研究成果の刊行に関する一覧表

1) Nakamura M, Koyama I, Iso H, Sato S, Okazaki M, Kiyama M, Shimamoto T, Konishi M: Measurement performance of reagent manufacturers by Centers for Disease Control and Prevention/Cholesterol Reference Method Laboratory Network lipid standardization specified for metabolic syndrome-focused health checkups program in Japan. ***J Atheroscler Thromb*** 2009; 16:756-763.

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Original Article

Measurement Performance of Reagent Manufacturers by Centers for Disease Control and Prevention/Cholesterol Reference Method Laboratory Network Lipid Standardization Specified for Metabolic Syndrome-Focused Health Checkups Program in Japan

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Aim: This study was designed to clarify the current measurement performance of 7 reagent manufacturers for high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and triglycerides (TG) specified for the metabolic syndrome (MetS)-focused health checkups program in Japan.
Methods: Twenty HDLC, 21 LDLC and 9 TG analytical reagent/instrument/calibrator systems (system), and combinations of reagent lots, instrument models and calibrator lots, underwent Centers for Disease Control and Prevention (CDC)/Cholesterol Reference Method Laboratory Network (CRMLN) lipid standardization. Eighty and 100% systems were requested to achieve an accuracy of within ±1% and ±2% of the reference value, so that a clinical laboratory can meet the CDC criteria.
Results: The CDC performance criteria of HDLC, LDLC and TG require an accuracy of within ±5%, ±4% and ±5%, respectively. For HDLC, all 20 systems met the criteria. Fourteen (70.0%) and 18 (90.0%) systems were within ±1% and ±2%, respectively. For LDLC, 14 (66.7%) of 21 systems met the criteria, but 7 (33.3%) failed. Five (23.8%) and 17 (81.0%) systems were within ±1% and ±2%, respectively. For TG, 8 of 9 systems met the criteria. Two (22.2%) and 4 (44.4%) systems were within ±1% and ±2%, respectively. The minimum and maximum differences of a specified sample among manufacturers were 1.6 and 11.0 mg/dL for HDLC, 7.8 and 33.0 mg/dL for LDLC, and 2.8 and 27.4 mg/dL for TG, respectively.
Conclusion: Homogeneous HDLC methods are acceptable for MetS, but further accuracy improvement of homogeneous LDLC and TG methods will be needed because of their poor performance.

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Key words; Metabolic syndrome, HDLC, LDLC, CDC/CRMLN

Introduction

The Japanese lifestyle, including eating habits, has become more westernized with marked post-war economic growth, and living and medical care levels have markedly improved, but the nation is also facing a new health problem: metabolic disorders constituting obesity, dyslipidemia, and abnormal glucose tolerance[1-3]. Moreover, dynamic changes in social and living environments have brought major quantitative increases in atherosclerotic cardiovascular diseases, malignant tumors, and their associated risk factors[4-6]. Under such circumstances, the Japanese government

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developed a standard program, The Health Checkups and Healthcare Advice with a Particular Focus on Metabolic Syndrome (MetS). In April 2008, the program enforced the provision of health checkups focusing on MetS for health-insured 40 -74 -year-old Japanese people. Three lipids, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and triglycerides (TG), were measured, excluding total cholesterol.

This study was designed, (1) to clarify the current measurement performance of 7 reagent manufacturers supplying homogeneous HDLC, LDLC and TG kits, and (2) to request their 80% and 100% systems to achieve an accuracy of within ±1% and ±2% of the reference value, respectively, so that a clinical laboratory can meet the Centers for Disease Control and Prevention (CDC) criteria and join the MetS program.

Materials and Methods

Standardization Items and Participating Reagent Manufacturers

HDLC, LDLC and TG were selected for standardization. Seven Japanese reagent manufacturers participated in the CDC/Cholesterol Reference Method Laboratory Network (CRMLN) standardization program: Serotec Co., Ltd. (Hokkaido), Denka Seiken Co. Ltd. (Niigata), Sekisui Medical Co. Ltd. (Tokyo), UMA Co. Ltd. (Tokyo), Kyowa Medex Co. Ltd. (Tokyo), Wako Pure Chemical Industries, Ltd. (Osaka) and Sysmex Co. Ltd. (Hyogo).

Standardization Protocols for HDLC, LDLC and TG

Standardization of HDLC, LDLC and TG for the manufacturers through the CDC/CRMLN followed the HDL Cholesterol Certification Protocol[7] for Manufacturers (November, 2002), the LDL Cholesterol Certification Protocol[7] for Manufacturers (June, 2006) and the Triglyceride Certification Protocol7) for Manufacturers (October, 2003), respectively (http://www.cdc.gov/labstandards/crmln.htm); however, the TG protocol has not yet been implemented by the CRMLN and TG was not certified.

Reference Methods for Establishing the Reference Value

The reference method for HDLC is the designated comparison method (DCM). Samples were precipitated for separation using dextran sulfate (50 kDa)-Mg^{2+} reagent and HDLC in the supernatant was measured by the Abell-Kendall (AK) reference method for cholesterol. The reference method for LDLC used the beta-quantification method (BQ)[8]. The BQ method uses a three-step procedure involving ultracentrifugation, precipitation of the bottom fraction (BF) with heparin-Mn^{2+} reagent, and cholesterol quantification of both the BF and high-density-lipoproteins fractions with the AK method. Samples were given 2 spins of ultracentrifugation, and the BF cholesterol and HDLC were measured. LDLC, as defined by the National Cholesterol Education Program (NCEP), includes intermediate-density lipoprotein cholesterol (IDLC) and lipoprotein (a) (Lp(a)) cholesterol. The reference method for TG is detailed in the Procedure for the Triglyceride DCM (November, 2001). Samples were extracted with activated silicic acid and methylene chloride, and then dehydrated. The extract was dried by evaporation, hydrolyzed with KOH alcohol, and glycerol was enzymatically analyzed. Since TG DCM undergoes standardization by the CDC reference method using the chromotropic acid method, the Osaka Medical Center for Health Science and Promotion (OMC) can determine the reference value.

Samples

The standardization protocol for HDLC and LDLC allows the use of fresh, individual and preferred fasting serum or mixtures of serum from a maximum of 2 persons with 40 or more specimens. HDLC samples containing TG of 200 mg/dL or lower[9] are specified so as to include at least 5 samples each containing HDLC at concentration ranges of 20–29, 30–39, 40–49, 50–59, and 60–69 mg/dL. LDLC samples are specified so that 20% of samples each contain LDLC at 100 mg/dL or lower and 161–400 mg/dL, and 30% each contain 100–130 and 131–160 mg/dL; however, for TG, frozen samples, the same as for the LDLC measurement were used. The concentration of the internal quality control material was 30–60 mg/dL for HDLC and 130–160 mg/dL for LDLC. All specimens were collected in turn at a manufacturer and provided to both other participating manufacturers and the OMC by overnight express delivery.

Measurements

Measurements were performed in duplicate once a week at both the reagent manufacturer and OMC. The manufacturers attempted to standardize 20 systems for HDLC, 21 systems for LDLC and 9 systems for TG. A system is a combination of the reagent lot, instrument model and calibrator lot. Each clinical laboratory selects any of the systems in routine work for patients. The instruments used were model series of

Table 1. Performance Criteria for CRMLN Laboratories, Manufacturers and Clinical Laboratories

Item	Performance Criteria for CRMLN Laboratories		Performance Criteria for Manufacturers and Clinical Laboratories	
	Accuracy Criterion	Imprecision Criterion	Accuracy Criterion	Imprecision Criterion
HDLC	Bias ≤1 mg/dL	SD ≤1 mg/dL	Bias ≤5%	CV ≤4%
LDLC	Bias ≤2%	CV ≤1.5%	Bias ≤4%	CV ≤4%
TG	Bias ≤2.5%	CV ≤2.5%	Bias ≤5%	CV ≤5%

Hitachi, Toshiba, Olympus and Japan Electron Optics Laboratory (JEOL).

