MSG measurement. The noteworthy finding was that our results contrast with those from the studies by He *et al.*[8,10]. Even though we used nearly same method as He *et al.* in the latter study[10], we could not find any significant association between MSG seasoning intake and overweight. In our survey, participants had their meals at home and the intake of MSG as seasoning was measured by the weighing method for every meal during three consecutive days; this may be a more reliable method than that used by He *et al.*, since they assessed the amount of MSG seasoning intake by 24 h recall[10] or asked users to demonstrate the amount of MSG seasoning added during food preparation[8]. Because overweight is a multi-influenced phenotype which is related to lifestyle, environment and genome, the relationship between MSG and overweight should be considered and studied not only in China and Vietnam, but also in different populations and ethnicities.

The question whether the MSG-enhanced taste of foods can lead to increased total energy intake, thus increasing overweight, is still controversial. It is well known that Asian countries have a higher intake of seasonings rich in MSG; however, these countries do not show higher BMI, while in Western countries the MSG intake is lower but the prevalence of overweight and obesity is higher[24,25,30]. In our participants, the prevalence of overweight was higher in urban areas, while MSG seasoning intake was not significantly different between rural and urban areas. Our literature reviews also found that several studies performed with animal models show either that MSG promotes overweight[31] or that it has no effect[32]. In addition, a recent study with human volunteers has shown that the subjective assessment of neither hunger nor fullness was affected by MSG supplementation[33]. Regarding to physiology of GLU, 'Glutamate salts such as MSG dissociates in the neutral area so that independent from origin and salt species free GLU is formed'. Also, GLU in food and GLU from MSG are similarly metabolized in the human body[1]. Beyreuther *et al.*[1] have shown that total intake of GLU from food in European countries was 5–12 g/d, while GLU in seasoning was only about 0·4 g/d. Our study also found that GLU in seasoning was 1·9 g/d, while GLU in food was estimated to be equal to 14·7 g/d. This means that GLU from MSG is indeed small when compared with GLU in food. For these reasons, we cannot say that only MSG (but not GLU in food) causes overweight/obesity. In our study, it is not likely due to chance that we found significant associations between overweight and intakes of energy, carbohydrate, saturated fat and animal protein separately, but not between overweight and MSG seasoning intake.

Our results also support the findings from the study carried out 5 years ago by Shi *et al.*[9], which found that there was no association between obesity and MSG seasoning intake in the Chinese population. However, in their study, the cut-off for overweight was BMI ≥ 25·0 kg/m^2, while in the present study the cut-off was BMI ≥ 23·0 kg/m^2, as defined by the WHO to identify risks of an undesirable state of health that warrants a public health or clinical intervention[17].

Despite being carried out in a large number of Vietnamese adults from three different areas (north, central and south), our study has several limitations. First, the survey was done in autumn and winter; therefore, dietary and MSG seasoning intake may not represent the full picture of the four seasons in Vietnam. Second, the participants in our study had meals at home; hence, adults who have meals away from home may have different characteristics from those presented in the current report. Third, due to the cross-sectional design, longitudinal studies using the same method of MSG measurement are needed to confirm our findings.

Conclusions

The present study demonstrates that the prevalence of overweight in Vietnam is relatively high compared with nearby countries. These findings suggest that overweight is becoming an alarming problem in Vietnam which requires great efforts to prevent. The associations between overweight and risk factors were assessed by using multiple logistic regression modelling. Although MSG intake was relatively high, we could not find a relationship between MSG intake and overweight. It is suggested that further longitudinal studies should be done in different populations and ethnicities to determine the association between MSG and overweight.

Acknowledgements

The study was supported by Professor Shigeru Yamamoto. All authors declare no conflict of interest. V.T.T.H. collected and analysed the data, interpreted the statistical analysis and wrote the manuscript with assistance from N.T.L. and S.Y. A.W. contributed to the writing of the paper and the literature review. N.T.L. and N.C.K. were responsible for quality control of the dietary data. S.Y. was responsible for data interpretation and all subsequent revision, as well as the financial support for the study. All authors reviewed the manuscript critically. The authors gratefully acknowledge the cooperation of all participants and the support of local authorities in each province/city. They are grateful to the staff of the Vietnam National Institute of Nutrition and local health staffs who helped in conducting the study; and thank Professor Andrew Durkin from Indiana University (Bloomington, IN, USA) for help with the manuscript.

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J Nutr Sci Vitaminol, 58, 113–117, 2012

Sugar Intakes from Snacks and Beverages in Japanese Children

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(Received November 1, 2011)

Summary While sugar intake is an important factor for obesity, diabetes and dental caries, sugars are also important energy sources, especially for rapidly growing children. Children like sugar-rich sweet foods. However, intake for Japanese children is not known due to a lack of studies and sugar composition data. This study was designed to determine sugar intakes from snacks and beverages in Japanese school children. A nutrition survey was conducted for 3 weekdays for 283 Japanese school children (7, 10 and 13 y old) in 8 prefectures from different areas of Japan. The methods for the survey were the weighing method for school lunches and the 24-h recall method for other foods. To estimate sugar intakes, the sugar composition table that was recently compiled by us for 135 beverages, cakes and other sweets was used. Height and weight were measured. They were similar to Japanese averages. Energy intakes were also similar to the results of the Japanese National Health and Nutrition Surveys. Sugar eaten outside meals was 24.7±15.5 g/d. From the National Health and Nutrition Surveys conducted in 2009, the mean sucrose intake from meals including some home-made cookies for 7–14-y-old children was 5.5 g/d, suggesting the mean total sugar intake of these children was about 30 g/d. This was within the range of FAO/WHO recommendation (less than 10% of energy intake, 49 g for these children. Mean intakes among age groups were not significantly different ($p>0.05$), but the intake for girls was lower than for boys in the oldest age group ($p<0.05$). Contributions of each sugar to total intake were sucrose 64%, fructose 14%, glucose 13% and lactose 9%. Fructose and glucose were mainly from isomerized sugar. Contributions of food groups to total intake were beverages 25%, baked goods 19% and ice cream 17%, respectively, covering 61% of all. In conclusion, we revealed that the average sugar intake of Japanese children was within the range of the FAO/WHO recommendation, though the effects of the kind of sugars on health remain to be clarified.

Key Words Japanese children, sucrose, fructose, glucose, lactose

Major sugars are sucrose, glucose, fructose and lactose. They convert to glucose, which plays an important role as the brain's energy source. Sugars are tasty and pleasurable, especially for growing children. Such characteristics easily induce people to consume more sugars than necessary and they can become a major factor in obesity, diabetes and dental caries. Sugars are also an important energy source, especially for rapidly growing children. However, intakes for Japanese children are not known due to the lack of a sugar composition table other than one in which foreign data are used (*1*). Japanese traditional foods and their sugar concentrations are quite different from those in other countries. We therefore recognized the importance of a sugar composition table for Japanese food. We have measured concentrations of sugars (sucrose, glucose, fructose and lactose) in 135 sugar-rich foods (42 commercial beverages and chilled snacks, 29 home-made cookies, and 64 commercial cakes and cookies). More than 5 samples from different manufacturers and home recipes of each food were analyzed and the average values were calculated and published (*2*, *3*). FAO/WHO recommend less than 10% of energy (about 50 g sugar), which is intended for the prevention of life-style related diseases and is used in 23 countries (*4*). Sheiham (*5*) recommends less than 40 g to prevent dental caries. The FAO/WHO definition of sugar is free sugar (sugars plus concentrated sugars in honey, syrups and fruit juices); the intrinsic sugars or milk sugars (lactose, galactose) are

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not included because they are not considered to have adverse health outcomes (*4*).

In this study we conducted a nutrition survey covering various areas of Japan and tried to estimate children's sugar intake.

METHODS

Subjects. The survey was conducted in about 350 children of 7, 10 and 13 y old in 8 prefectures from north to south in Japan. The final available number was for 283. We tried random sampling as much as possible in selecting areas, schools and classes so that the subjects would be close to representative for the country as a whole.

