0.33	0.41	<0.003	0.27	0.29	0.37	0.33	<0.003	0.18	0.26	0.34	0.44	<0.003	0.19	0.29	0.34	0.40	<0.001
S-HDL, mmol/L	0.29	0.39	0.40	0.48	<0.004	0.40	0.38	0.43	0.38	0.79	0.27	0.36	0.42	0.51	<0.004	0.23	0.34	0.41	0.54	<0.002
Triglycerides, mmol/L	1.77	1.52	1.13	1.00	<0.005	1.77	1.4	1.13	0.98	<0.001	1.47	1.40	1.29	1.10	0.001	1.50	1.31	1.23	1.21	0.02
Impaired glucose tolerance, %	12	12	14	9	0.64	14	12	14	7	0.97	9	11	15	11	0.32	16	9	12	12	0.87
Diabetes mellitus, %	14	7	5	6	0.04	11	10	5	5	0.04	12	5	9	5	0.23	9	7	9	6	0.84

Triglycerides are expressed as geometric mean

BP indicates blood pressure; HDL, high-density lipoprotein; L-HDL, large high-density lipoprotein; M-HDL, medium high-density lipoprotein; and S-HDL, small high-density lipoprotein.

Table 2. Odds Ratios (95% Confidence Interval) of Stroke and Subtypes According to High-density Lipoprotein Cholesterol Levels by High-density Lipoprotein Subclass

	Total HDL cholesterol					L-HDL quartiles				
	1	2	3	4	OR per 1 SD increment	1	2	3	4	OR per 1 SD increment
Total stroke										
No of cases	70	62	54	55		55	67	56	63	
No of controls	51	58	67	65		65	53	66	57	
Age-, sex-, and community-matched OR	1.00	0.67 (0.39–1.17)	0.46 (0.25–0.84)*	0.47 (0.26–0.88)*	0.85 (0.69–1.06)	1.00	1.54 (0.91–2.62)	1.04 (0.62–1.76)	1.38 (0.78–2.41)	1.09 (0.89–1.32)
Multivariable OR[a]	1.00	0.60 (0.33–1.10)	0.41 (0.21–0.81)*	0.40 (0.19–0.80)†	0.79 (0.61–1.02)	1.00	1.78 (1.00–3.16)	1.25 (0.70–2.23)	1.57 (0.81–3.05)	1.13 (0.89–1.44)
Ischemic stroke										
No of cases	49	43	28	35		38	42	37	38	
No of controls	38	39	39	39		44	38	40	33	
Age-, sex-, and community-matched OR	1.00	0.77 (0.41–1.44)	0.46 (0.22–0.96)*	0.57 (0.27–1.18)	0.89 (0.69–1.15)	1.00	1.30 (0.70–2.44)	1.11 (0.58–2.11)	1.40 (0.69–2.83)	1.11 (0.87–1.41)
Multivariable OR[a]	1.00	0.66 (0.33–1.35)	0.37 (0.15–0.87)*	0.47 (0.20–1.12)	0.85 (0.62–1.16)	1.00	1.70 (0.83–3.48)	1.35 (0.65–2.80)	1.80 (0.76–4.24)	1.19 (0.87–1.62)
Lacunar infarction										
No of cases	40	30	21	25		31	34	21	30	
No of controls	23	31	30	32		30	26	33	27	
Age-, sex-, and community-matched OR	1.00	0.50 (0.23–1.07)	0.29 (0.12–0.72)†	0.33 (0.14–0.79)*	0.82 (0.61–1.10)	1.00	1.25 (0.60–2.61)	0.58 (0.26–1.30)	1.00 (0.44–2.25)	1.02 (0.78–1.34)
Multivariable OR[a]	1.00	0.52 (0.22–1.21)	0.23 (0.08–0.67)†	0.27 (0.10–0.75)*	0.75 (0.53–1.06)	1.00	1.77 (0.75–4.16)	0.62 (0.24–1.60)	0.97 (0.35–2.69)	1.02 (0.72–1.44)
Hemorrhagic stroke										
No of cases	21	19	26	20		17	25	19	25	
No of controls	13	19	28	26		21	15	26	24	
Age-, sex-, and community-matched OR	1.00	0.46 (0.14–1.47)	0.39 (0.13–1.20)	0.30 (0.09–1.00)	0.75 (0.50–1.13)	1.00	2.24 (0.83–6.06)	1.00 (0.40–2.51)	1.46 (0.56–3.84)	1.04 (0.74–1.46)
Multivariable OR[a]	1.00	0.46 (0.13–1.64)	0.44 (0.12–1.62)	0.35 (0.09–1.35)	0.79 (0.49–1.29)	1.00	2.71 (0.83–8.84)	1.43 (0.45–4.56)	1.87 (0.57–6.13)	1.17 (0.76–1.80)
Intraparenchymal hemorrhage										
No of cases	13	18	17	16		12	18	16	18	
No of controls	9	14	21	20		17	10	17	20	
Age-, sex-, and community-matched OR	1.00	0.66 (0.15–2.99)	0.36 (0.08–1.57)	0.33 (0.07–1.52)	0.74 (0.45–1.22)	1.00	2.85 (0.86–9.47)	1.49 (0.49–4.54)	1.45 (0.44–4.81)	1.08 (0.72–1.62)
Multivariable OR[a]	1.00	0.80 (0.13–4.95)	0.52 (0.07–3.89)	0.32 (0.05–2.16)	0.68 (0.37–1.28)	1.00	4.06 (0.74–22.2)	3.71 (0.61–2.60)	1.60 (0.25–10.4)	1.06 (0.60–1.87)

BMI indicates body mass index; HDL, high-density lipoprotein; L-HDL, large high-density lipoprotein; M-HDL, medium high-density lipoprotein; OR, odds ratio; and S-HDL, small high-density lipoprotein.

*$P<0.05$, †$P<0.01$, ‡$P<0.001$.