Performance Criteria in the CRMLN Reference Laboratory and Reagent Manufacturer

The performance criteria[10, 11] required for the lipid reference laboratory of the CRMLN, reagent manufacturers and clinical laboratories are shown by accuracy and imprecision in **Table 1**. The performance criteria of TG required for the lipid reference laboratory of the CRMLN are tentative. There are 9 grades to determine the pass or fail status for HDLC standardization: the square of the correlation coefficient, ≥0.975 [1], the %bias accuracy is ≤5% at concentrations of 40 [2] and 60 [3] mg/dL, the average relative %bias accuracy [4] and average absolute %bias accuracy [5] are ≤5%, the among-run coefficient of variation (CV) as precision is ≤4% [6], no significant difference is present at a significance level of α=5% on a *t*-test of bias (*t*-value) [7], only one within-method outlier is acceptable [8], and there are no between-method outliers [9]. There are 10 grades for LDLC standardization. the square of the correlation coefficient, ≥0.975 [1], the %bias is ≤4% at concentrations of 100 [2], 130 [3], and 160 [4] mg/dL, the average relative % bias [5] and average absolute %bias [6] are ≤4%, the among-run CV is ≤4% [7], there is no significant difference at a significance level of α =5% on a *t*-test of bias (*t*-value) [8], only one within-method outlier is acceptable [9], and there are no between-method outliers [10]. There are 4 tentative grades for TG standardization: the square of the correlation coefficient, ≥0.975 [1], the average %bias [2] and average absolute %bias [3] are ≤5%, and the among-run CV is ≤5% [4].

Statistical Analysis

The assayed results were compared using a CDC spreadsheet for HDLC and LDLC, but TG results were only evaluated statistically. The *t*-value is based on CV 4% and an allowable difference of 5%, assuming n-1 degrees of freedom. Among-run CV was calculated from the 20-day values of the internal quality control sample. For within-method outliers, tests of absolute and relative differences were performed. Only samples indicated by both absolute and relative tests were within-method outliers. For between-method outliers, tests of absolute and relative differences between the test method and the reference method were performed. Only samples that did not pass either test were between-method outliers.

Electrophoresis

All samples were analyzed by agarose and polyacrylamide gel electrophoresis.

Results

Measurement Performance of OMC

CRMLN lipid reference laboratories are evaluated bimonthly by the CDC. When the criteria are met, they are qualified to certify reagent manufacturers and clinical laboratories. The OMC measurement performance demonstrated that the accuracy for HDLC in March 2008 was +0.3 mg/dL in bias and the SD was 0.23 mg/dL, and the accuracy for LDLC in April 2008 was +1.6% in bias and the CV was 0.6%, while the accuracy for TG was +0.9% in bias and the CV was 0.4%. These findings were acceptable for standardization by OMC.

Measurement Performance of HDLC by Analytical Reagent/Instrument/Calibrator Systems

HDLC was measured for February 27, 2008 to April 2, 2008 with 6 different runs using 50 samples. **Table 2** shows the performance of 20 systems by the 9 grades for HDLC criteria. All systems demonstrated traceability to the HDLC accuracy base. The mean precision of all systems was 0.9% in among-run CV, and mean accuracy was 0.5% for average %bias and 2.4% for absolute %bias. The predicted bias at 40 mg/dL is at the specific medical decision point of Japan Atherosclerosis Society (JAS) Guidelines for the diagnosis and treatment of atherosclerotic cardiovascular diseases.

Table 2. HDLC Performance of 20 Analytical Reagent/Instrument/Calibrator Systems by 9 Grades

Analytical system	[1] R-square	[2] Predicted at 40 mg/dL		[3] Predicted at 60 mg/dL		[4] Avg %bias	[5] Avg abs %bias	[6] Among-Run CV	[7] t-value	[8] Within-method Outliers	[9] Between-method Outliers
		mg/dL	%bias	mg/dL	%bias						
1	0.9818	38.9	−2.7	58.6	−2.4	−2.7	3.5	0.5	0.95	0	0
2	0.9872	41.3	3.3	61.5	2.6	3.1	3.7	0.9	1.09	1	0
3	0.9898	40.8	2.0	60.4	0.7	1.8	2.7	0.7	0.63	1	1
4	0.9924	39.8	−0.4	58.9	−1.8	−0.7	2.1	0.5	0.25	0	0
5	0.9915	40.2	0.6	59.1	−1.5	0.1	2.2	0.7	0.03	0	0
6	0.9792	39.8	−0.5	59.4	−0.9	−0.7	3.1	0.7	0.25	1	0
7	0.9911	40.4	0.9	60.5	0.8	0.8	2.4	0.9	0.28	0	0
8	0.9917	40.4	1.1	60.1	0.1	0.9	2.1	0.5	0.32	0	0
9	0.9910	40.4	1.0	60.6	1.0	0.9	2.4	0.8	0.32	0	0
10	0.9910	40.5	1.2	61.2	2.0	1.2	2.7	1.1	0.42	0	0
11	0.9940	40.3	0.8	60.2	0.3	0.7	1.9	1.0	0.25	0	0
12	0.9937	40.1	0.2	59.9	−0.2	0.0	1.7	1.4	0.00	0	0
13	0.9921	40.5	1.3	60.1	0.2	1.0	2.1	2.1	0.35	0	0
14	0.9924	40.6	1.5	60.5	0.8	1.3	2.2	0.4	0.46	0	0
15	0.9934	40.2	0.4	60.0	−0.1	0.3	2.0	0.7	0.11	0	0
16	0.9927	39.8	−0.6	59.2	−1.4	−0.8	2.1	0.9	0.28	0	0
17	0.9916	40.1	0.3	59.7	−0.5	0.1	2.1	1.5	0.03	0	0
18	0.9927	40.7	1.7	60.6	1.0	1.5	2.2	0.3	0.53	0	0
19	0.9903	40.4	1.0	59.8	−0.3	0.7	2.3	1.5	0.25	0	0
20	0.9924	40.5	1.2	59.7	−0.4	0.8	2.2	0.4	0.28	0	0

Avg %bias: Average %bias
Avg abs %bias: Average absolute %bias

Measurement Performance of LDLC by Analytical Reagent/Instrument/Calibrator Systems

LDLC was measured for April 8, 2008 to May 27, 2008 with 6 different runs using 51 samples. Table 3 shows the performance of 21 systems by the 10 grades for LDLC criteria. Seven (33.3%) of all systems were smaller than 0.975 in the r-square, indicating poor stability and reproducibility in different runs. Increased absolute %bias found in systems 2, 3 and 4 is considered to be non-specific affinity to TG-rich lipoproteins, such as intermediate-density lipoproteins, very-low-density lipoproteins and chylomicron remnant from the analysis of electrophoresis. In system 7, both average %bias and average absolute %bias were over 4%, which is considered to be poor value assignment in the calibrator. The mean precision of 14 standardized systems was 0.9% in among-run CV, mean accuracy was 1.3% for average %bias and 2.9% for absolute %bias. Three (42.9%) of 7 manufacturers and 7 (33.3%) of 21 systems failed in terms of traceability to the LDLC accuracy base. The predicted biases at 2 concentrations, 130 and 160 mg/dL, are both ends of the medical decision point, 140 mg/dL, of the JAS Guidelines.