Survey. The nutrition survey was conducted for 3 weekdays. An investigation of height and weight was conducted before the commencement of the survey. Regarding the intake of school lunches, researchers measured the actual amount of each student's portion, while that of the other meals, including in-between snacks, was measured by the 24-h recall method with the cooperation of the students' guardians. In the case of incomplete items or unclear descriptions on the form, the researchers confirmed details directly with the students or asked their guardians to fill out the items.

Estimation of sugar and energy intakes. Calculations of energy intake were made in accordance with the data listed in "Standard Tables of Food Composition in Japan, 5 revised and enlarged ed" and the table of processed foods data (*6–8*). Sucrose, glucose, fructose and lactose intakes from foods other than meals were calculated by using the sugar composition table constructed by us previously (*2, 3*).

Ethical considerations. This study was conducted with the approval of the Ethics Committee of Ochanomizu University, and in accordance with the Declaration of Helsinki: Ethical principles for research involving human subjects with special attention paid to the following: To prevent the identification of individuals, each subject's personal information was carefully coded and obtained data were strictly managed. We obtained a statement that participation in the research was by free will on the part of the participants and their guardians by providing explanations about the objectives and details of the investigation and the intention to use the results for oral and written presentations. Even after commencement, explanations were provided whenever subjects dropped out of the study, either of their own volition or at the guardian's behest; no subjects were penalized in any way.

Statistical analysis. Analysis of the data was carried out with SPSS (version 17.0) statistical software. Data were assessed by one-way ANOVA and then Tukey's multiple comparison test and p values less than 0.05 were considered statistically significant. Correlation between body weight and sugar intake was assessed by Pearson's correlation coefficient test.

RESULTS

Table 1 shows the height, weight and energy intake of the children in this study. All values were similar between our subjects and the Japanese average (*9*),

Table 1. Characteristics of the subjects and energy intake.

Age (y)	Gender	n	Height (cm)	Weight (kg)	Energy/d (kcal)
7	boy	33	122.5±5.4^{a}	23.6±3.7^{a}	1,843±310^{a}
	girl	42	120.9±5.2^{a}	22.8±3.5^{a}	1,668±238^{a}
10	boy	64	139.8±6.1^{b}	34.8±8.4^{b}	2,081±385^{b}
	girl	64	140.4±6.9^{b}	32.6±6.1^{b}	1,896±282^{a}
13	boy	42	162.2±8.4^{c}	52.5±11.5^{c}	2,340±446^{c}
	girl	38	156.2±5.3^{d}	47.7±7.4^{d}	1,871±287^{a}

Values are mean±SD.
Figures with different superscript letters in the same column are significantly different as assessed by one-way ANOVA and then Tukey's multiple comparison test ($p<0.05$).

Table 2. Sugar intakes of Japanese children (g/d).

Age (y)	Gender	n	Glucose (A)	Fructose (B)	Sucrose (C)	Lactose (D)	Total sugar (A+B+C+D)
7	boy	33	3.1±2.6^{abc}	3.5±3.3^{abc}	15.9±9.4	2.5±2.3	25.1±14.6^{ab}
	girl	42	3.5±3.0^{abc}	3.8±3.6^{abc}	17.7±11.7	2.4±1.6	27.4±15.9^{a}
10	boy	64	3.2±3.4^{abc}	3.4±3.7^{abc}	16.8±9.8	2.2±1.9	25.7±14.2^{ab}
	girl	64	3.4±2.4^{abc}	3.6±2.8^{abc}	16.6±9.5	2.4±1.9	26.0±12.7^{ab}
13	boy	42	4.0±4.2^{b}	4.4±4.6^{b}	14.6±12.7	2.1±3.1	25.0±20.7^{ab}
	girl	38	2.0±2.4^{c}	2.1±2.7^{c}	12.0±11.2	1.4±1.7	17.5±14.3^{b}
Average			3.2±3.1	3.5±3.5	15.8±10.7	2.2±2.1	24.7±15.5

Values are mean±SD.
Figures with different superscript letters in the same column are significantly different as assessed by one-way ANOVA and then Tukey's multiple comparison test ($p<0.05$).

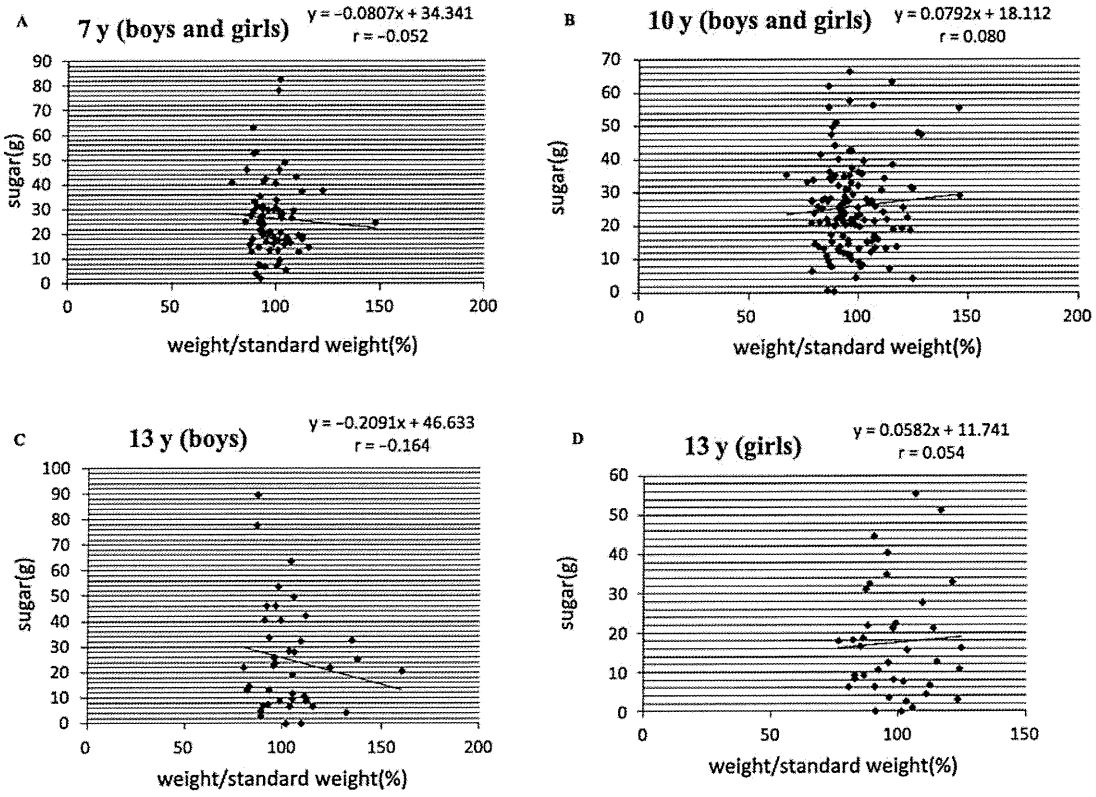

Fig. 1. Relationships between body weight and sugar intake for 7-(A) and 10-(B) y-old children and 13-y-old boys (C) and girls (D).

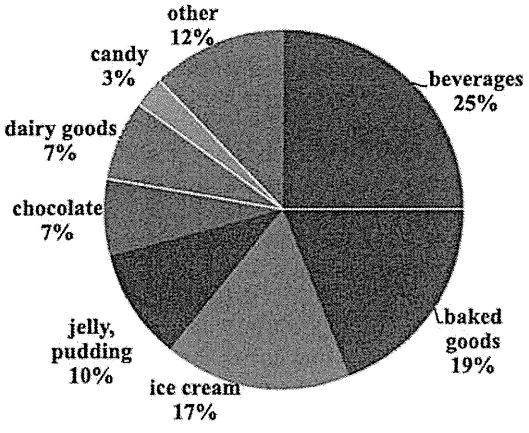

Fig. 2. Contributions of various food groups to total sugar intake.

indicating that our subjects were similar to the country representative. Energy intakes were higher in boys than in girls in all the age groups and increased by age except for girls of 13 y old.