[a]Adjusted for hypertension status BMI, current alcohol intake, cigarette smoking status, cholesterol-lowering medication, log-transformed triglyceride levels, serum glucose category, and matching for sex, age, community, year of serum stored, and fasting status.

with risk of total stroke and lacunar infarction but not of hemorrhagic stroke. S-HDL cholesterol levels were strongly and inversely associated with risk of total stroke, ischemic stroke, particularly lacunar infarction, and hemorrhagic stroke, specifically intraparenchymal hemorrhage. These associations remained statistically significant after further adjustment for cardiovascular risk factors. Moderate inverse associations were observed between M-HDL cholesterol levels and risk of total stroke and its subtypes. No associations were found between L-HDL cholesterol levels and risk of total stroke or its subtypes.

Discussion

The present study is the first study to show that higher cholesterol levels in small HDL and medium HDL particles were associated with lower risk of total stroke, either ischemic or hemorrhagic stroke even after adjustment for known cardiovascular risk factors and matching variables of age, sex, years of serum

M-HDL quartiles					S-HDL quartiles				
1	2	3	4	OR per 1 SD increment	1	2	3	4	OR per 1 SD increment
68	67	55	51		75	64	53	49	
52	54	66	69		45	57	68	71	
1.00	0.77 (0.42–1.39)	0.41 (0.21–0.80)†	0.35 (0.17–0.69)†	0.64 (0.49–0.83)‡	1.00	0.36 (0.18–0.73)†	0.14 (0.06–0.33)‡	0.08 (0.03–0.21) ‡	0.39 (0.27–0.57)‡
1.00	0.66 (0.34–1.28)	0.31 (0.15–0.64)†	0.23 (0.11–0.51)‡	0.56 (0.41–0.75)‡	1.00	0.37 (0.17–0.78)†	0.18 (0.06–0.34)‡	0.05 (0.02–0.15) ‡	0.34 (0.23–0.52) ‡
46	41	35	33		50	40	32	33	
37	33	41	44		31	36	42	46	
1.00	0.84 (0.41–1.73)	0.51 (0.24–1.10)	0.44 (0.12–0.97)*	0.72 (0.54–0.96)*	1.00	0.33 (0.13–0.85)*	0.14 (0.05–0.42)‡	0.09 (0.03–0.30) ‡	0.44 (0.28–0.69) ‡
1.00	0.72 (0.33–1.61)	0.39 (0.17–0.90)*	0.30 (0.12–0.76)*	0.63 (0.45–0.88)†	1.00	0.34 (0.13–0.89)*	0.14 (0.05–0.44)‡	0.07 (0.02–0.25)‡	0.38 (0.23–0.63)‡
33	32	26	25		36	30	26	24	
26	23	33	34		20	30	30	36	
1.00	0.92 (0.40–2.15)	0.45 (0.18–1.12)	0.40 (0.16–1.01)	0.68 (0.49–0.95)*	1.00	0.26 (0.09–0.79)*	0.13 (0.04–0.47))†	0.08 (0.02–0.31)‡	0.36 (0.21–0.63)‡
1.00	0.70 (0.27–1.80)	0.35 (0.13–0.94)*	0.30 (0.11–0.86)*	0.59 (0.40–0.87)†	1.00	0.29 (0.09–0.93)*	0.15 (0.04–0.57)†	0.07 (0.02–0.32)‡	0.33 (0.18–0.61)‡
22	26	20	18		25	24	21	16	
15	21	25	25		14	21	26	25	
1.00	0.58 (0.19–1.74)	0.21 (0.05–0.83)*	0.17 (0.04–0.72)*	0.43 (0.24–0.78)†	1.00	0.40 (0.14–1.13)	0.12 (0.03–0.52)†	0.04 (0.01–0.28)‡	0.30 (0.15–0.61)‡
1.00	0.30 (0.07–1.34)	0.11 (0.02–0.62)*	0.09 (0.02–0.53)†	0.41 (0.21–0.80)†	1.00	0.45 (0.13–1.54)	0.12 (0.02–0.66)*	0.02 (0.002–0.20)‡	0.30 (0.14–0.65)‡
14	23	17	10		17	21	16	10	
10	19	17	18		9	18	20	17	
1.00	0.64 (0.19–2.22)	0.31 (0.06–1.58)	0.14 (0.03–0.82)*	0.36 (0.17–0.75)†	1.00	0.42 (0.13–1.36)	0.15 (0.03–0.68)*	0.04 (0.005–0.35)†	0.29 (0.13–0.66)†
1.00	0.25 (0.04–1.72)	0.18 (0.02–1.60)	0.06 (0.005–0.62)*	0.38 (0.16–0.90)*	1.00	0.46 (0.11–2.00)	0.16 (0.03–1.02)	0.02 (0.001–0.29)†	0.30 (0.12–0.77)*

storage, fasting status, and community. There was no association between L-HDL cholesterol levels and risk of total stroke and its subtypes. Risk of total stroke was ≈90% lower among persons at the highest quartile of S-HDL cholesterol levels or M-HDL cholesterol levels than among those at the lowest quartile. S-HDL and M-HDL cholesterol levels were not associated with age, sex, blood pressure levels, body mass index, smoking, and diabetes mellitus. Taken together, higher cholesterol levels in S-HDL and M-HDL are suggested to reduce risk of stroke beyond the effects of other conventional risk factors. Small HDL and medium HDL cholesterol levels can be increased by increasing dietary intake of carbohydrate[19] and use of fenofibrate.[20] These nonpharmacological and pharmacological interventions may increase hepatic triglyceride lipase activity that promotes the conversion of large HDL particles into small HDL particles via cholesteryl ester transfer protein.[21,22]