Measurement Performance of TG by Analytical Rea gent/Instrument/Calibrator systems

Table 4 shows the performance of 9 systems by the 4 grades of TG criteria. Eight of 9 systems demonstrated traceability to the TG accuracy base, while system 5 failed in terms of average absolute %bias. The difference between the maximum and minimum average %bias reached 9.2%. The mean precision of 8 standardized systems was 0.4% in among-run CV, and mean accuracy was 1.3% for average %bias and 2.9% for average absolute %bias. Two (22.2%) and 4 (44.4%) systems achieved within ±1% and ±2% from the reference value, respectively. Systems 7 and 8 were products from the same manufacturer. System 7 was calibrated with triolein as the standard and system 8 was calibrated with glycerol. There was a 6.1% difference in accuracy between the 2 systems and it was close to the theoretical value.

Achievement Rate of Manufacturer Systems

Table 5 shows the achievement rates of systems

Table 3. LDLC Performance of 21Analytical Reagent/Instrument/Calibrator Systems by 10 Grades

Analytical system	[1] R-square	[2] Predicted at 100 mg/dL		[3] Predicted at 130 mg/dL		[4] Predicted at 160 mg/dL		[5] Avg %bias	[6] Avg abs %bias	[7] Among-run CV	[8] t-value	[9] Within-method Outliers	[10] Between-method Outliers
		mg/dL	%bias	mg/dL	%bias	mg/dL	%bias						
1	0.9853	102.7	2.7	132.3	1.8	161.8	1.1	2.0	2.9	0.7	0.88	0	0
2	0.9702	101.0	1.0	134.2	3.2	167.3	4.6	2.5	5.4	0.4	1.10	0	0
3	0.9616	100.0	0.0	132.7	2.1	165.5	3.4	1.4	5.5	0.9	0.62	0	0
4	0.9501	101.2	1.2	133.9	3.0	166.7	4.2	2.5	6.2	0.5	1.10	0	0
5	0.9836	100.8	0.8	132.2	1.7	163.6	2.2	1.4	3.1	0.5	0.62	0	0
6	0.9841	101.9	1.9	133.8	2.9	165.6	3.5	2.6	3.7	0.5	1.15	0	0
7	0.9649	108.6	8.6	139.4	7.3	170.3	6.4	7.6	7.9	1.0	3.36	1	0
8	0.9698	97.6	−2.4	128.5	−1.1	159.4	-0.4	−1.5	3.8	0.7	0.66	0	0
9	0.9701	97.7	−2.3	128.6	−1.0	159.6	-0.3	−1.4	3.8	0.9	0.62	0	0
10	0.9700	98.6	−1.4	129.7	−0.3	160.7	0.4	−0.6	3.5	0.8	0.27	0	0
11	0.9874	100.0	0.0	131.2	0.9	162.4	1.5	0.7	2.4	1.5	0.31	0	0
12	0.9878	100.9	0.9	132.2	1.7	163.6	2.3	1.5	2.8	0.8	0.66	0	0
13	0.9864	99.5	−0.5	130.3	0.2	161.1	0.7	0.0	2.4	1.5	0.00	0	0
14	0.9860	101.1	1.1	132.6	2.0	164.2	2.6	1.8	3.0	1.1	0.80	0	0
15	0.9849	100.7	0.7	132.7	2.0	164.7	2.9	1.6	3.0	1.1	0.71	0	0
16	0.9872	101.1	1.1	132.9	2.3	164.8	3.0	1.9	3.1	1.0	0.84	0	0
17	0.9821	100.2	0.2	131.8	1.4	163.5	2.2	1.1	2.9	1.6	0.49	0	0
18	0.9817	100.8	0.8	133.2	2.5	165.6	3.5	2.0	3.3	0.5	0.88	0	0
19	0.9828	101.4	1.4	131.6	1.2	161.7	1.1	1.2	2.6	1.1	0.53	0	0
20	0.9864	101.1	1.1	130.3	0.2	159.5	−0.3	0.5	2.5	0.6	0.22	0	0
21	0.9868	99.6	−0.4	129.3	−0.5	159.0	−0.6	−0.5	2.5	0.5	0.22	0	0

Avg %bias: Average %bias
Avg abs %bias: Average absolute %bias

Table 4. TG Performance of 9 Analytical Reagent/Instrument/Calibrator Systems by 4 Grades

Analytical system	[1] R-square	[2] Avg %bias	[3] Avg abs %bias	[4] Among-Run CV
1	0.9940	4.9	4.9	0.3
2	0.9985	3.7	3.8	0.3
3	0.9985	0.0	1.3	0.3
4	0.9993	1.1	1.4	0.2
5	0.9963	4.9	5.2	0.2
6	0.9991	2.4	2.5	0.7
7	0.9988	1.8	2.4	0.6
8	0.9988	−4.3	4.5	0.7
9	0.9991	0.5	2.1	0.2

Avg %bias: Average %bias
Avg abs %bias: Average absolute %bias

which met the accuracy criteria for HDLC, LDLC and TG. The pass rate for HDLC was very accurate and acceptable for MetS, but LDLC and TG were not accurate for MetS at the manufacturers' sites.

Value Differences Among Reagent Manufacturers

The minimum and maximum values for HDLC, LDLC and TG assayed in the same samples among manufacturers are shown in Table 6. Values were calculated based on all values assayed by the same type of instrument in all systems. These findings suggest differences among the reagent manufacturers.

Value Differences by Instrument

The minimum and maximum values for HDLC and LDLC measured in the same samples among analytical instruments are shown in Table 7. Values were calculated based on all values measured by the same reagent and calibrator using instruments from Hitachi, Toshiba, Olympus and JEOL. The TG values were not calculated. The difference in values among instruments suggest that it is a factor in the evaluation of HDLC and LDLC.

Table 5. Achievement Rate of Analytical Reagent/Instrument/Calibrator System met the Accuracy Criteria

Item	Analytical System	Accuracy					
		Within ± 1%	Within ± 2%	Within ± 3%	Within ± 4%	Within ± 5%	± 5% Over
HDLC	20	70.0%	90.0%	95.0%	100.0%		
LDLC	21	23.8%	81.0%	95.2%			100.0%
TG	9	22.2%	44.4%	55.6%	66.7%	100.0%	

Table 6. Minimum and maximum values in the same samples among reagent manufacturers

Item	Minimum value	Maximum value
HDLC	1.6 mg/dL	11.0 mg/dL
LDLC	7.8 mg/dL	33.0 mg/dL
TG	2.8 mg/dL	27.4 mg/dL

Table 7. Minimum and maximum values in the same samples among analytical instruments

Item	Minimum value	Maximum value
HDLC	0.0 mg/dL	4.4 mg/dL
LDLC	0.2 mg/dL	10.4 mg/dL