Table 2 shows sugar intakes. Sugar intakes in both sexes in all the age groups were similar except the low intake in 13-y-old girls. The average intake for all the children was 24.7±15.5 g/d.

As shown in Fig. 1, no significant relationship was observed between body weight and sugar intakes in 3 age groups. The data for 13-y-olds were separated into boys and girls because only in this age group the height, weight and sugar intake were different in the two genders. The standard weight was established from the health statistics of school children in 2000 (*10*).

Figure 2 shows the contribution of major foods to sugar intake. Sugars from beverages contributed about 25% and baked goods and ice cream each about 19% and 17%, respectively. These 3 items contributed about 61% of the sugar intake.

DISCUSSION

We found in this study that average sugar intake of Japanese children from snacks and beverages was about 25 g. To our knowledge, this is the first study to estimate sugar intake in Japanese children. The limitation of this study was that the sugar intake from meals could not be estimated, because for estimating sugar content of a meal, another study in which the amount of sugar used before cooking would have to be measured. However, in Japan, luckily the nationwide Health and Nutrition Survey has been conducted every year since 1946 and the amount of sugar used in meals and some homemade cakes were estimated, although sugar-rich snacks and beverages are shown only by weight but not by their sugar content. According to the most recent survey in 2009 (*9*), mean sugar intake from meals in children aged 7–14 y was reported to be 5.5 g/d. From the result of the present study and that of Japanese Health and Nutrition Survey, the sum of sugar intake was estimated as 30 g. However, the sugar from some homemade cakes is doubly calculated in both studies, suggesting the sugar intake may be less than 30 g/d.

The FAO/WHO recommendation of sugar intake is less than 10% of energy (*4*). In this study we also mea-

sured the energy intake. It was 1,960 kcal/d and 10% of it (196 kcal) was equivalent to 49 g of sugar. The standard deviation of sugar intake was about 15 g. Thus the mean sugar intake+1SD is about 45 g which is smaller than 49 g. When the distribution is normal, mean+1SD covers about 85% of the age population. Therefore, sugar intake for most of the Japanese children may be within a desirable level.

There are reports about sugar intake from various countries: American male children of 6–12 y old consume 124 g/d (*11*), English 84 g/d (*12*), Dutch 135 g/d (*13*), South African 43 g/d (*14*) and Filipino 60 g/d (*15*). These data are much higher than our present result. Nutrition surveys often underestimate intakes (*16*). To prevent such problems, in this study we used the weighing method for school lunches and carefully checked other meals and foods. There are seasonal variations in the intakes; however, this study was conducted in summer and in this season sugar intake from beverages is thought to be usually higher than in other seasons because people drink more cold sweet beverages, which decreases the underestimation problem. To verify the reliability of the present nutrition survey, we compared the energy intakes in the present subjects with those reported by the National Health and Nutrition Survey (*9*). Energy intake was 1,960±388 kcal in our subjects and 1,936 kcal in the children reported in the nationwide nutrition survey (*9*). Both sets of data were similar.

Our present result is also supported by the FAO report on sugar intakes by countries estimated from each country's annual supply of sugar (*17*). From the reports, the sugar intakes per person (g/d) were 48.8 g for Japanese, 84.5 g for Americans, 139.4 g for Cubans, 138.6 g for Brazilians, 100.4 g for British, 96.2 g for Germans, 47.1 g for Koreans, 17.2 g for Chinese, 79.6 g for Thais, 31.7 g for Vietnamese, and 66.0 g for Filipinos.

The contribution of each sugar to the total sugar intake (about 24.7 g) in the present study was 64% for sucrose, 14% for fructose, 13% for glucose and 9% for lactose (Table 2). From the data shown in Fig. 2, sugar from beverages was 25% and the highest. These results indicate that fructose and glucose (total 27%) were mainly from beverages. From our finding, most of the beverages contain isomerized sugar, commonly called high-fructose corn syrup. It is produced by the hydrolysis of corn starch and other starches and is cheaper than sucrose. It is in soluble form and is usually used in beverages. The different physiological effect of glucose and fructose are known. For example, fructose increases blood triglyceride concentration and causes obesity more than glucose (*18–21*). Therefore, we may have to determine what kind of sugar we are taking and the effects of them on health.

The lower sugar intake by 13-y-old girls was perhaps due to over-concerns about body weight. In Japan most adolescent girls think that a slim body is beautiful. Such an image on the part of Japanese girls seems to be more serious than for girls in other countries (*22*).

In conclusion, the sugar intake of most Japanese children is supposed to be within the proper range.

Acknowledgments

The authors wish to thank the subjects and their family members for their cooperation in participating in this study, as well as everyone in the institutions for their kind help. The research for this paper was supported by a Grant-in-Aid for Scientific Research, as a part of the project in 2008 "Implementation of investigation of eating habits of schoolchildren at school and at home" (Principal Researcher: Shigeru Yamamoto) commissioned by the Ministry of Education, Culture, Sports, Science and Technology in Japan.

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Clinical Nutrition xxx (2012) 1–6

Contents lists available at SciVerse ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

A new equation to estimate basal energy expenditure of patients with diabetes☆

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ARTICLE INFO

Article history:
Received 17 February 2012
Accepted 22 November 2012

Keywords:
Basal metabolic rate
Resting metabolic rate
Indirect calorimetry
Prediction equation
Diabetes
Medical nutrition therapy

SUMMARY

Background & aims: Predictive equations for basal energy expenditure (BEE) derived from Caucasians tend to overestimate BEE in non-Caucasians. The aim of this study was to develop a more suitable method to estimate BEE in Japanese patients with diabetes using indices readily measured in clinical practice.

Methods: BEE was measured by indirect calorimetry under a strict basal condition in 68 Japanese patients with type 1 or type 2 diabetes. The best fitting equation was investigated by multiple regression analysis using of age, sex, and anthropometric indices. The resultant new equation was tested in a separate group of 60 Japanese patients with type 1 or type 2 diabetes, and the accuracy compared with existing equations.

Results: The best-fit equation was BEE [kcal/day] = 10 × (body weight)[kg] − 3 × (age)[y] + 125 (if male) + 750. Adjusted coefficient of determination was 81.0%. Root mean squared errors and accurate prediction in the validation set were 103 kcal/day and 78% for the new equation; 184 and 50 for Harris-Benedict; 209 and 38 for Oxford; 205 and 42 for Liu; and 140 and 63 for Ganpule.

Conclusions: This new equation is simpler and estimates BEE more accurately in Japanese patients with diabetes than the presently used equations do.

1. Introduction

Diet is the most fundamental and initial treatment for all patients with diabetes, and poor dietary management alone predicts poor subsequent glycemic control.[1] Estimation of daily energy expenditure for each patient is necessary for effective individualized diabetic meal planning. Resting energy expenditure (REE) or basal energy expenditure (BEE) is defined as the energy expended to maintain minimal metabolic activities, and is the main component of total daily energy expenditure. To estimate daily energy expenditure, REE or BEE is multiplied by a number specific to the various daily activities.

In healthy subjects, 65–90% of inter-individual variation in REE is explained by fat-free mass (FFM).[2] In patients with diabetes, FFM is also the main factor in REE and BEE,[3–5] and there is no difference in FFM-adjusted REE between mildly hyperglycemic patients and controls.[6] In clinical practice, BEE or FFM are not usually available. Equations factoring body weight, height, age and sex are widely used for clinical estimation of the daily energy requirement of patients with diabetes.[7] However, there has been little investigation of the comparative validity of these equations.