Mechanisms for HDL subpopulation in protection against cardiovascular disease are complex and not fully understood. The ATP-binding cassette transporter A1 (ABCA1) mediates the efflux of cellular cholesterol and phospholipids to lipid-poor apolipoproteins.[23,24] Because smaller HDL particles contained phospholipid and more apoA-1 compared with large HDL particles, they have a larger capacity to remove cholesterol from membranes of peripheral cells, particularly macrophages and form cells.[2] Our result is in line with this mechanism, supporting those subjects with higher cholesterol levels in small HDL or medium HDL particle subclasses may protect against atherosclerosis and atherosclerotic cardiovascular disease. The inverse association between cholesterol levels in smaller HDL particles and risk of cardiovascular disease was observed in the Epic-Norfolk prospective population study,[8] Lopid Coronary Angiography Trial Study[6], and Caerphilly Study.[7] The ATP-binding cassette transporter G1 (ABCG1) stimulates the cholesterol efflux to larger HDL particles[25] because larger HDL particles are the preferred acceptor of ABCG1-mediated cholesterol efflux.[26] This may explain findings of a previous study that large HDL particles were inversely associated with risk of cardiovascular disease, including myocardial infarction and ischemic stroke in women.[11] ABCA1 mediates cholesterol and phospholipid efflux to lipid-poor apoA-I but not to mature HDL. ABCG1 mediates macrophage cholesterol efflux to mature HDL, which might explain mechanism of the relationship of HDL to atherosclerosis risk.[25] However, a study with mice suggested that both ABCA1 and ABCG1 contribute to macrophage reverse cholesterol transport. That study showed a greater decrease in macrophage reverse cholesterol transport from cells where both ABCA1 and ABCG1 expressions were knocked down than from ABCG1-knockdown cells.[27] Another study also indicated that ABCA1 may lipidate lipid-poor apoA-I to generate nascent HDL, which can then act as acceptor for ABCG1-mediated cholesterol efflux.[28]

The present study first showed that cholesterol levels in smaller HDL particles were inversely associated with risk of lacunar infarction. The mechanism for a protective effect of small HDL particles on lacunar infarction is unknown. Anti-inflammatory effects of smaller HDL particles[5,29] may reduce risk of lacunar infarction by inhibiting angionecrosis[30,31] or microatheroma formation[32] in cerebral vessels.

An inverse association was found between smaller HDL cholesterol levels and risk of hemorrhagic stroke, primarily intraparenchymal hemorrhage. One mechanism to explain the protective effect of smaller HDL particles might be their enriched apolipoprotein and enzymes with antioxidative activities.[4] Reduced LDL oxidation may contribute to inhibition of microatheroma formation in small cerebral vessels.[31,33,34]

The strength of the present study is the large number of strokes confirmed by imaging studies, which allowed investigation of the association between S-HDL, M-HDL, and L-HDL cholesterol levels and risk of total stroke and its subtypes. There are several limitations. First, we used frozen serum to estimate HDL cholesterol levels and did not examine long-term changes in HDL cholesterol levels in stored serum samples. A previous study on frozen storage (−70°C) of serum samples for up to 7 years showed no significant change in HDL cholesterol.[35] Second, the frozen serum samples used in the present study had been thawed once. However, a previous study reported that freezing and thawing of HDL have no effect on HDL particle size.[36]

In conclusion, the present study showed that cholesterol levels in small HDL and medium HDL particles were inversely associated with risks of total stroke, either ischemic or hemorrhagic stroke, whereas those in large HDL particles were not associated with risk of total stroke or any subtypes.

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Disclosure

None.

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High-density Lipoprotein Subclasses and Risk of Stroke and its Subtypes in Japanese Population: The Circulatory Risk in Communities Study

Choy-Lye Chei, Kazumasa Yamagishi, Akihiko Kitamura, Masahiko Kiyama, Hironori Imano, Tetsuya Ohira, Renzhe Cui, Takeshi Tanigawa, Tomoko Sankai, Yoshinori Ishikawa, Shinichi Sato, Shinichi Hitsumoto, Hiroyasu Iso and on behalf of the CIRCS Investigators
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Lipoprotein particle profiles compared with standard lipids in association with coronary artery calcification in the general Japanese population

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ABSTRACT

Objective: The utility of lipoprotein particle profiles measured by nuclear magnetic resonance (NMR) spectroscopy beyond standard serum lipids remains inconclusive. Furthermore, few studies have compared NMR measurements with standard lipids in association with coronary artery calcification (CAC) in Japanese, where the coronary atherosclerotic burden is low. We examined whether NMR-based lipoprotein particle profiles are associated with CAC, and compared them with standard lipid and lipid ratios in the Japanese general population.

Methods and Results: We conducted a cross-sectional study in 851 men aged 40–79 years without cardiovascular diseases and lipid-lowering therapies. Adjusted odds ratios (ORs) (95% confidence intervals) for the top versus the bottom quartile of NMR-measured particle concentrations were 2.01 (1.24–3.23) for low-density lipoprotein (LDL-P), 1.04 (0.62–1.75) for high-density lipoprotein (HDL-P), 1.82 (1.13–2.95) for very-low-density lipoprotein (VLDL-P), and 1.92 (1.18–3.17) for LDL-P/HDL-P ratio. Similarly adjusted ORs of NMR-measured particle sizes were 0.59 (0.36–0.97) for LDL-P, 0.66 (0.40–1.10) for HDL-P, and 0.67 (0.40–1.12) for VLDL-P. The corresponding ORs were 1.82 (1.14–2.90) for total cholesterol (TC), 2.06 (1.28–3.30) for low-density lipoprotein cholesterol (LDL-C), 0.56 (0.34–0.91) for high-density lipoprotein cholesterol (HDL-C), 2.02 (1.24–3.29) for triglycerides, 2.08 (1.29–3.36) for non-high-density lipoprotein cholesterol (non-HDL-C), 2.27 (1.37–3.78) for TC/HDL-C ratio, and 1.73 (1.06–2.85) for LDL-C/HDL-C ratio. After mutual adjustment for total LDL-P concentration and TC/HDL-C ratio or non-HDL-C, LDL-P was no longer associated, whereas TC/HDL-C ratio remained significantly associated with CAC.

Conclusions: In community-based Japanese men, the overall association of CAC with NMR-measured lipoprotein indices is comparable, but not superior, to that with standard lipids.