Discussion

The MetS-focused health checkups program indicates that "the same value is obtained at any clinical laboratory in the same patient when the reference material is used". For this purpose, we requested that the achievement rate of systems at the manufacturer level should be at least 80% for within ± 1% and 100% for within ± 2% from the reference value. Otherwise, accuracy control in clinical laboratories adopting a heterogeneous system in which they can freely select and change reagents, instruments, calibrators or analytical parameters will be impossible. We assumed that this requirement is achievable because all manufacturers had previously undergone standardization once every two years 5–6 times over 10 years. Seven manufacturers demonstrated traceability to the HDLC accuracy base using healthy fresh samples, which did not contradict the results of the past 6 standardizations conducted from 1996 to 2006. Based on these findings, we found that HDLC can be adopted for the MetS-focused health checkups program. However, we think that there are two potential difficulties in standardizing homogeneous methods. First is the bias associated with the different assay principles of the various methods. Absorbance from the LDLC and very-low-density lipoprotein cholesterol (VLDLC) is differentiated from the absorbance used to quantify HDLC, resulting in different correlation relationships with the reference method. Second is the altered matrix characteristics of calibrators and controls[12]. The only reliable approach to establishing appropriate calibration for acceptable accuracy must be based on comparison studies conducted with fresh specimens. Calibrators must be assigned set points that result in accurate results on fresh patient samples. Regarding the variation in HDLC values among manufacturers, the minimum and maximum differences were 1.6 and 11.0 mg/dL, respectively, showing a comparatively larger bias than expected. This variation should be considered when evaluating HDLC values in clinical trials and epidemiological studies[13-15]. Only 4 companies established traceability to the LDLC accuracy base and 3 companies failed. Five (23.8%) systems achieved within ± 1% and 17 (81.0%) systems achieved within ± 2% of the reference value. Failures in terms of accuracy suggest a problem in value assignment in the calibrator or setting parameters in the analytical instrument. The CRMLN reference laboratory can assist in the value assignment of calibrators and control of manufacturers and in the performance of LDLC standardization every year[16]. Failures in the correlation coefficient suggested a problem regarding reproducibility among runs. Care will be necessary to incorporate LDLC into the MetS-focused health checkups[17]. After clarifying these problems, the 3 failed companies underwent re-standardization for about one month from November to December 2008, and all met the LDLC requirement. Considering the measurement limit of TG, the merit of a homogenous LDLC method is greater than Friedewald's calculation equation. Regarding the variation in LDLC values among manufacturers, the minimum and maximum differences were 7.8 and 33.0 mg/dL, respectively, showing a large bias. Eight systems for TG met the precision and accuracy criteria; however, the large bias suggested an accuracy problem in TG. Caution is necessary when incorporating TG into MetS-focused

health checkups. Additionally, for the TG calibrator, conversions to triolein and glycerol is used, i.e., double standards, in clinical laboratories. Theoretically, there should be an 8% difference between the 2 converted values. Variation in TG tended to be overlooked, but confirmation of the conversion method is necessary to compare the values among studies and laboratories. To further improve TG accuracy, new reference methods using isotope dilution/gas chromatography/mass spectrometry are being established in CDC and OMC. Iso *et al.* reported that nonfasting serum TG levels predicted the incidence of coronary heart disease among Japanese 4,452 men and 6,616 women aged 40-69 years in a 15.5-year prospective study ending in 1997[18]. This result suggests that it is very important for TG to be measured more accurately[19, 20]. There are 3 main factors leading to variation among the values: reagent, instrument and calibrator. Since the reagent and calibrator are derived from reagent manufacturers, many standardization problems are attributed to reagent manufacturers and variations due to the instruments are overlooked in many cases. We found differences in HDLC and LDLC among 4 instruments in systems using the same reagent and calibrator. The maximum difference accounted for 40% of the variation among instrument manufacturers in HDLC and 32% in LDLC. Based on these findings, not only variation among reagent manufacturers, but also among instrument manufacturers, should be considered when evaluating results. According to the lipid matrices of samples confirmed by the two electrophoresis methods, 80% of all samples used for HDLC and LDLC were healthy with regard to the electrophoresis pattern, while the remaining 20% did not show sufficient morbidity to be regarded as dyslipidemia, although a high VLDL-TG level or mid-band was noted. Samples with biases of within ± 1% and more than ± 1% from the reference value were compared. No marked difference was noted in HDLC between groups, but the presence or absence of a mid-band and the VLDLC level may have affected the degree of bias. The high-performance liquid chromatography (HPLC) method based on particle sizes can give useful qualitative and quantitative information about abnormal lipoproteins[21]. We examined the assessment of between-instrument variations in the HPLC method for serum lipoproteins and reported good traceablity to CDC reference methods for total cholesterol and HDLC[22]. We reported several discrepancies in LDLC levels using the HPLC method and the CDC reference method using lipoprotein abnormalities, such as lipoprotein lipase deficiency, E2/2 type III hyperlipidemia, cholesteryl ester transfer protein deficiency and hyper Lp(a) lipoproteinemia[23, 24]. Another HPLC method with anion-exchange chromatography has been developed by Hirowatari *et al.*[25] who reported large discrepancies in LDLC values between anion-exchange HPLC and a homogeneous assay for cholestasis patients, including lipoprotein X[26].

In conclusion, we clarified the current measurement performance of HDLC, LDLC and TG in systems at the manufacturer level specified for a MetS-focused health checkups program in Japan. The results suggest: (1) homogeneous HDLC methods can be adopted in the program because of high accuracy, but (2) homogeneous LDLC methods are still poor in terms of both accuracy and stability, and (3) TG methods require further improvement in accuracy. We demonstrated that LDLC accuracy can be improved by re-evaluation of traceability to the BQ method through a fresh split sample comparison by value assignment of the calibrator and control serum.

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Hemoglobin A1c in predicting progression to diabetes☆

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ABSTRACT

The predictive value of hemoglobin A1c (HbA1c) in comparison to fasting plasma glucose (FPG) is evaluated for 5-year incident diabetes (DM), as HbA1c may be more practical than FPG in the screening for DM in the future. Of 1189 non-DM subjects aged 35–89 years old from the Funagata Study, 57 subjects (4.8%) had developed DM on the WHO criteria at 5-year follow-up. The odds ratio (95% confidence interval: CI) for a one standard deviation increase in FPG/HbA1c was 3.40 (2.44–4.74)/3.49 (2.42–5.02). The area under the receiver operating characteristic curve for FPG/HbA1c was 0.786 (95% CI: 0.719–0.853)/0.785 (0.714–0.855). The HbA1c corresponding to FPG 5.56 mmol/l was HbA1c 5.3%. There was no statistical difference in sensitivity between FPG 5.56 mmol/l and HbA1c 5.3% (61.4% vs. 56.1%), while specificity was higher in HbA1c 5.3% than FPG 5.56 mmol/l (87.8% vs. 82.5%, p-value < 0.001). 0.001). The fraction of incident case from those with baseline IGT was similar between the groups, however the fraction of people above the cut-off was significantly lower in HbA1c 5.3% than FPG 5.56 mmol/l (14.3% vs. 19.6%, p-value < 0.001). HbA1c is similar to FPG to evaluate DM risk, and HbA1c could be practical and efficient to select subjects for intervention.

1. Introduction

The prevalence of type 2 diabetes (T2DM) is increasing rapidly worldwide, and emerging as a serious health issue [1]. Recent clinical trials have demonstrated that lifestyle or pharmacological interventions in subjects with impaired glucose tolerance (IGT) can delay or prevent T2DM [2–4]. More recent epidemiological study [5] and clinical trial [6] have shown that aggressive glycemic control should be started as early as possible to delay or prevent serious diabetes-related complications in subjects with DM. Thus, high-risk subjects for T2DM should be identified at early stage of the disease for intensive interventions.

In Japan, people with possible (hemoglobin A1c [HbA1c] 5.6–6.0%) and probable (HbA1c ≥6.1% and under treatment of diabetes) DM increased from 16.2 million in 2002 to 22.1 million in 2007 among the general population over 20 years old, representing an average 7.3% increase in rate per year [7]. The high-risk approach where either FPG or HbA1c is

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Abbreviations: ADA, American Diabetes Association; CI, confidence intervals; BMI, body mass index; DM, diabetes mellitus; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; IGT, impaired glucose tolerance; JDS, Japan Diabetes Society; OGTT, oral glucose tolerance test; OR, odds ratio; ROC, receiver operating characteristic; Wc, Waist circumference; WHO, World Health Organization; 2 h PG, 2 h plasma glucose.

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incorporated into the general health check targeted future lifestyle-related diseases including DM has been launched in 2008 [8]. Although 2 h plasma (2 h PG) on an oral glucose tolerance test (OGTT) is a better predictor of DM than FPG [9,10], an OGTT is abandoned at opportunistic screening for DM. The simple and inexpensive substitutes would be required at primary health care. To date, both HbA1c and FPG are significant predictors of DM in some studies [11,12]. However, these studies used the American Diabetes Association (ADA) criteria [13] for the diagnosis of DM and the impact of HbA1c on incident DM based on 2 h PG was not taken into account. Thus, the aim of the current study was to assess the predictabilities of baseline FPG and HbA1c for DM based on the World Health Organization (WHO) criteria [14] at 5-year follow-up, by comparing baseline 2 h PG on an OGTT. Moreover, the cut-off points on baseline HbA1c were examined with respect to the prediction of DM at 5-year follow-up.