The existing predictive equations derived from Caucasians are unevenly applied to non-Caucasians, tending to overestimate energy expenditure.[8–11] This accords with the recent finding from the basal metabolic rate database that BEE is higher in Caucasians than in non-Caucasians.[12] However, REE is similar in Asians and Caucasians after adjustment for FFM, and BEE in Indians and Australians is similar after adjustment for FFM and fat mass.[13,14] To date, there are few equations to estimate energy expenditure specifically in Asian populations.[10,15]

Differences in the measurement technique of REE can cause biases.[12] In most studies evaluating energy expenditure, REE has been used rather than BEE. However, REE is defined less rigorously

☆ A part of this study was presented in abstract form at the annual meeting of American Diabetes Association, New Orleans, Louisiana, 5–9 June 2009.

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http://dx.doi.org/10.1016/j.clnu.2012.11.017

than BEE and is influenced by physical and psychological stress and ambient and body temperature.[16–18] Since BEE is measured early in the morning before the subject begins any physical activity and at least 10 h after ingestion of any food, drink, or nicotine, it remains remarkably constant on a daily basis.[16,18]

In the present study, by measuring BEE under strict conditions, we developed a new equation for estimation of BEE in Japanese patients with diabetes for use in a clinical setting.

2. Patients, materials and methods

2.1. Patients

Japanese patients with type 1 or type 2 diabetes admitted to the Department of Diabetes and Clinical Nutrition, Kyoto University Hospital, Kyoto, Japan for diabetes self-management education during the period of December 2007 through September 2009 were recruited for derivation study. Written, informed consent was obtained from all participants. During hospital stay, the participants had a prescribed diet with or without medications including oral hypoglycemic agents and insulin according to the treatment guide for diabetes of the Japan Diabetes Society.[19] Their physical activity was not restricted, but they did not engage in vigorous exercise. Participants were screened by medical history, physical examination, and laboratory testing to assure the absence of hepatic, pulmonary, thyroid, cardiac and renal dysfunction, macroalbuminuria, inflammatory diseases, and malignant tumors. Those who took steroids or beta blockers or had physical disabilities were excluded. The study protocol was approved by Kyoto University Graduate School and Faculty of Medicine, Ethics Committee.

2.2. Indirect calorimetry

Basal energy expenditure (BEE) was measured in the morning under glycemic control with prescribed diet (29.1 ± 2.5 kcal/kg of standard body weight per day consisting of 52% carbohydrate, 20% protein, and 28% fat in energy component) and with medications when needed. Standard body weight (kg) was calculated by multiplying 22 (kg/m^2) by square of height (m). Whole-body oxygen consumption (VO_2) and carbon dioxide production (VCO_2) was measured for more than 10 min with indirect calorimetry (AE300S, Minato Medical Science, Osaka, Japan) by one investigator (KI) at the bedside of each patient under the strict condition described previously.[5,16,17] Briefly, an afebrile patient in a post-absorptive state after an overnight fast (14 h) with <180 mg/dL capillary plasma glucose remained in a supine position after waking on the bed in the ward without smoking or taking caffeine, and the measurements were performed at room temperature between 22 °C and 27 °C. After discarding the initial 5 min of recording, we took 5-min of data, in accord with the steady state definition,[17] during which the coefficient of variation for VO_2 per minute and VCO_2 per minute was achieved ≤10%, and applied them to the Weir formula with 24-h urinary urea nitrogen.[20]

2.3. Anthropometry and body composition

Height was measured on the day of admission. Body weight, skinfold thickness, and waist circumference were measured immediately after the measurement of BEE by one investigator (KI). Triceps-skinfold thickness (TSF) and mid-upper arm circumference (MAC) were measured in the non-dominant arm with the elbow bent at 90°. The physical markers were measured at least twice, and their respective mean values expressed according to Japanese standard method.[21] Arm muscle circumference (AMC) and arm muscle area (AMA) were calculated; AMC [cm] = MAC [cm] − π × TSF [mm]/10, AMA [cm^2] = (AMC [cm])^2 / 4π. Waist circumference was measured at the mid-point between the lowest rib and the iliac crest in a standing position at the end of gentle expiration keeping the measuring tape horizontal and just fitted to the skin. Hip circumference was measured at the widest part of the hip while standing. FFM and fat mass were measured by dual energy X-ray absorptiometry scanner (Discovery, Hologic, Bedford, MA, USA) within 3 days before and after measurement of BEE.

2.4. Other measurements

Glycated hemoglobin was measured by use of HPLC (ADAMS™ A1C HA8180, Arcray, Kyoto, Japan) and expressed as a National Glycohemoglobin Standardization Program (NGSP) equivalent value [%] calculated by the formula HbA1c [%] = HbA1c [Japan Diabetes Society (JDS)] [%] + 0.4 [%], which considers the relational expression of HbA1c (JDS) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP).[22] Capillary glucose before each meal was measured by glucose meter (One Touch Ultra™, Johnson & Johnson, New Brunswick, NJ, USA) and expressed as capillary plasma glucose (PG). As a parameter of glycemic control, mean preprandial PG for three consecutive days before the measurement of BEE and fasting PG (FPG) just before the measurement of BEE are shown.

2.5. Testing the new equation

A separate data set of Japanese patients with type 1 or type 2 diabetes admitted to the same department for the same purpose during the period of June 2005 through December 2007 was drawn from the medical records for validation study. Inclusion/exclusion criteria and dietary condition during hospital stay were similar to that of the derivation sample.

Whole-body VO_2 and VCO_2 was measured after an overnight fast (14–16 h) for more than 15 min with the same calorimetry by one investigator (MI) on the same condition. Each patient was conveyed from their ward to the examination room by a healthcare staff member in a wheel chair and they rested in bed in a supine position for 30 min before the measurement of BEE. BEE was calculated from VO_2 and VCO_2 by use of Elwyn formula (BEE [kcal/day] = 3.581 × VO_2 [L/day] + 1.448 × VCO_2 [L/day] − 32.4).[16] Body weight was measured on the day of calorimetry.

The protocol of this validation study was also approved by Kyoto University Graduate School and Faculty of Medicine, Ethics Committee.

2.6. Statistical analysis

Numerical data are summarized as means ± SDs. Categorical data were treated as dummy variables.

We first explored good estimators for FFM and fat mass in anthropometric indices, such as body weight, height, TSF, AMA, waist circumference and hip circumference, because FFM and fat mass are known as two major estimators of BEE. Correlations between these variables were evaluated by Pearson's correlation analysis. Multiple linear regression analysis was then performed to evaluate the contribution of anthropometric indices, age, and sex to FFM and fat mass. Next, a best-fit equation to estimate BEE from anthropometric indices, age, and sex was explored by multiple linear regression analysis with consideration of estimators of FFM and fat mass.

For testing the validity of our new equation and comparing it with existing prediction equations, we calculated measures of accuracy. The mean percentage difference between BEE estimated and measured (bias) was considered systematic error. The root

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mean squared error (RMSE) was considered to reflect each individual's error range unrelated to whether it was over or under estimation. The proportion of patients with BEE estimated within ±10% of BEE measured was considered another measure of accuracy.[23]

Data were analyzed by use of Stata 11.0 (Stata Corporation, College Station, TX, USA). Statistical significance was set at $P < 0.05$ (2-tailed).

3. Results

Data were obtained and analyzed in 68 patients, of which 7 had type 1 diabetes and 61 had type 2 diabetes. Mean glycated hemoglobin (HbA1c) on admission was as high as 10.5%, but mean fasting plasma glucose just before the measurement of BEE (FPG) was as low as 113.7 mg/dL due to the treatments during hospital stay (Table 1). Additional characteristics of patients in the derivation set and the results of measurement are shown in Table 1.

Body weight had the highest correlation with FFM ($r = 0.90$), followed by arm muscle area (AMA), height and hip circumference ($r = 0.84$, 0.75 and 0.73, respectively) (Table 2). Waist circumference had the highest correlation with fat mass ($r = 0.91$), followed by hip circumference, triceps-skinfold thickness (TSF) and body weight ($r = 0.79$, 0.78 and 0.75, respectively).