1. Introduction

Lipoprotein particle profiles assessed by proton nuclear magnetic resonance (NMR) spectroscopy [1] are heterogeneous with respect to size and density, having a differential effect and strong

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connection with their atherogenic properties [2,3]. NMR-based lipoprotein profiles have thus been suggested as alternative lipoprotein measures for improved risk assessment of cardiovascular disease (CVD). These profiles have also been reported to be associated with an increased risk of CVD outcomes or subclinical atherosclerosis [4–8].

However, how well NMR-based lipoprotein indices predict CVD or subclinical atherosclerosis compared with standard serum lipids remains uncertain. Only a few prospective population-based studies have directly compared the predictability of clinical CVD risk between various NMR-based lipoprotein profiles and standard lipids including ratio measures [5,6]. In those studies, the association of CVD risk with NMR measures was statistically significant, yet of a lesser magnitude than that with standard lipids. Interestingly, the studies that compared these indices in association with subclinical atherosclerosis reported a stronger relation of NMR-based indices than standard lipids [7,8]. Additionally, these epidemiological studies were mainly conducted in Western countries.

The burden of coronary atherosclerosis in Japan has remained lower compared with that in Western populations. This has been confirmed with multiple levels of evidence, including comparative studies for clinical coronary artery disease (CAD) [9], an autopsy study [10], and studies on the subclinical stage of coronary atherosclerosis as measured by coronary artery calcification (CAC) [11–13]. More recently, however, the overall levels of total cholesterol (TC) in Japan have now become similar to or even higher than those in US [9,14,15]. The level of high-density lipoprotein (HDL) cholesterol (HDL-C) among Japanese adults is higher than that of the US [16], and has increased in recent years [17,18]. Literature is scarce on comparison of NMR-based lipid profiles among community-based samples from Japan and other countries. However, we have reported considerable differences in this profile among samples of Japanese men and Caucasians from the US [19]. To the best of our knowledge, no studies have examined the association of NMR-measured lipoproteins with subclinical atherosclerosis in the general Japanese population.

Therefore, we conducted a cross-sectional study of the general Japanese population to examine whether NMR-based lipoprotein particle profiles are associated with CAC, a marker of subclinical atherosclerosis, and compared them with standard lipid and lipid ratios. A community-based Japanese population tends to have a lower burden of CAD and subclinical atherosclerosis compared with Western populations. Therefore, this Japanese population would be useful for examining lipid profiles for CVD assessment in low-risk populations.

2. Methods

2.1. Participants and risk factor measurement

Participants eligible for the present study were 1094 Japanese men enrolled at baseline (May 2006 to March 2008) in the Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA). Detailed methods are described elsewhere [20,21]. In brief, SESSA participants were community-dwelling men aged 40–79 years, who were selected based on an age-stratified random sample from the Basic Residents' Register of the city, which includes information on the name, age, and sex of residents. The present study was approved by the Institutional Review Board of Shiga University of Medical Science (No. 17–19, 17–83).

A total of 243 men were excluded from this analysis for the following reasons: history of CVDs ($n = 103$), use of lipid-lowering medications ($n = 119$), missing information for lipid parameters ($n = 9$), and participants with a triglyceride (TG) level at or above 400 mg/dl ($n = 12$). The last criterion was used to adequately estimate low-density lipoprotein (LDL) cholesterol (LDL-C) levels following Friedewald's formula [22–24]. Therefore, 851 participants were finally included in the present analyses (mean [SD] age, 63.4 [10.0] years).

2.2. CAC

We assessed CAC either by electron-beam computed tomography (EBCT) ($n = 593$, 75.1%) using a C-150 scanner (Imatron, South San Francisco, CA, USA) or 16-channel multidetector-row computed tomography (MDCT, $n = 258$) scans using an Aquilion scanner (Toshiba, Tokyo, Japan). Images were obtained from the level of the root of the aorta through the heart at a slice thickness of 3 mm, with a scan time of 100 ms (EBCT) or 320 ms (MDCT). We acquired images at 70% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Quantification of CAC was performed using a DICOM workstation and AccuImage software (AccuImage Diagnostics, South San Francisco, CA, USA). The presence of CAC was defined as a minimum of 3 contiguous pixels (area = 1 mm^2) with a density $\geq$ 130 Hounsfield units (HU). We placed a region of interest around each high-density lesion in the epicardial coronary arteries. Peak density (HU) and area (mm^2) of the individual coronary calcifications were measured, and then the CAC score was calculated according to the Agatston method [25]. All computed tomography (CT) images were read by one physician who was trained in CT reading at the Cardiovascular Institute of the University of Pittsburgh, and who was blinded to participants' demographics. The protocol described above was adopted from a separate cohort study performed by our research group [21], in which the reproducibility of the scans showed an intraclass correlation of 0.98 [11]. In stratified analysis by type of CT, we found that trends were similar between participants assessed by EBCT and those by 16-channel MDCT (data not shown). Additionally, CAC assessment by EBCT and MDCT has been reported to be comparable [26]. Therefore, we presented the combined results. To define the presence of CAC, a CAC score >0 was used [7].

2.3. Assays for lipids, lipoproteins, and other variables

Venipuncture was performed early in a clinical visit after a 12-h fast. We separated serum by centrifugation (3000 revolutions per min, for 15 min) at 4 °C within 90 min. Samples were sent for routine laboratory tests, including those for lipids and glucose. Plasma glucose levels were determined from NaF-treated plasma using a hexokinase glucose-6-phosphate-dehydrogenase enzymatic assay. Serum TC and TG were determined using enzymatic assays, and HDL-C was measured using a direct method (Determiner-C-TC, Determiner-C-TGL, Determiner-L HDL-C, respectively; Kyowa Medix, Tokyo, Japan). Serum lipids were determined at a single laboratory (Shiga Laboratory; MEDIC, Shiga, Japan) that has been certified for standardized lipid measurements according to the protocol of the Centers for Disease Control and Prevention/US Collaborating Center for Reference Method Laboratory Network Research in Blood Lipids (CDC/CRMLN) [27]. We used Friedewald's formula to estimate LDL-C levels [28]. Non-high-density lipoprotein cholesterol (non-HDL-C) was calculated by subtracting HDL-C from TC.