2. Subjects and methods

Funagata Study has been described previously [15]. Briefly, the Funaga Study is a population-based study conducted in an agricultural area 400 km north of Tokyo to clarify the risk factors, related conditions, and consequence of type 2 DM. The baseline data from the 2nd survey performed between 18th June1995 and 6th July 1997 consisted of 2154 subjects aged 35–89 years (participation rate: 48.4%). Of those, 1189 subjects without DM on the 1999-WHO criteria [14] were repeatedly performed an OGTT at the 3rd survey conducted between 16th June 2000 and 7th June 2002.

In both baseline and 5-year follow-up, blood samples were drawn from the antecubital vein after overnight fasting for measurement of FPG and lipids (enzymatic and direct methods) followed by an 75 g OGTT (Trelan-G®, Shimizu Pharmaceutical, Shimizu) in subjects without a treatment of DM. HbA1c was measured after the calibration standardized of the Japan Diabetes Society (JDS) [16,17] and the JDS assigned HbA1c values, which is 0.3% lower than the National Glycoprotein Standardization Program assigned values [18], were used in the present study. Intra-assay coefficient of variation for HbA1c was 1.0% at values 5.2% and 10.5%. Waist circumference (Wc) was measured at the navel level at the end of expiration under normal breathing in a standing position. Systolic and diastolic blood pressures were measured in the sitting position after a 5 min rest using a mercury sphygmomanometer. All participants were questioned about their smoking and alcohol habits.

2.1. Statistical analyses

McNemar's test was used to compare proportions between dependent samples. The 5-year cumulative incidence of DM was calculated as the number of subjects who developed DM at 5-year follow-up divided by the sum of duration of follow-up for each subject, in the three glucose categories for FPG, 2 h PG and HbA1c, respectively, as follows: FPG <5.05, 5.05–5.55, 5.56–6.99 mmol/l, 2 h PG <5.60, 5.60–7.79, 7.80-11.09 mmol/l, and HbA1c <5.0, 5.0–5.2, ≥5.3%. The FPG 5.56 mmol/l and 2 h PG 7.80 mmol/l were chosen, as they are defined as the lower limit of abnormal glucose metabolism in non-DM glucose range [14]. The HbA1c 5.3% was chosen, as it corresponds to FPG 5.56 mmol/l in the receiver operating characteristic (ROC) curve analysis [19] described below. The below these cut-offs, subjects were equally divided into cited group for FPG, 2 h PG and HbA1c, respectively.

Odds ratios (ORs) for the presence of DM at 5-year follow-up were estimated by using logistic regression analysis and reported with their 95% confidence intervals (CIs). The model adjusted for age (continuous), sex (categorical), Wc (continuous), FPG, 2 h PG or HbA1c (categorical) was made and tested by one by one for following explanatory variables: systolic blood pressure (continuous), cholesterol (continuous), triglyceride (continuous), high density lipoprotein cholesterol (continuous), smoking status (categorical, none/past smoker/current smoker), alcohol habits (categorical, none/drink occasionally/drink regularly) and family history of DM (categorical, none/present in first degree relatives). A variable of family history of DM, which came out to be significant in the former model, was fitted in a final model with age, sex, Wc and variables for FPG, 2 h PG or HbA1c. The subsequent logistic regression model, in which a continuous variable for a one standard deviation increase in FPG (0.58 mmol/l), 2 h PG (1.83 mmol/l) or HbA1c (0.4%) was entered, was fitted to see which of the glucose index has the strongest impact on the development of DM.

2.1.1. *Performance of three glucose indices as screening tests for DM at 5-year follow-up*

The ability of baseline FPG, 2 h PG and HbA1c to predict the incidence of DM at 5-year follow-up was determined by computing sensitivity and specificity and plotting them in a ROC curve [19]. The optimal cut-off maximizing sum of sensitivity plus specificity was explored for each glucose indicator. The sensitivity, specificity, positive predictive value (PPV) and false negative predictive value (NPV) for DM at 5-year follow-up and the proportion of subjects above the cut-off were calculated at baseline FPG 5.56 mmol/l and 2 h PG 7.80 mmol/l. The same calculation was made for HbA1c 5.1%, 5.2%, 5.3% and 5.4%.

The study was approved by the Institutional Review Board of Yamagata University and the informed consent to participate was obtained from all participants. All statistical analyses were performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL, USA). A p-value < 0.05 was considered as statistically significance.

3. Results

During a 5-year follow-up period, 34 men (6.8% [95% CI: 4.6–9.0]) and 23 women (3.3% [2.0–4.7]) developed DM. The overall cumulative 5-year incidence density of DM was 12.1 (95% CI: 8.9–15.2) per 1000 person years of follow-up for men and women combined (Table 1).

3.1. *Incidence density and risk prediction of DM at 5-year follow-up from baseline FPG, 2 h PG, or HbA1c*

The 5-year cumulative incidence density and the multivariate ORs of DM at 5-year follow-up were significantly higher in subjects with the highest glucose category than the lowest

Table 1 – Incidence density and adjusted odds ratios for the presence of DM at 5-year follow-up according to baseline glucose categories.

	Number of subjects (%)	Number of incident case (incident case from IGT)	Incidence density of DM 1000 person-years (95% CI)	[a] Adjusted ORs for DM (95% CI)
Fasting plasma glucose (mmol/l)				
<5.05	507 (42%)	8 (1)	4.0 (1.2–6.7)	1.00
5.05–5.55	449 (38%)	14 (9)	7.9 (3.8–12.0)	1.72 (0.71–4.19)
5.56–6.99	233 (20%)	35 (25)	37.8 (25.5–50.1)	7.53 (3.35–16.93)
2 h plasma glucose (mmol/l)				
<5.60	512 (43%)	6 (0)	3.0 (0.6–5.3)	1.00
5.60–7.79	541 (46%)	16 (0)	7.5 (3.8–11.1)	2.38 (0.91–6.26)
7.80–11.09 (IGT)	136 (11%)	35 (35)	64.8 (44.1–85.6)	20.64 (8.13–52.37)
HbA1c (%)				
<5.0	559 (47%)	8 (2)	3.6 (1.1–6.1)	1.00
5.0–5.2	460 (39%)	17 (7)	9.3 (4.9–13.7)	2.14 (0.91–5.05)
≥5.3	170 (14%)	32 (26)	47.4 (31.4–63.4)	10.06 (4.44–22.79)
Total				
	1189 (100%)	57 (35)	12.1 (9.0–15.2)	

[a] Adjusting for age, sex, waist circumference, and family history of DM.

glucose category for FPG, 2 h PG and HbA1c (Table 1). There was no difference in the 5-year cumulative incidence density between three glucose indicators for each of the lowest, middle and the highest glucose category.

Modeling with continuous FPG, 2 h PG or HbA1c, the risk for DM at 5-year follow-up related to a one standard deviation increase in FPG, 2 h PG and HbA1c were 3.40 (2.44–4.74), 4.76 (3.30–6.86) and 3.49 (2.42–5.02), respectively.

3.2. *ROC curve analyses predicting DM from baseline FPG, 2 h PG, or HbA1c*

The area under the ROC curve for DM at 5-year follow-up was not statistically different across three glucose indicators: 0.830 (0.767–0.893) for 2 h PG, 0.786 (0.719–0.853) for FPG and 0.785 (0.714–0.855) for HbA1c (Fig. 1). The optimal cut-offs for FPG, 2 h PG and HbA1c were 5.36 mmol/l, 7.52 mmol/l and 5.1%, respectively. The HbA1c 5.3% gave the same sum of sensitivity plus specificity as FPG 5.56 mmol/l/l.