In regression analysis for FFM, we selected body weight, AMA, height and hip circumference as potent estimators together with other plausible estimators, age and sex. As both AMA and hip circumference were strongly correlated with body weight and AMA was also strongly correlated with hip circumference, to analyze these three variables separately, we used three sets of independent variables, (body weight, height, age and sex), (AMA, height, age and sex), and (hip circumference, height, age and sex). The regressions revealed that all four variables were significant estimators for FFM in the first analysis (model 1 in Table 3), that AMA and height were significant in the second analysis (model 2) and that hip circumference, height and sex were significant in the third analysis (model 3). The first four variables accounted for 95% of variation in FFM, the second two variables 84%, and the third three variables 87%. For fat mass, we selected another three sets of independent variables, (waist circumference, age and sex), (hip circumference, TSF, age and sex) and (body weight, TSF, age and sex) because waist circumference had a strong correlation with hip circumference, TSF and body weight, and hip circumference also had a strong correlation with body weight. In the first analysis, only waist circumference and sex were significant estimators for fat mass, accounting for 86% of fat mass (model 4). In the second analysis, hip circumference, TSF and age were significant, accounting for 84% of fat mass (model 5). In the third analysis, body weight, TSF, age and sex were significant, accounting for 87% of fat mass (model 6).

We performed regression analysis to determine BEE with the most influential estimators (FFM and fat mass) and plausible additional estimators (age and sex), which together explained 81% of the variation (model 7 in Table 3). We then performed backward stepwise estimation, using three sets of variables, (significant variables in model 1 and 6; body weight, height, TSF, age and sex), (significant variables in model 2 and 4 plus age; AMA, height, waist, sex and age), and (significant variables in model 3 and 5; hip circumference, height, TSF, age and sex). The best fitting regression for BEE consisted of body weight, age and sex in the first analysis (model 8), height, waist, age and sex in the second analysis (model 9), and hip circumference, height, TSF and sex in the third analysis (model 10). The adjusted coefficient of determination in model 8 was 81%, which was larger than the 73% in model 9 and the 77% in model 10. The detailed results of model 8 are shown in Table 4.

We then simplified the resultant equation of model 8 to make it easy to use in clinical practice.

$$\mathrm{BEE} = 10 \times \text{body weight} - 3 \times \text{age} + 125(\text{if male}) + 750.$$

[BEE (kcal/day), body weight (kg), age (year)]

The bias of this equation in the derivation set was −1.2 ± 6.4%; RMSE was 94 kcal/day; accurate estimation was 91%.

We then tested this new equation in a separate validation data set comparing it with existing equations (Table 5). Characteristics of patients in the validation set are shown in Table 6. The ratio of patients with type 1 and 2 diabetes was almost the same as in the derivation set. Mean age was similar to that in the derivation set,

Table 1
Characteristics of patients (derivation set).

	All	Male	Female
No. of patients	68	39	29
Type of diabetes (type1/type2) (n)	7/61	4/35	3/26
Age (years)	59.8 ± 11.2 (range 19–78)	58.3 ± 10.3	61.8 ± 12.2
Height (cm)	161.3 ± 9.5	167.6 ± 6.0	152.9 ± 6.3
Body weight (kg)	62.8 ± 14.7 (range 34.6–113.6)	67.3 ± 16.0	56.7 ± 10.2
BMI (kg/m^2)	24.0 ± 4.7	23.9 ± 5.3	24.2 ± 3.8
FFM (kg)	47.7 ± 10.6	53.4 ± 9.4	39.9 ± 6.5
Fat mass (kg)	16.0 ± 7.0	14.8 ± 8.0	17.8 ± 4.9
TSF (mm)	15.9 ± 7.8	13.1 ± 6.5	19.8 ± 7.8
AMA (cm^2)	44.6 ± 10.2	48.9 ± 9.7	38.8 ± 7.9
Waist (cm)	86.5 ± 12.4	86.2 ± 14.0	86.9 ± 10.3
Hip (cm)	91.3 ± 7.8	92.4 ± 8.5	89.8 ± 6.7
BEE (kcal/day)	1290 ± 217	1395 ± 210	1149 ± 130
FPG (mg/dL)	113.7 ± 25.8	113.3 ± 25.5	114.3 ± 26.6
PPPG (mg/dL)	143.5 ± 35.9	146.5 ± 39.7	139.3 ± 30.3
HbA1c (%)	10.5 ± 2.5	10.3 ± 2.4	10.8 ± 2.7
Duration of diabetes (years)	9.3 ± 7.8	10.9 ± 8.9	7.1 ± 5.5
Treatment			
Diet (kcal/SBW/day)	29.1 ± 2.5	28.9 ± 2.1	29.3 ± 3.0
Medications			
Ins only (n)	34	21	13
Ins + Met (n)	10	4	6
Ins + SU (n)	3	2	1
Ins + SU + Met (n)	1	0	1
SU (n)	8	5	3
SU + Met (n)	5	2	3
Met only (n)	4	3	1
None (n)	3	2	1

Data are means ± SD. BMI, body mass index; FFM, fat-free mass; TSF, triceps-skinfold thickness; AMA, arm muscle area; Waist, waist circumference; Hip, hip circumference; BEE, basal energy expenditure; FPG, fasting plasma glucose just before the measurement of BEE; PPPG, mean preprandial plasma glucose for three consecutive days before the measurement of BEE; HbA1c, glycated hemoglobin; SBW, standard body weight; Ins, insulin; SU, sulfonylurea; Met, metformin.

Table 2
Correlations between FFM, fat mass and anthropometric indices.

	FFM	FM	Ht	Wt	TSF	AMA	Waist	Hip
FFM	1.00	–	–	–	–	–	–	–
FM	0.38‡	1.00		–	–	–	–	–
Ht	0.75‡	−0.12	1.00	–	–	–	–	–
Wt	0.90‡	0.75‡	0.49‡	1.00	–	–	–	–
TSF	0.13	0.78‡	−0.30*	0.46‡	1.00	–	–	–
AMA	0.84‡	0.48‡	0.50‡	0.83‡	0.07	1.00	–	–
Waist	0.56‡	0.91‡	0.02	0.83‡	0.70‡	0.60‡	1.00	–
Hip	0.73‡	0.79‡	0.28*	0.90‡	0.50‡	0.73‡	0.83‡	1.00

Pearson's correlation coefficients ($n = 68$): *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$. FFM, fat-free mass; Ht, height; Wt, weight; TSF, triceps-skinfold thickness; AMA, arm muscle area; Waist, waist circumference; Hip, hip circumference.

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Table 3
Results of multiple regressions for FFM, FM and BEE.

	Adj. R^2		Model
FFM = −26.9 + 0.5 × Wt + 0.3 × Ht − 0.1 × Age + 3.9 × Sex[a]	0.95		1
FFM = −60.8 + 0.6 × AMA + 0.5 × Ht[b]	0.84		2
FFM = −102.8 + 0.8 × Hip + 0.5 × Ht + 4.5 × Sex[c]	0.87		3
FM = −26.3 + 0.5 × Waist − 2.6 × Sex[c]	0.86		4
FM = −45.4 + 0.5 × Hip + 0.4 × TSF + 0.1 × Age[d]	0.84		5
FM = −14.3 + 0.4 × Wt + 0.2 × TSF + 0.1 × Age − 5.1 × Sex	0.87		6
BEE = 691.6 + 11.6 × FFM + 8.9 × FM − 2.6 × Age + 106.7 × Sex	0.81		7
BEE = 748.4 + 10.4 × Wt − 3.0 × Age + 125.4 × Sex[e]	0.81	Model (1 + 6)	8
BEE = −332.3 + 6.1 × Ht + 9.5 × Waist − 4.6 × Age + 147.1 × Sex[f]	0.73	Model (2 + 4)	9
BEE = −1139.3 + 13.8 × Hip + 6.1 × Ht + 5.6 × TSF + 157.9 × Sex[c]	0.77	Model (3 + 5)	10

FFM, fat-free mass (kg); FM, fat mass (kg); BEE, basal energy expenditure (kcal/day); Wt, body weight (kg); Ht, height (cm); AMA, arm muscle area (cm^2); Hip, hip circumference (cm); Waist, waist circumference (cm); TSF, triceps-skinfold thickness (mm); Adj. R^2, adjusted coefficient of determination.