Serum samples were stored at −80C° and shipped on dry ice to LipoScience Inc, (Raleigh, NC) for lipoprotein particle analysis. NMR spectroscopy [1] was performed to quantify the particle concentrations of very-low-density lipoprotein (VLDL), LDL, and HDL [29]. Additionally, particle concentrations were further determined for 3 VLDL subclasses (large, >60 nm; medium, 35–60 nm; and small, 27–35 nm), 3 LDL subclasses (intermediate-density lipoprotein [IDL], 23–27 nm; large, 21.3–23 nm; small, 18.3–21.2 nm), and 3

HDL subclasses (large, 8.8–13 nm; medium, 8.2–8.8 nm; and small, 7.3–8.2 nm) [30]. We calculated weighted average particle sizes of VLDL, LDL, and HDL.

2.4. Statistical analysis

Analyses were performed using the Statistical Package for the Social Sciences, version 18.0J (SPSS Inc., Chicago, IL, USA) and SAS version 9.3 (SAS Institute, Cary, NC, USA). Two-tailed *P* values <0.05 were considered significant. Participants' characteristics are shown using means and standard deviations (SDs) for continuous variables with approximately normal distributions or medians and interquartile ranges for continuous variables with skewed distributions, and percentages for categorical variables according to the absence or presence of CAC. Differences in characteristics were evaluated using the unpaired Student's *t* test, Mann–Whitney *U*-test, or χ^2 test, as appropriate. We calculated age-adjusted Spearman's rank correlation coefficients between the measured lipid levels. We divided each index of lipid into quartiles, and conducted logistic regression to estimate multivariable adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the presence of CAC (CAC score >0) according to the quartiles. In most analyses, the adjusted covariates were as follows: age, smoking status (former, current), ethanol consumption (g/day), BMI, blood glucose, systolic blood pressure, medication status (hypertension and diabetes), [above these covariates were defined as "non-lipid risk factors"], and type of CT. Further adjustment for exercise (defined as participants who regularly exercised ≥1 h/week) and a family history of coronary heart disease did not substantially affect the findings. Therefore, we did not include these variables in the model. The *P* value for linear trend across quartile was obtained with the median value for each quartile. We also calculated adjusted ORs and 95% CIs per 1-SD increase in lipid indices for the presence of CAC. The following lipid indices were log-transformed due to their skewed distributions: TG, small LDL particle (LDL-P), IDL, large and medium HDL particles (HDL-P), and total, large, medium, and small VLDL particles (VLDL-P). Furthermore, we analyzed non-HDL-C, total LDL-P concentration, and TC/HDL-C ratio in multivariable logistic regression models that were adjusted for other lipids according to previous studies. [7,8].

3. Results

The characteristics of the participants according to CAC are shown in Table 1. Participants with CAC tended to be older and to have less favorable risk factor distributions than those without CAC, including BMI, blood glucose, systolic and diastolic blood pressure, and medication status (hypertension and diabetes). Participants with CAC had higher levels of TG, non-HDL-C, NMR-measured lipoprotein particle concentrations of total LDL-P, small LDL-P, and all of the ratios than those without CAC. Participants with CAC also had lower levels of HDL-C, total and small HDL-P concentrations, and had a smaller size of LDL-P compared with those without CAC.

Supplementary table I shows the age-adjusted Spearman's correlation coefficients between NMR-based indices and standard lipid-based indices. Total LDL-P concentration was strongly correlated with LDL-C and non-HDL-C (r = 0.806 and 0.815, respectively). Large HDL-P concentration was positively correlated with HDL-C (r = 0.877), but inversely correlated with TC/HDL-C ratio (r = −0.813). Moreover, total and medium VLDL-P concentrations were strongly correlated with TG (r = 0.856 and 0.804, respectively). Additionally, LDL-P/HDL-P ratio was strongly correlated with TC/HDL-C and LDL-C/HDL-C ratios (r = 0.828 and 0.872, respectively).

Table 1
Characteristics of the participants according to the absence or presence of CAC in apparently healthy Japanese men aged 40–79 years: SESSA, Shiga, 2006–2008.

	CAC (−) (n = 328)	CAC (+) (n = 523)	*P* value
Age—years	58.4 (11.3)	65.9 (7.8)	<0.001
Smokers—%			
Current	32.9	33.8	0.783
Former	47.3	51.2	0.258
Ethanol consumption—g/day	26.8 (28.0)	33.5 (27.9)	<0.001
Body mass index—kg/m^2	22.9 (2.8)	23.6 (3.1)	<0.001
Blood glucose—mg/dl	98.5 (17.3)	103.9 (23.1)	<0.001
Blood pressure—mmHg			
Systolic	129.7 (17.4)	139.7 (19.6)	<0.001
Diastolic	77.7 (10.6)	80.9 (11.1)	<0.001
Exercise—%	37.2	46.3	0.009
Medication for hypertension—%	13.1	31.4	<0.001
Medication for diabetes—%	2.7	10.3	<0.001
Family history of coronary heart disease—%	11.3	11.1	0.932
Standard lipids—mg/dl			
TC	208.5 (32.6)	209.7 (35.0)	0.489
LDL-C	125.0 (31.2)	127.2 (31.9)	0.292
HDL-C	61.8 (16.2)	57.8 (17.6)	<0.001
TG[a]	94.0 (70.0, 133.5)	105.0 (77.0, 152.0)	0.001
Non-HDL-C	146.6 (34.4)	152.0 (35.3)	0.019
Lipoprotein particle concentrations			
LDL-P—nmol/l			
Total	1242.7 (371.2)	1320.8 (391.6)	0.003
Large	645.6 (282.8)	635.1 (290.9)	0.673
Small[a]	471.0 (94.0, 756.3)	559.0 (107.0, 888.0)	0.007
IDL[a]	100.0 (44.8, 169.0)	84.0 (40.0, 161.0)	0.276
HDL-P—μmol/l			
Total	35.1 (6.2)	33.5 (6.7)	<0.001
Large[a]	6.8 (4.8, 10.1)	6.4 (4.1, 9.6)	0.055
Medium[a]	7.4 (4.8, 10.6)	7.3 (4.9, 10.3)	0.566
Small	19.4 (5.3)	18.5 (5.1)	0.004
VLDL-P—nmol/l			
Total[a]	47.3 (27.3, 76.4)	51.2 (27.5, 87.8)	0.064
Large[a]	0.6 (0.3, 1.6)	0.5 (0.2, 1.6)	0.151
Medium[a]	16.7 (6.9, 33.6)	18.7 (9.1, 38.1)	0.184
Small[a]	24.1 (13.0, 42.3)	27.2 (12.2, 48.2)	0.289
Lipoprotein particle size—nm			
LDL-P	21.0 (0.5)	20.9 (0.6)	0.013
HDL-P	9.3 (0.5)	9.3 (0.5)	0.717
VLDL-P	44.7 (5.9)	44.4 (5.8)	0.590
Ratios			
TC/HDL-C	3.6 (1.0)	3.9 (1.1)	<0.001
LDL-C/HDL-C	2.2 (0.8)	2.4 (0.9)	0.001
LDL-P/HDL-P	36.9 (14.2)	41.4 (16.1)	<0.001