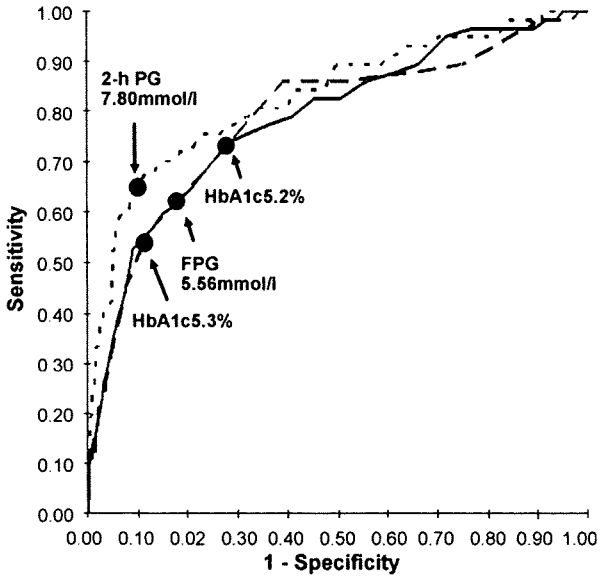

Fig. 1 – Receiver operating characteristic curves for incident diabetes at 5-year follow-up: baseline FPG (solid line), 2 h PG (dotted line) and HbA1c (solid and dotted line) among 1189 non-diabetes subjects at baseline.

3.3. *Performance as the screening test for future DM at various Pre-DM glucose cut-offs*

There was no statistical difference in sensitivity and 100-PPV between FPG 5.56 mmol/l, 2 h PG 7.80 mmol/l, HbA1c 5.2% and HbA1c 5.3%. The specificity was the highest in 2 h PG 7.80 mmol/l, the second highest in HbA1c 5.3%, followed by FPG 5.56 mmol/l, and the lowest in HbA1c 5.2% (all p-values <0.01). There was a precise reverse order in the proportion of subjects above the cut-off (all p-values <0.05).

The distribution of incident case of DM from subjects with baseline IGT was almost similar between the categories for baseline FPG and baseline HbA1c (Table 1). The proportion of incident case of DM from subjects with baseline IGT was significantly higher in those with baseline HbA1c 5.2% (89%, 31/35)(p-values <0.001) than that in those with baseline FPG 5.56 mmol/l (71%, 25/35) or baseline HbA1c 5.3% (74%, 26/35).

4. Discussion

The FPG is an established predictor of DM and considered as a relevant screening test for DM in the future [9–12]. However, blood sampling at fasting state in the morning is oftentimes difficult to perform in general population. Our study has shown that HbA1c has a similar ability to FPG for evaluating future DM risk and for detecting incident cases of DM, especially from the group of subjects with IGT at baseline. Obtained data also demonstrated that 2 h PG on an OGTT had a slightly better predictability for future DM than FPG or HbA1c, which is partly accordance with European reports [9,10]. However, its use as an initial screening test is unrealistic. In the screening at non-fasting state, HbA1c could be practically

and efficiently used to identify subjects at high-risk for DM who should be targeted for intensive prevention intervention.

The 2 h PG depends on insulin secretary capacity of pancreatic beta cells, peripheral insulin sensitivity, and hepatic glucose output and uptake whereas FPG largely depends on hepatic glucose production. While HbA1c reflects glucose metabolism over the past 1–2 months [16], can be converted into the estimated average glucose levels [20], has smaller variability than FPG and 2 h PG [21], and is closely correlated with post-load glucose in its low range and correlated with FPG in its high range [22]. Thus, HbA1c could cover a wider range of pathophysiological processes of DM than FPG. In our study, HbA1c showed almost the same overall predictability for DM in the future as FPG. In some previous studies, HbA1c seemed to be inferior to FPG with respect to the risk prediction and detection [11,12]. This might partly be due to the application of ADA criteria for the diagnosis of DM [11,12]. In our data, 70% of new cases of DM was identified by isolated 2 h PG (data not shown) and these subjects would not be identified as DM by the ADA criteria. In our country, HbA1c ≥6.5% has been used as a supportive test for the diagnosis of DM for past 10 years [23]. The International Expert Committee appointed by the ADA, the European Diabetes Association for the Study of Diabetes, and the International Diabetes Federation has recommended diagnosing DM by using HbA1c, since June 2009 [24]. Moreover, HbA1c has been provided a treatment target for patients with DM in many organizations including JDS [23]. Thus, HbA1c could be used in different stages of the diseases: screening, diagnosis and treatment. Meanwhile, HbA1c measurement by enzymatic method (Arkray, Kyoto) has become possible at a reasonable cost [25]. This satisfactorily correlates with HbA1c measurement by the HPLC method, does not need standardization, and is more economical than it measurement by HPLC method. This might be a rationale for recommending HbA1c in evaluating future DM risk.

Recently, we have shown that FPG ≥5.56 mmol/l is the better predictor than metabolic syndrome or a constellation of cardiovascular risk factors except for FPG ≥5.56 mmol/l regardless of abdominal adiposity in the Funagata Study [26]. The same trend was obtained when HbA1c ≥5.3% replaced FPG ≥5.56 mmol/l (data not shown). This highlighted glucose itself as the screening test for DM in the future. In our data, HbA1c 5.3% corresponded to FPG 5.56 mmol/l for predicting DM (Fig. 1 and Table 2) and both cut-offs identified similar risk of DM (Table 1) and had equal detection rate of DM, especially from the group of subjects with baseline IGT (Tables 1 and 2). On the other hand, the proportion of people above the cut-off was significantly lower in HbA1c 5.3% than FPG 5.56 mmol/l. Thus, HbA1c 5.3% rather than FPG 5.56 mmol/l might be efficient to identify those targeted for intensive intervention. Since the decision of the screening cut-off is tentative, the cut-off for HbA1c applied in Japan of 5.2% [8] might be too low in our study subjects. Since HbA1c 5.2% could identify significantly more incident cases from those with IGT than FPG 5.56 mmol/l or HbA1c 5.3%, the use of HbA1c 5.2% would make markedly high proportion of subjects (= one third of the entire screened population) who would be followed by intensive intervention.

There are limitations in our study. First, despite concerted efforts to maximize follow-up, the participation rate at 5-year of follow-up was 60%, which, although comparable to other studies of this nature, could potentially bias our results. When comparing baseline characteristics between those who did and did not participate in follow-up, the participants were younger and were healthier than non-participants (data not shown). This is in line with the frequent observation of "healthy participants' effect", which has also been reported in other studies [27]. This would lead to an underestimation of the true cumulative incidence in the general population, and thus our results are conservative. Second, the study population is approximately 10-years older than the representative sample of the Japanese general population [7], and this may have influenced our results. The relevance of Japanese cut-off of 5.2% for HbA1c to screen subjects requiring health guidance in the screening program [8] should be further examined in other Japanese studies. Third, FPG and 2 h PG in this population were assessed only once at both baseline and follow-up. The inter- and intra-coefficients of variations in glucose values may have caused some random misclassification in glucose categories [21], and thereby influenced our results. Fourth, the total number of incident cases is too small to obtain conclusive cut-off discriminating risks and performance as the screening between different strata. Fifth, we did not run sex-stratified analysis due to limited number of incident cases but did adjustment by sex. Since the crude proportion of incident case in men was double-folds higher than women, the overall predictabilities of DM based on ROC curve analysis did not differ between sexes for each glucose indicators or not differ across three glucose indicators in both

Table 2 – Performance (%) [95% confidence interval] of cut-offs on three glucose indicators for predicting DM at 5-year follow-up.