[a] Male = 1, female = 0.
[b] Age and sex were not significant determinants when added to this model.
[c] Age was not a significant determinant when added to this model.
[d] Sex was not a significant determinant when added to this model.
[e] Height and TSF were not significant determinants when added to this model.
[f] AMA was not a significant determinant when added to this model.

but there were more obese people in the validation set. FPG and PPPG, which represent the glycemic levels around the time of measurement of BEE, were higher, but HbA1c on admission was lower than that in the derivation set. Mean duration of diabetes was similar to that in the derivation set. Prescribed diet was almost the same as in the derivation set, but treatment with insulin was more common in the derivation set. The bias of the new equation was 4.8 ± 7.7%, RMSE was 103 kcal/day, and the percent of patients estimated within ±10% of measured value was 78%. The new equation had better validity than Harris and Benedict equation, Oxford equation, or the Liu equation and Ganpule equation (Table 7).

4. Discussion

We report a new equation to estimate BEE in Japanese patients with diabetes with higher accuracy compared to existing equations. As in other BEE estimation equations, the main estimator was FFM and additional estimators were fat mass, age and sex.[2–4,24] Stepwise estimation analysis of the estimators of FFM and fat mass in the present study revealed that no other indices improved fitting of the equation for BEE except body weight, age and sex. Although anthropometric indices are good estimators for body composition and they improve predictability of certain equations for BEE,[25,26] they were not as effective as body weight in the present study. This accords with the finding that the standard error of the estimate of REE prediction by weight, height, sex and age was well within the range of the standard error of estimates from other FFM-derived prediction equation.[27] Since ethnic difference in BEE is derived from differences in body composition,[13] an ethnicity-specific constant term could more precisely estimates BEE,[4,12] but an ethnicity-specific coefficient of anthropometry is also valid.

We compared our new equation with existing equations such as Harris and Benedict, Oxford, Liu, and Ganpule because the Harris and Benedict equation is widely known in clinical practice in Japan, the Oxford equation was recently developed from a large number of subjects including many ethnicities, and the Liu equation and Ganpule equations were derived from Chinese and Japanese subjects, respectively.[7,10,12,15] The validation analysis revealed better validity of the new equation in Japanese patients with diabetes than any of the other equations.

BEE was measured under strictly controlled conditions in the present study. In addition, we confirmed the FPG of the patients to be < 180 mg/dL just before the measurement of BEE, since BEE is unaffected by the glucose level when its value is < 180 mg/dL.[5,6] As the mean FPG of patients in the derivation set was improved to 114 mg/dl just before the measurement of BEE due to the prescribed diet and medications during hospital stay, in contrast to the poor mean FPG level as high as 170 mg/dl just after admission, clinical application of this equation to patients with stable glycemic control is recommended.

There are potential weaknesses of the present study. First, only a small number of patients with type 1 diabetes was included. However, no difference in the value of BEE between patients with type 1 and type 2 diabetes has been described to date. In type 1

Table 4
Detailed result of model 8.

Dependent variable BEE[a]	Coef.[b]	95% CI[c]		Std. coef.[d]	$P > t$	Adj. R^2[e]
Independent variables						
Intercept	748.4	562.6	934.1		<0.001	0.810
Wt (kg)	10.4	8.6	12.1	0.70	<0.001	
Age (year)	−3.0	−5.2	−0.9	−0.16	0.007	
Sex (male = 1, female = 0)	125.4	75.6	175.1	0.29	<0.001	

[a] BEE, basal energy expenditure (kcal/day).
[b] Coef., partial regression coefficient.
[c] CI, confidence interval.
[d] Std. coef., standardized coefficient.
[e] Adj. R^2, adjusted coefficient of determination.

Table 5
Equations to estimate BEE.[a]

	Formula	Reference
New equation	10 W − 3 A + 125 (if male) + 750[b,c]	
Harris and Benedict (1919)	Male: 13.75 W + 5.00 H − 6.76 A + 66.47[d] Female: 9.56W + 1.85 H − 4.68 A + 655.10	7
Oxford (2005)	Male: 18–30 years; 16.0 W + 545 30–60 years; 14.2 W + 593 60 + years; 13.5 W + 514 Female: 18–30 years; 13.1 W + 558 30–60 years; 9.74 W + 694 60 + years; 10.1 W + 569	12
Liu (1995)	13.88 W + 4.16 H−3.43 A − 112.40 (if female) + 54.34	10
Ganpule (2007)	(48.1 W + 23.4 H−13.8 A−547.3(if female) − 423.5)/4.186	15

[a] BEE, basal energy expenditure (kcal/day).
[b] W, weight (kg).
[c] A, age (year).
[d] H, height (cm).

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Table 6
Characteristics of patients (validation set).

	All	Male	Female
No. of patients	60	36	24
Type of diabetes (type1/type2) (n)	6/54	3/33	3/21
Age (years)	58.9 ± 13.3 (range 21–82)	55.8 ± 13.5	63.6 ± 11.8
Body weight (kg)	66.9 ± 18.2 (range 41.1–138.0)	70.0 ± 19.2	62.2 ± 15.8
BMI (kg/m^2)	25.7 ± 6.7	24.6 ± 6.2	27.5 ± 7.2
BEE (kcal/day)	1260 ± 219	1342 ± 225	1137 ± 141
FPG (mg/dL)	132.1 ± 20.8	130.8 ± 20.5	133.9 ± 21.6
PPPG (mg/dL)	157.6 ± 32.3	156.7 ± 34.8	159.0 ± 28.9
HbA1c (%)	9.3 ± 1.5	9.5 ± 1.8	9.0 ± 1.1
Duration of diabetes (years)	10.0 ± 8.8	9.3 ± 8.4	11.0 ± 9.5
Treatment			
Diet (kcal/SBW/day)	29.4 ± 2.8	29.4 ± 3.0	29.4 ± 2.5
Medications			
Ins only (n)	28	15	13
Ins + Met (n)	2	1	1
Ins + SU (n)	2	2	0
SU (n)	13	9	4
SU + Met (n)	4	4	0
Met only (n)	3	1	2
None (n)	8	4	4

Data are means ± SD. BMI, body mass index; BEE, basal energy expenditure; FPG, fasting plasma glucose just before the measurement of BEE; PPPG, mean preprandial plasma glucose for three consecutive days before the measurement of BEE; HbA1c, glycated hemoglobin; SBW, standard body weight; Ins, insulin; SU, sulfonylurea; Met, metformin.

diabetes, the elevated energy expenditure is observed only during insulin deprivation, and it returns to normal level by insulin treatment.[28] In type 2 diabetes, there is no difference in FFM-adjusted REE between mildly hyperglycemic patients and controls.[6] Thus, when they are under treatment, BEE in both type 1 and type 2 diabetes patients can be assumed comparable to that in healthy people. In addition, our validation data set has more background in common with the derivation set than the general population of Japanese patients with diabetes. We also did not measure BEE of healthy Japanese for comparison. It remains to be established whether or not the difference in BEE between Japanese patients with diabetes and healthy Japanese is insignificant when FPG of patients are <180 mg/dL.

The values estimated from the proposed equation in the present study are well matched to the reference values for Japanese BEE (Dietary reference intakes) reported in healthy Japanese as values per body weight among different groups for age and sex.[29] In addition, when mean BEE values were calculated by the proposed equation from mean body weight and age reported in other studies including healthy Japanese and Chinese, estimated BEE values were in good agreement with measured values.[10,15,30]

We report a new equation using parameters readily available in clinical practice to estimate BEE of patients with diabetes in an Asian population. Further studies are required to in a wide range of populations to determine its usefulness in Asian clinical settings.

Table 7
Evaluation of equations in validation set.