Values are expressed as mean (standard deviation) for continuous variables with approximately normal distributions and as % for categorical variables.
Abbreviations: CAC, coronary artery calcification; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle; IDL, intermediate-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle; non-HDL-C, non-high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; VLDL-P, very-low-density lipoprotein particle.
[a] Continuous variables with skewed distributions are expressed as median (interquartile range). The presence of CAC was defined as a CAC score >0. Participants who exercised were defined as those who regularly exercised ≥1 h/week.

Table 2 shows the ORs of CAC across quartiles for each lipid index adjusted for non-lipid risk factors and type of CT. Of the NMR measures, the association of total LDL-P concentration with CAC was strong, but not greater than associations of standard lipids or ratios. Small LDL-P, but not large LDL-P, concentration was associated with CAC. None of the NMR-measured HDL-P concentrations were significantly associated with CAC. Total, medium, and small VLDL-P concentrations were significantly associated with CAC. Among the three particle sizes that we studied (i.e., LDL-P, HDL-P, and VLDL-P), only LDL-P size was significantly and inversely

Table 2
Association of lipid measures with the presence of CAC (CAC score >0), adjusted for non-lipid risk factors in apparently healthy Japanese men aged 4079 years: SESSA, Shiga, 2006–2008.

	First quartile	Second quartile	Third quartile	Fourth quartile	*P* For trend
Standard lipids					
TC					
Range, mg/dl	<187	187–206	207–230	≥231	
Adjusted OR (95% CI)	1.00	0.98 (0.62–1.53)	1.19 (0.76–1.89)	1.82 (1.14–2.90)	0.006
LDL-C					
Range, mg/dl	<106.0	106.0–123.3	123.4–146.5	≥146.6	
Adjusted OR (95% CI)	1.00	1.47 (0.94–2.31)	1.35 (0.86–2.13)	2.06 (1.28–3.30)	0.005
HDL-C					
Range, mg/dl	<47	47–56	57–68	≥69	
Adjusted OR (95% CI)	1.00	0.83 (0.52–1.32)	0.50 (0.31–0.81)	0.56 (0.34–0.91)	0.011
TG					
Range, mg/dl	<74	74–99	100–144	≥145	
Adjusted OR (95% CI)	1.00	1.17 (0.75–1.83)	1.03 (0.66–1.61)	2.02 (1.24–3.29)	0.004
Non-HDL-C					
Range, mg/dl	<126	126–147	148–171	≥172	
Adjusted OR (95% CI)	1.00	1.43 (0.91–2.23)	1.53 (0.96–2.42)	2.08 (1.29–3.36)	0.003
Lipoprotein particle concentrations					
LDL-P					
Total					
Range, nmol/l	<1010	1010–1269	1270–1540	≥1541	
Adjusted OR (95% CI)	1.00	1.50 (0.95–2.35)	1.59 (1.00–2.5 s)	2.01 (1.24–3.23)	0.004
Large					
Range, nmol/l	<453	453–633	634–830	≥831	
Adjusted OR (95% CI)	1.00	0.79 (0.50–1.24)	0.92 (0.58–1.46)	0.91 (0.57–1.44)	0.847
Small					
Range, nmol/l	<103	103–516	517–841	≥842	
Adjusted OR (95% CI)	1.00	1.17 (0.75–1.84)	1.41 (0.88–2.25)	1.87 (1.14–3.05)	0.008
IDL					
Range, nmol/l	<41	41–90	91–162	≥163	
Adjusted OR (95% CI)	1.00	0.85 (0.54–1.32)	0.81 (0.52–1.27)	0.89 (0.56–1.41)	0.788
HDL-P					
Total					
Range, μmol/l	<29.8	29.8–33.3	33.4–37.4	≥37.5	
Adjusted OR (95% CI)	1.00	1.13 (0.71–1.81)	0.66 (0.42–1.06)	1.04 (0.62–1.75)	0.766
Large					
Range, μmol/l	<4.3	4.3–6.5	6.6–9.7	≥9.8	
Adjusted OR (95% CI)	1.00	0.74 (0.47–1.18)	0.64 (0.39–1.05)	0.68 (0.41–1.12)	0.185
Medium					
Range, μmol/l	<4.9	4.9-7.2	7.3-10.2	≥10.3	
Adjusted OR (95% CI)	1.00	1.23 (0.78–1.94)	1.27 (0.81–1.99)	1.36 (0.85–2.17)	0.238
Small					
Range, μmol/l	<15.4	15.4-18.7	18.8-22.1	≥22.2	
Adjusted OR (95% CI)	1.00	1.14 (0.71–1.82)	0.97 (0.61–1.54)	0.66 (0.42–1.05)	0.060
VLDL-P					
Total					
Range, nmol/l	<27.5	27.5–49.7	49.8–83.9	≥84.0	
Adjusted OR (95% CI)	1.00	1.06 (0.68–1.66)	1.17 (0.74–1.84)	1.82 (1.13–2.95)	0.009
Large					
Range, nmol/l	<0.3	0.3-0.5	0.6-1.5	≥1.6	
Adjusted OR (95% CI)	1.00	0.99 (0.63–1.55)	0.72 (0.46–1.13)	0.98 (0.61–1.57)	0.743
Medium					
Range, nmol/l	<7.7	7.7-17.9	18.0-35.4	≥35.5	
Adjusted OR (95% CI)	1.00	1.28 (0.82–2.00)	1.21 (0.78–1.89)	1.70 (1.06–2.73)	0.042
Small					
Range, nmol/l	<12.6	12.6-25.4	25.5-46.5	≥46.6	
Adjusted OR (95% CI)	1.00	0.74 (0.47–1.15)	1.52 (0.96–2.41)	1.46 (0.92–2.34)	0.017
Lipoprotein particle size					
LDL-P					
Range, nm	<20.6	20.6–20.9	21.0–21.2	≥21.3	
Adjusted OR (95% CI)	1.00	0.66 (0.41–1.06)	0.69 (0.44–1.09)	0.59 (0.36–0.97)	0.041
HDL-P					
Range, nm	<8.8	8.8–9.2	9.3–9.6	≥9.7	
Adjusted OR (95% CI)	1.00	0.70 (0.44–1.12)	0.64 (0.40–1.01)	0.66 (0.40–1.10)	0.115
VLDL-P					
Range, nm	<40.4	40.4–43.3	43.4–47.3	≥47.4	
Adjusted OR (95% CI)	1.00	0.95 (0.58–1.56)	1.21 (0.72–2.03)	0.67 (0.40–1.12)	0.120
Ratios					
TC/HDL-C					
Range	<2.93	2.93–3.63	3.64–4.45	≥4.46	
Adjusted OR (95% CI)	1.00	1.26 (0.81–1.96)	1.67 (1.05–2.64)	2.27 (1.37–3.78)	0.001
LDL-C/HDL-C					
Range	<1.65	1.65–2.19	2.20–2.88	≥2.89	
Adjusted OR (95% CI)	1.00	1.21 (0.78–1.89)	1.62 (1.02–2.56)	1.73 (1.06–2.85)	0.016