Variables	Cut-offs	Number (%)	% Sensitivity	% Specificity	100-Positive predictive value (%)	100-Negative predictive value (%)
FPG	5.56 mmol/l	233 (19.6)	61.4 [48.8–74.0]	82.5 [80.3–84.7]	85.0 [80.4–89.6]	2.3 [1.4–3.3]
2 h PG	7.80 mmol/l	136 (11.4)	61.4 [48.8–74.0]	91.1 [89.4–92.7]	74.3 [66.9–81.6]	2.1 [1.2–3.0]
HbA1c	5.1%	490 (41.2)	86.0 [76.9–95.0]	61.0 [58.2–63.9]	90.0 [87.3–92.7]	1.1 [0.4–1.9]
	5.2%	360 (30.3)	73.7 [62.3–85.1]	71.9 [69.3–74.5]	88.3 [85.0–91.6]	1.8 [0.9–2.7]
	5.3%	170 (14.3)	56.1 [43.3–69.0]	87.8 [85.9–89.7]	81.2 [75.3–87.1]	2.5 [1.5–3.4]
	5.4%	113 (9.5)	45.6 [32.7–58.5]	92.3 [69.3–74.5]	77.0 [69.2–84.8]	2.9 [1.9–3.9]

FPG: fasting plasma glucose, 2 h PG: 2 h plasma glucose.

sexes (data not shown). Sixth, the application of micro- and macro-vascular complication as the hard end point was not unable in the current study. However, notwithstanding the limitations, our study has notable strengths, being population-based, consisting of both men and women, having FPG and 2 h PG to enable rigorous biochemical diagnosis of DM based on either FPG or 2 h PG criteria and a well-phenotyped sample at baseline and follow-up.

In conclusion, HbA1c can be practically used to screen high-risk of future DM in a general Japanese population. It could also effectively be used in association with IGT who could be targeted for intensive prevention intervention.

Conflicts of interest

The authors declare that they have no conflict of interest.

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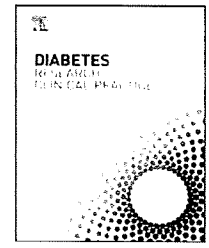

Brief report

Raised fasting plasma glucose a better predictor of diabetes than the IDF definition of the metabolic syndrome☆

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ABSTRACT

Raised FPG was better at identifying future diabetes than either IDF MetS or a constellation of risk factors except for raised FPG with and without abdominal adiposity. This should shed light on a screening program for future DM in Japan.

The metabolic syndrome (MetS) concept has been used as a screening test for preventing cardiovascular diseases (CVD) and type 2 diabetes mellitus (DM) in Japan since April 2008 [1]. Abdominal adiposity is a prerequisite of MetS, and in association with a cluster of metabolic abnormalities can be followed to identify subjects at high risk for developing DM [1]. This study aimed at clarifying the extent to which either the MetS or a constellation of risk factors except glucose with or without abdominal adiposity, can be used to identify future DM in comparison with raised fasting plasma glucose (FPG).

The study enrolled 779 non-diabetic subjects aged 40–74 years without a prior CVD event or risk factors, and followed them for 5 years, with 3092 person years accumulated, from the Funagata Diabetes Study [2]. World Health Organization criteria [3] were used to diagnose DM. The FPG $\geq$ 5.56 mmol/l was used to define raised FPG. The International Diabetes Federation (IDF) definition [4] with three different cut-offs of waist circumference (Wc) for abdominal adiposity was used to identify MetS. The cut-offs were as follows: IDF cut-offs for Japanese (m/f = 90/80 cm) [4]; current cut-offs in Japan

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Table 1 – Incidence density of DM (per 1000 person years) and its 95% confidence interval in subjects with different cluster of metabolic abnormalities using three cut-offs of waist circumference (WC) for diagnosis of abdominal adiposity.

	Abnormal obesity							
	Absent				Present			
	≤1[a]		≥2[a]		≤1[a]		≥2 (MetS)[a]	
	Absent[b]	Present[b]	Absent[b]	Present[b]	Absent[b]	Present[b]	Absent[b]	Present[b]
IDF cut-offs for Japanese								
Number of subjects	461	88	52	12	102	24	23	17
Number of incident case	7	12	0	1	0	4	1	1
Incidence density	3.8 (1.0–6.7)	34.3 (15.2–53.4)	–	20.9 (−19.7–61.5)	–	42.0 (1.7–82.3)	10.9 (−10.4–32.3)	14.7 (−14.0–43.6)
Current Japanese cut-offs								
Number of subjects	494	86	61	16	69	26	14	13
Number of incident case	5	10	1	2	2	6	0	0
Incidence density	2.6 (0.3–4.8)	29.3 (11.4–47.2)	4.1 (−3.9–12.2)	31.4 (−11.4–74.2)	7.3 (−2.8–7.4)	58.1 (13.0–103.2)	–	–
Proposed Japanese cut-offs								
Number of subjects	419	73	45	9	144	39	30	20
Number of incident case	5	9	0	1	2	7	1	1
Incidence density	3.0 (0.4–5.6)	31.0 (11.1–51.0)	–	27.9 (−26.0–81.8)	3.5 (−1.3–8.3)	45.2 (12.5–78.0)	8.4 (−8.0–24.8)	12.6 (−11.9–37.1)

Metabolic abnormalities include: serum triglycerides ≥1.7 mmol/l; high-density cholesterol <1.03/1.29 mmol/l in *m/f*; systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg. Raised FPG: FPG ≥5.56 mmol/l.

[a] Number of metabolic abnormalities except raised FPG.

[b] Condition of raised FPG.

(*m/f* = 85/90 cm) [1], and proposed cut-offs which corresponded to ≥2 non-essential risk factors (*m/f* = 85/80 cm) [5,6].

The 5-year cumulative incidence of DM was calculated as the number of subjects who developed DM from non-DM divided by the sum of the durations of follow-up for each subject among those with different clusters of metabolic abnormalities.

The prevalence of the MetS according to IDF Wc cut-offs, current and proposed Japanese cut-offs were 5.1, 3.5 and 6.4%, respectively. The prevalence of raised FPG was 18.1% and was higher in subjects with abdominal adiposity than those without, regardless of any of the three Wc cut-offs used (*p*-values <0.05).

Overall, 26 non-DM subjects developed DM within the next 5 years. Raised FPG identified absolute high risk of future DM whereas the constellation of risk factors except raised FPG with or without abdominal adiposity, using any of the three Wc cut-offs, did not (Table 1). As a screening test for future DM, the MetS defined by Wc according to IDF or current and proposed Japanese cut-offs had a significantly lower sensitivity (7.7, 0 and 7.7% vs. 69.2%, all *p*-values <0.001) and false positive test rate (95.0, 100 and 96.0% vs. 87.2%, all *p*-values <0.001) and higher specificity (95.0, 96.4 and 93.6% vs. 83.7%, all *p*-values <0.001) than raised FPG.

A recent meta-analysis has reported that the MetS, regardless of definition, is a significant predictor of incident DM in many populations and the average estimated relative risk of IDF MetS for incident DM was 4.42 [7]. Analysis using a multivariate regression model was not performed in this study due to the limited number of cases, however, the MetS with abdominal adiposity as an essential component, regardless of which cut-off was applied, did not identify a high absolute risk of future DM. Among the MetS components, raised FPG is thought to be the strongest predictor of DM in many studies [8–10], and this was the case in our study. Raised FPG, regardless of the number of other risk factors with and without abdominal adiposity defined by different Wc cut-offs, was a better predictor of DM than the MetS. In contrast, the MetS provided additional prediction beyond that provided by raised FPG in Non-Hispanic White and Mexican Americans [11]. The different characteristics of Japanese in the pathogenesis of type 2 DM, where decreased β-cell function is a major factor rather than insulin resistance, might cause the different impact of raised FPG on the relation between MetS and incident DM. Moreover, the very low sensitivity of the IDF MetS seen in our study has not always been shown in other studies [7]. In contrast, high specificity was commonly observed in many studies [7] including ours. This may suggest the need for population specific prediction models for future type2 DM in Japan.