Equation	Estimated BEE per body[a]	Estimated BEE per kg Wt[b]	Bias[c]	RMSE[d]	Accurate estimation[e]
New equation	1317 ± 227	20.2 ± 2.3	4.8 ± 7.7	103	78
Harris and Benedict	1388 ± 309	21.1 ± 2.2	9.8 ± 9.4	184	50
Oxford	1420 ± 309	21.6 ± 2.3	12.3 ± 9.5	209	38
Liu	1407 ± 321	21.3 ± 2.1	11.1 ± 10.9	205	42
Ganpule	1323 ± 295	20.1 ± 2.4	4.5 ± 10.5	140	63

n = 60. Data are means ± SD.
[a] Estimated BEE per body, mean basal energy expenditure estimated per body (kcal/day).
[b] Estimated BEE per kg Wt, mean basal energy expenditure estimated per kg body weight (kcal/kg/day).
[c] Bias, mean percentage error between estimated and measured BEE ((BEE estimated − BEE measured)/BEE measured) (%).
[d] RMSE, root mean squared error (kcal/day).
[e] Accurate estimation, percent of the patients estimated by each equation within ±10% of measured value (%).

Statement of authorship

The authors' responsibilities were as follows: KI, SF, MG, and TK designed research; KI, CY, AH, MI, KN and KS conducted research; KI, MG, and SF analyzed data; KI and SF wrote the paper; and NI supervised research. All authors read and approved the final manuscript.

Conflict of interest

None of the authors had any conflict of interest.

Acknowledgements

This study was supported by a research grant from the Ministry of Health, Labour and Welfare of Japan and also by Kyoto University Global COE program "Center for Frontier Medicine".

The authors thank Masayuki Yokode from Kyoto University Hospital Translational Research Center Department of Clinical Innovative Medicine for his general assistance in conducting this research.

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Please cite this article in press as: Ikeda K, et al., A new equation to estimate basal energy expenditure of patients with diabetes, Clinical Nutrition (2012), http://dx.doi.org/10.1016/j.clnu.2012.11.017

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Please cite this article in press as: Ikeda K, et al., A new equation to estimate basal energy expenditure of patients with diabetes, Clinical Nutrition (2012), http://dx.doi.org/10.1016/j.clnu.2012.11.017

DOI: 10.1111/j.1365-2362.2012.02687.x

ORIGINAL ARTICLE

Correlation of fasting serum apolipoprotein B-48 with coronary artery disease prevalence

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ABSTRACT

Background Postprandial hyperlipidemia partially refers to the postprandial accumulation of chylomicrons and chylomicron remnants (CM-R). Many *in vitro* studies have shown that CM-R has highly atherogenic properties, but consensus is lacking on whether CM-R accumulation correlates with the development of atherosclerotic cardiovascular diseases. We investigated the correlation between CM-R accumulation and the prevalence of coronary artery disease (CAD).

Design Subjects who received a coronary angiography and did not take any lipid-lowering drugs (n = 189) were enrolled. Subjects with coronary artery stenosis (≥ 75%) were diagnosed as CAD. Biochemical markers for glucose and lipid metabolism including fasting apolipoprotein (apo) B-48 concentration were compared between CAD patients (n = 96) and age-, sex-, and body mass index (BMI)-matched non-CAD subjects without overt coronary stenosis (< 75%) (n = 67). We tried to determine which metabolic parameters were correlated with the prevalence of CAD by multiple logistic regression analysis, and whether or not the combination of high apo B-48 and other coronary risk factors (high triglyceride, low HDL-C, high HbA1c or low adiponectin levels) increased the prevalence of CAD.

Results Fasting serum apo B-48 levels were significantly higher in CAD patients than in non-CAD subjects (3·9 ± 2·4 vs. 6·9 ± 2·6 μg/mL, P < 0·0001) and had the most significant correlation with the existence of CAD. The clustering of high fasting apo B-48 levels (> 4·34 μg/mL, the cut-off value) and other coronary risk factors were found to be associated with a stronger risk of CAD compared with single high fasting apo B-48 levels.

Conclusion Fasting serum apo B-48 levels significantly correlated with the prevalence of CAD.

Keywords Apolipoprotein B-48, chylomicrons, coronary artery disease, postprandial hyperlipidemia, remnant lipoproteins.

Eur J Clin Invest 2012; 42 (9): 992–999

Introduction

Fasting hypertriglyceridaemia and postprandial hyperlipidaemia (PH) are both closely related to the development of atherosclerotic cardiovascular diseases [1,2]. PH is characterized by postprandial accumulation of triglyceride (TG)-rich lipoproteins and their partially hydrolysed products, 'remnant lipoproteins', as suggested by Zilversmit [3] and supported by numerous subsequent studies [4,5]. Remnant lipoprotein cholesterol levels proved to be closely correlated with the prevalence of coronary artery disease (CAD) [6,7]. The atherogenicity of remnant lipoproteins has been the subject of numerous studies [5]. However, the atherogenicity of chylomicron remnants (CM-R) has been investigated less frequently than that of very-low-density lipoproteins (VLDL) remnants (VLDL-R) or intermediate-density lipoprotein (IDL). Investigators have developed an assay system for measuring serum apolipoprotein B-48 (apo B-48) concentration, which represents the

number of chylomicrons (CMs) and CM-R in the serum [8]. Fasting apo B-48 levels ranged from 0 to 25 μg/mL (the mean ± SD value was 5·2 ± 3·8 μg/mL) and were significantly higher in hyperlipidaemic patients with supposed accumulation of CMs and CM-R [8] as well as in patients with metabolic syndrome (MetS) [9] than in healthy subjects.

Several clinical studies have suggested a correlation between serum apo B-48 levels and atherosclerosis [10,11]. In our recent study, high levels of fasting apo B-48 significantly correlated with intima-media thickness (IMT) in subjects with normal but relatively high TG levels (100 < TG≤150 mg/dL) [12]. Emerging evidence from *in vivo* [13–15] and *in vitro* studies [5,16] suggests that CM-R might have atherogenic features and may be responsible for the initiation of atherogenesis. However, one report suggested that there was no significant correlation between fasting apo B-48 levels and CAD [17], emphasizing also that no consensus existed as to whether high levels of fasting apo B-48 were correlated with the prevalence of CAD. Moreover, it remained uncertain whether the prevalence of CAD in subjects with high levels of CAD would increase or not in combination with other metabolic disorders such as insulin resistance of MetS.

In this study, we attempted to investigate whether fasting serum levels of apo B-48 correlated with the prevalence of CAD and whether these correlations were stronger than other metabolic parameters recognized as coronary risk factors.

Subjects and methods

Subjects

A consecutive series of patients with suspected CAD were hospitalized in Osaka University Hospital and National Hospital Organization Kure Medical Center from January 2002 to December 2003. Patients who needed emergency care, had acute coronary syndrome or were already being treated with lipid-lowering drugs were eliminated. As a result, 189 subjects (120 men and 69 women) undergoing quantitative coronary angiography (CAG) were enrolled in this study. Height and weight were measured and (BMI, kg/m^2) was calculated. During hospitalization, all patients adhered to a standard diet which contained 25 kcal/kg standard body weight (BMI = 22 kg/m^2) per day (patients with hypertension took a sodium-restricted diet with the same calorie intake), and their blood pressure (BP) was measured in a supine position. The presence of hypertension was assessed by systolic BP ≥ 135 mmHg and/or diastolic BP ≥ 85 mmHg (based on the Guideline for the Management of Hypertension from the Japanese Society of Hypertension) or by intake of anti-hypertensive drugs (Ca blockers were mainly used, beta-blockers were used in only two patients in both CAD and non-CAD groups and no woman used contraceptives or received hormone replacement therapy). Angiographically significant coronary stenosis was defined as 75% or more luminal diameter stenosis by CAG. Those who had significant stenosis in the left anterior descending artery, left circumflex artery and/or right coronary artery were treated as CAD patients (n = 96, 71 men and 25 women). Age-, sex- and BMI-matched subjects who did not have significant stenosis were regarded as non-CAD subjects (n = 67, 49 men and 18 women).