Table 2 (*continued*)

	First quartile	Second quartile	Third quartile	Fourth quartile	*P* For trend
LDL-P/HDL-P					
Range	<0.029	0.029–0.036	0.037–0.048	≥0.049	
Adjusted OR (95% CI)	1.00	1.28 (0.82–2.01)	1.86 (1.17–2.96)	1.92 (1.18–3.17)	0.004

Odds ratios were adjusted for age, smoking status (former, current), ethanol consumption (g/day), body mass index, blood glucose, systolic blood pressure, medication status (hypertension, diabetes), and type of CT. Abbreviations: CI, confidence interval; OR, odds ratio; other abbreviations are as in Table 1.

associated with CAC. Among single standard lipids, non-HDL-C had a large OR for the presence of CAC. All of the standard lipids were also significantly associated with CAC. As shown in Table 2, ORs for NMR measures were of approximately similar magnitude to those for standard lipids or ratios. However, the TC/HDL-C ratio had the largest OR for the presence of CAC among all of the indices of lipid or lipoprotein that we studied.

The ORs of CAC per 1-SD increase in lipid indices adjusted for non-lipid risk factors and type of CT are shown in the Fig. 1. Among the NMR-measured lipoproteins, the strength of association with CAC was largest for total LDL-P concentration, although it was not predominant over that for standard lipids. Among the single standard lipids, non-HDL-C had a large OR per 1-SD increase for the presence of CAC. TC/HDL-C ratio had the largest OR per 1-SD increase for the presence of CAC among all of the indices of lipid or lipoprotein that we studied.

In the model that included non-lipid risk factors plus other lipids (as continuous variables), the association of total LDL-P concentration with CAC was attenuated (Supplementary Table II). In particular, no significant association of total LDL-P concentration was found after adjustment for TC/HDL-C ratio or non-HDL-C when LDL-P was examined either as a quartile (highest quartile OR, 1.37; 95% CI, 0.74–2.54 and highest quartile OR, 1.36; 95% CI, 0.66–2.80, respectively) or a continuous variable (OR per 1-SD increase, 1.07; 95% CI, 0.84–1.35 and OR per 1-SD increase, 1.03; 95% CI, 0.76–1.39, respectively). Whereas, TC/HDL-C ratio was still significantly associated with CAC after adjustment for total LDL-P concentration when TC/HDL-C ratio was examined both as a quartile (highest quartile OR, 2.01; 95% CI, 1.04–3.89) and a continuous variable (OR per 1-SD increase, 1.30; 95% CI, 1.01–1.68).

4. Discussion

In this cross-sectional study among an apparently healthy Japanese population, we found that lipoprotein particle profiles assessed by NMR were significantly associated with the presence of CAC after adjustment for non-lipid risk factors. This magnitude of association was comparable, but not superior, to that of standard lipids. Among the NMR-measured lipoproteins, the strength of association with CAC was largest for total LDL-P concentration. However, this association was weaker than that of either non-HDL-C or TC/HDL-C. To the best of our knowledge, this is the first study to directly compare NMR-measured lipoproteins with standard lipids and ratios in relation to CAC within a Japanese or an Asian population.