Conflict of interest

The authors state that they have no conflict of interest.

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ORIGINAL ARTICLE

Demographics, health-related behaviors, eating habits and knowledge associated with vegetable intake in Japanese adults

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Objectives: To analyze demographic, health-related behaviors, eating habit and knowledge associated with vegetable intake.
Methods: Secondary analyses using the dataset from the National Health and Nutrition Survey 2003. Food intake data measured by the food-weighing method in one-day and a questionnaire assessed the dietary intake and health-related behaviors, eating habit and knowledge. This study was made in Japan. The data of 1742 men and 2519 nonpregnant/nonlactating women, aged 20–69 years, energy intake between percentiles 1 and 99 were included. Vegetable intake was analyzed according to the Japanese vegetable recommendation ($\geqslant$350 g/day) after age adjustment.
Results: Average of VI was 307 g/day in men and 297 g/day in women. Only 35% of men and 31% of women met the recommended amount of vegetable intake. Japanese from city areas, aged 60–69 years, had the highest vegetable intake and subjects from metropolitan areas had the lowest vegetable intake. Depending on the age groups, risks for low vegetable intake in Japanese were found in subjects with skipping meals, alcohol intake and history of smoking.
Conclusions: To increase vegetable intake, it is necessary to provide more nutritional education and lifestyle-related diseases education.
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Keywords: behaviors; vegetable intake; Japan

Introduction

There is strong and consistent epidemiological evidence that vegetable intake (VI) is beneficial to health, decreasing the risk for a range of chronic diseases and many cancers (Mozaffarian *et al.*, 2003; Sauvaget *et al.*, 2003; World Health Organization, 2004; Pomerleau *et al.*, 2006). As a consequence, in many countries, dietary guidelines include recommendations for vegetable consumption. In Japan, a VI of $\geqslant$350 g/day is recommended. However, most Japanese consume less than this amount. The National Health and Nutrition Survey of 2003 (NHNS, 2003) showed that the average daily VI was 278 g. The highest intake was among the Japanese aged between 60 and 69 years, though it was still below the recommended amount (Ministry of Health, Labour and Welfare, 2005). Therefore, health authorities are making an effort to develop dietary programs, such as the Food Guide Spinning Top (Yoshiike *et al.*, 2007), to encourage people to eat a balanced diet by choosing enough servings of vegetable dishes. Many research studies have been carried out in a number of countries to ascertain the determinants of fruit and vegetable consumption. The majority of the studies have focused on household income (Kirkpatrick and Tarasuk, 2003; Laaksonen *et al.*, 2003), regional differences (Pollard *et al.*, 2001; Papadaki and Scott, 2002) and understanding the psychosocial and sociodemographic determinants of fruit and VI. These include knowledge, perception of benefits and barriers, food preparation skills, gender and social status (Satia *et al.*, 2002; Friel *et al.*, 2004;

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Larson *et al.*, 2006). Furthermore, a higher VI is associated with eating homegrown vegetables (Billson *et al.*, 1999), eating vegetables at lunch or dinner, eating salads (Satia *et al.*, 2002) and skill in preparing vegetables (Larson *et al.*, 2006; Crawford *et al.*, 2007). In addition, other studies showed that distress was associated with unhealthy habits, such as low VI (Unusan, 2006). In Japan, very little research has been conducted on these topics. In terms of initiatives to promote increased consumption, a more thorough understanding of the behavioral correlates of VI is likely to be important; thus, the aim of this paper is to analyze demographics, health-related behaviors, eating habits and the knowledge associated with VI among Japanese adults, because such information could be beneficial for developing effective dietary interventions.

Methods

NHNS 2003 data

NHNS 2003 data is a cross-sectional survey of a nationally representative sample of the noninstitutionalized population of Japan. It includes (1) a physical examination (anthropometry measurements, blood pressure, blood test, a questionnaire on medication, smoking status, alcohol intake, exercise and number of steps measured by a pedometer), (2) a dietary survey that involves weighing the amount of food consumed over a day by a household and individual household members and (3) a questionnaire on health-related behaviors, eating habits and knowledge (Iwaoka *et al.*, 2001).

The design and protocol of the national survey conducted by the Ministry of Health, Labour and Welfare of Japan (MHLWJ) were thoroughly reviewed by the technical committee on the survey in the ministry, and also approved by the Ministry of Internal Affairs and Communications in the government office, including the ethical issues. Detailed explanation was made to the households selected as the survey sample by the dietitians of the local public health centers and informed consent was obtained from the households participating in the survey.

Subjects

We performed a secondary analysis of the dataset from NHNS 2003 with the permission of the MHLWJ. From 11 105 subjects, 1742 men and 2519 women were selected according to the following inclusion criteria: age (between 20 and 69 years), energy intake (between percentiles 1 and 99 according to sex cutoff (910–4015 kcal for men and 726–3079 kcal for women)) and those who completed the health-related behaviors, eating habits and knowledge questionnaire. The inclusion criteria for energy were restricted to percentiles 1–99 to exclude outlying data for the analyses. The exclusion criteria were pregnant/lactating women.

Vegetable classification

Green-yellow vegetables, light color vegetables, pickles made from vegetables, vegetable juice. Mushrooms, seaweeds and plants foods that contain variable amounts of starch, such as potato, were not included in vegetable groups.

Demographics, health-related behaviors, eating habits and knowledge

A questionnaire about demographic factors, health-related behaviors, eating habits and knowledge were carried out. It was assessed as follow:

- Demographic factors: age, sex, region of residence (12 regions: Hokkaido, Tohoku, Kanto I, Kanto II, Hokuriku, Tokai, Kinki I, Kinki II, Chugoku, Shikoku, Northern Kyushu and Southern Kyushu), area of residence (metropolitan: more than 150 000 people, city: between 50 000 and 150 000 people and town/rural: less than 50 000 people).
- Health-related behaviors: exercise habit (yes, no), perception of overall health status (very good, good, not good), average hours of sleep (less than 6 h or more than 9 h, 7–9 h), feeling dissatisfaction, distress or burden and others (very often, sometimes, never), history of smoking (yes, no), frequency of alcohol intake (every day, 6 days/week to 1 day/month, gave up/rarely).
- Eating habits: skipping meals (yes, no), eating snacks (yes, no).
- Knowledge: knowledge about 'Health Japan 21' (yes, no), 'lifestyle-related diseases (LSRD)' (I know the content, I have heard about it, I do not know what it is), attendance at health-related education programs (yes, no).

Statistical analysis

All analyses were performed using the Statistical Package for Social Science (version 11; SPSS Inc., Chicago, IL, USA). Data were expressed as mean and 95% confidence interval (CI) according to sex and age groups (20–39, 40–59 and 60–69 years).

Two approaches were used to assess the relationship between vegetable consumption and other factors. First, we used a general linear model (GLM) with VI as the dependent variable, and all other independent factors as categorical variables: demographics, health-related behaviors, eating habits and knowledge factors. We determined age-adjusted means of VI across factors and correlations with P values <0.05 to be significant.

In the second approach, we computed the proportion of subjects who ate a total of $\geqslant 350$ g/day of vegetables, the intake recommended for adults by 'Health Japan 21'. Factors correlated with meeting the recommendation of VI were assessed the odd ratio (OR), with 95% CI, estimated from a multiple logistic regression after age adjustment.