Our results support 2 prospective population-based studies, which reported that routine measurement of NMR lipoproteins is similar in incident CVD risk prediction to non-HDL-C and TC/HDL-C ratio, and is not recommended when a standard lipid panel is available [5,6]. However, in these previous studies, one was conducted among only women [5], and the other was a nested case–control study design [6]. Surprisingly, 2 cross-sectional studies that compared these lipid indices in association with subclinical atherosclerosis reported a stronger relation of NMR-based indices than standard lipids [7,8]. Therefore, only a limited number of Western studies directly compared the risk estimates of clinical CVD or subclinical atherosclerosis between NMR-based lipoprotein profiles and standard lipids, and showed conflicting results. Some prospective studies of the Japanese and US general populations also suggested NMR-based lipoprotein profiles as alternative lipid measures for improved risk assessment of CVD, but this was not assessed in comparison with non-HDL-C or lipid ratios [31–33]. Consistent with the prospective Western studies with hard

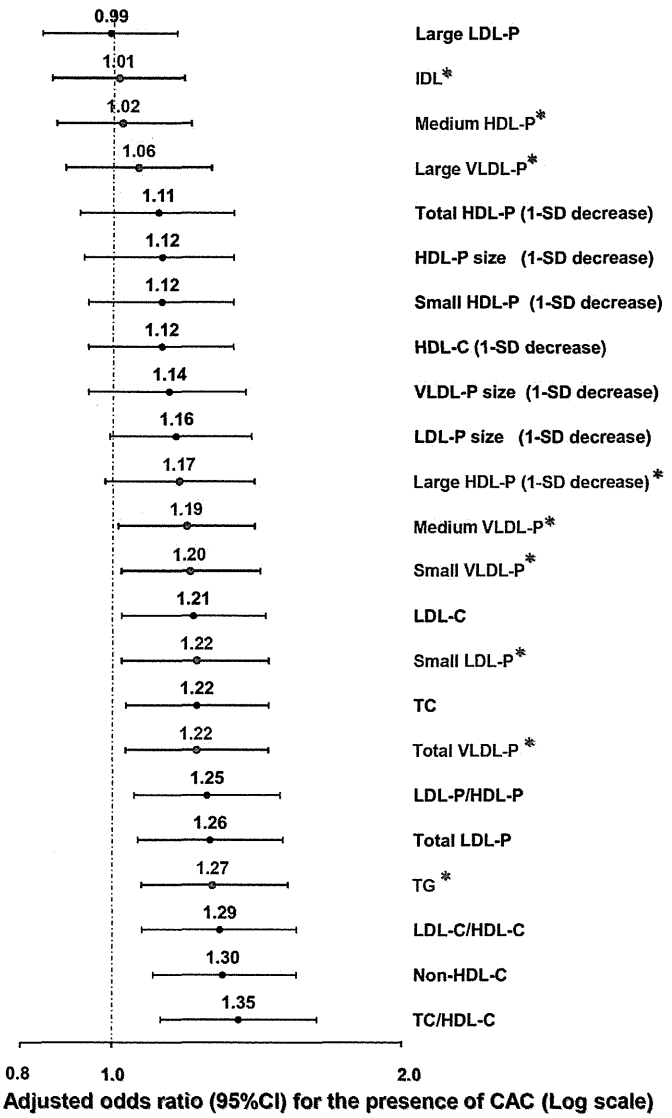

Fig. 1. Adjusted odds ratios and 95% CIs for the presence of coronary artery calcification (CAC score >0) per 1-standard deviation (SD) increase in lipid indices (per 1-SD decrease in the case of large HDL-P, total HDL-P, HDL-C, small HDL-P, HDL-P size, VLDL-P size, and LDL-P size) in apparently healthy Japanese men aged 40–79 years: SESSA. Adjustment for age, smoking status (former, current), ethanol consumption (g/day), body mass index, blood glucose, systolic blood pressure, medication status (hypertension, diabetes), and type of CT was performed. *Lipid indices with skewed distributions were log-transformed. Abbreviations: all abbreviations are as in Table 2.

endpoints [5,6], the association of CAC with NMR measures was comparable, but not predominant, over that with standard lipids in our study. This was the case in the Japanese general population where the burden of CAD, subclinical atherosclerosis, and lipid profiles are quite different compared with Western populations.

Non-HDL-C, defined as TC minus HDL-C, includes all of the potentially atherogenic lipid fraction (LDL-C, lipoprotein [a], IDL cholesterol, and VLDL remnants). TC/HDL ratio gives identical results to non-HDL-C/HDL-C ratio (note that TC/HDL ratio = 1 plus non-HDL-C/HDL-C ratio), and thus exclusively consists of non-HDL-C divided by an anti-atherogenic denominator (HDL-C). Inclusion of all of the atherogenic lipid fractions in the calculation of non-HDL-C and TC/HDL-C ratio may then provide an improved assessment of CVD risk. In fact, a prospective cohort study in the Japanese population showed that the predictive value of non-HDL-C for incident CAD is almost similar to that of LDL-C and recommended non-HDL-C as an alternative screening marker for primary prevention of CAD in the Japanese community [34].

Our data also support current guidelines that recommend the use of a standard lipid panel, especially non-HDL-C or TC/HDL-C ratio, for CVD risk assessment in clinical practice. [23,24,35,36] European, North American, and Japanese guidelines recommend the use of standard lipids for CVD risk assessment in asymptomatic individuals. Non-HDL-C is introduced as another means to refine risk estimation beyond LDL-C from Friedwald's formula, especially in combined hyperlipidemia, diabetes, metabolic syndrome, or chronic kidney disease [23,24,36]. TC/HDL ratio is useful for CVD risk estimation [23,24]. By contrast, a statement involving an international panel of lipid experts proposed that CVD risk may be more closely related to atherogenic lipoprotein particle concentrations than to LDL-C [37].

Our study has several limitations. First, the study design was cross-sectional. Therefore, causal and longitudinal relationships were not addressed. Second, we did not examine CVD outcome, although our data are consistent with large prospective studies with assessment of clinical CVD. Finally, our analyses were limited to apparently healthy Japanese men, and our results should not be generalized to women, other races, and patient populations.

In conclusion, the overall association of CAC with NMR-measured lipoprotein indices is comparable, but not superior to, that with standard lipids in a community-based sample of asymptomatic Japanese men who do not use lipid-lowering medication. Non-HDL-C and TC/HDL ratio are strongly associated with the presence of CAC. Therefore, our data support the clinical use of standard lipids, particularly non-HDL-C and TC/HDL ratio, which are highly effective and readily available for routine CVD risk assessment.

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Conflict of interest

There is no potential conflict of interest that relates to the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can http://dx.doi.org/10.1016/j.atherosclerosis.2014.07.019.

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