Laboratory measurements and diagnosis of coronary risk factors

Immediately after blood samples were collected in the morning of CAG after an overnight fast, serum and plasma were separated by centrifugation (2000 g, 15 min, 4 °C) and stored at −80 °C until measurement. Serum total cholesterol (TC) and TG levels were determined by enzymatic methods, serum LDL-C and HDL-C levels by the direct method (Sekisui Medical Co., Ltd., Tokyo, Japan) and plasma adiponectin levels by ELISA (Otsuka Pharmaceuticals, Tokyo, Japan). The presence of dyslipidaemia was assessed by LDL-C ≥ 140 mg/dL, TG ≥ 150 mg/dL and/or HDL-C < 40 mg/dL [18]. Fasting plasma glucose (FPG) was measured by the enzymatic method, and HbA1c by ion-exchange high performance liquid chromatography (HPLC) (Sekisui Medical Co.). The presence of high fasting glucose was assessed by FPG ≥ 126 mg/dL (Japan Diabetes Society) or by intake of anti-diabetic drugs. The presence of MetS was diagnosed according to the criteria of the Japanese Society of Internal Medicine [19] and National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III), USA [20]. Serum apo B-48 levels were determined by the chemiluminescent enzyme immunoassay system (Fujirebio Inc., Tokyo, Japan) [21] which was modified from the sandwich ELISA system [8]. All samples were treated in accordance with the Helsinki Declaration.

Statistical analyses

Apo B-48, BMI and adiponectin levels were normalized by logarithmic transformation. The statistical significance of differences in TC, TG, HDL-C, LDL-C, systolic BP, diastolic BP, FPG, HbA1c, Log-apo B-48 and Log-adiponectin between CAD and non-CAD subjects was determined by Mann–Whitney *U*-test, by frequency of smoking, as well as by prevalence of dyslipidaemia, hypertension and high fasting glucose. The prevalence of Mets was then compared by chi-square test. The correlations between metabolic parameters and CAD were analysed by Pearson's correlation coefficients, and stepwise multiple logistic regression analysis was used to determine independent predictors of CAD. Age, sex, Log-BMI, smoking, TC, LDL-CADL-C, TG, systolic BP, diastolic BP, FPG, HbA1c, Log-apo B-48 and Log-adiponectin were included as explanatory variables in the

method. Receiver-operating characteristic (ROC) curves were used to examine the apo B-48 values for categorizing subjects on the basis of the presence of CAD, and the cut-off value was identified.

We compared the effect of different metabolic parameters of MetS (TG, HDL-C, HbA1c or plasma adiponectin; classified as low and high) on CAD prevalence in patients with low or high levels of apo B-48. The cut-off value of apo B-48 level in CAD patients was 4·34 μg/mL. We divided subjects (n = 163) into two groups according to their apo B-48 levels: low (≤ 4·34 μg/mL) and high (> 4·34 μg/mL). Both groups were also divided into low (< 150 mg/dL) or high TG levels (≥ 150 mg/dL), high (≥ 40 mg/dL) or low HDL-C levels (< 40 mg/dL), low (< 5·8%) or high HbA1c levels (≥ 5·8%) and high (≥ 4·0 μg/mL) or low plasma adiponectin levels (< 4·0 μg/mL) [22]. The statistical significance of differences in the prevalence rates of CAD was determined among these four groups by chi-square test. The data were analysed with JMP8 software (SAS Institute, Cary, NC, USA). All statistical significance were accepted at $P < 0·05$.

Results

Comparison of clinical profiles between coronary artery disease patients and non- coronary artery disease subjects

Serum FPG and HbA1c levels were significantly higher, whereas serum levels of HDL-C and adiponectin were significantly lower in patients with CAD (n = 96) than in age-, sex- and BMI-matched non-CAD subjects (n = 67) (Table 1). Fasting apo B-48 and TG levels were significantly higher in CAD patients than in non-CAD subjects ($P < 0·0001$), and the statistical significance of difference was the highest for these parameters (Table 1). Fasting serum apo B-48 levels ranged from 0 to 13 μg/mL in non-CAD subjects, and from 0 to 19 μg/mL in patients with CAD (Fig. 1). The fasting apo B-48 concentration in a large majority of CAD patients and non-CAD subjects was 10 μg/mL or less, but the peak and average of fasting apo B-48 levels were higher in patients with CAD than in non-CAD subjects (Fig. 1 and Table 1). The ROC curve analysis showed that the AUC-ROC value was 0·79, and the cut-off value of apo B-48 was identified as 4·34 (overall sensitivity, 0·82; 1-specificity, 0·33; predictive positive value, 79; predictive negative value, 61).

Correlations between the existence of CAD and metabolic parameters

The correlations between the existence of CAD and metabolic parameters related to coronary risk were analysed by logistic regression analysis in these subjects (Table 2). By Pearson's correlation analysis, significant correlations with the existence of CAD were observed in smoking, HDL-C, TG, FPG, HbA1c, Log-apo B-48 and Log-adiponectin levels. Multiple regression analysis indicated that only log-apo B-48 was a significant determinant of the existence of CAD ($P < 0·0001$) among the various metabolic parameters related to coronary risk (Table 2).

Prevalence of coronary artery disease in subjects with high Apo B-48 levels and other metabolic parameters of abnormal levels

The clustering of metabolic parameters is a high risk state for CAD. We compared the prevalence of CAD in patients with low (≤ 4·34 μg/mL) and high (> 4·34 μg/mL) levels of apo B-48 when their metabolic parameters of MetS (TG, HDL-C, HbA1c or plasma adiponectin levels) were in high risk status (detailed in *Subjects and Methods*). CAD was significantly more prevalent in subjects with high levels of apo B-48 than in subjects with low levels of apo B-48, irrespective of TG, HDL-C, HbA1c or plasma adiponectin levels (in Fig. 2). The prevalence of CAD was significantly higher in subjects with high levels of apo B-48 and high TG, low HDL-C, high HbA1c or low plasma adiponectin levels, compared with that in subjects with low levels of apo B-48 and normal TG, HDL-C, HbA1c or plasma adiponectin levels.

Discussion

This study demonstrated that fasting levels of apo B-48 were higher in patients with CAD than in those with non-CAD, and that high levels of fasting apo B-48 were definitely correlated with the prevalence of CAD among other metabolic biomarkers related to coronary risk. The combination of high fasting apo B-48 levels and other metabolic disorders represented a stronger risk state for CAD.

High fasting serum apo B-48 levels in patients with coronary artery disease compared with non-coronary artery disease subjects

The fasting apo B-48 concentration in a large majority of CAD patients and non-CAD subjects was 10 μg/mL or less, and the peak and average of fasting apo B-48 levels were higher in patients with CAD than in non-CAD subjects (Fig. 1 and Table 1). In CAD patients, MetS components, such as dyslipidaemia, hypertension, high fasting glucose and low adiponectin levels, were more clustered than in non-CAD subjects (Table 1), implying that CAD patients tended to have a pathophysiological background of MetS. The presence of insulin resistance leads to a deterioration of postprandial remnant metabolism [23]. Impaired clearance of lipoproteins is related to the accumulation of CM-R in the postprandial serum and the increase in fasting apo B-48 concentrations [24]. In this study, a high prevalence of CAD was observed in patients with high levels of apo B-48 (Fig. 2). This may indicate that the

Table 1 Clinical Profiles of the Non-CAD subjects and the patients with CAD

	non-CAD (n = 67)	CAD (n = 96)
Age (years)	62·7 ± 10·8	65·1 ± 9·9
Sex[†] (m vs. w)	49 vs. 18	71 vs. 25
Smoking (%)	48·2	60·4
BMI (kg/m^2)	24·1 ± 3·6	24·4 ± 2·8
Prevalence of Dyslipidaemia[‡] (%)	40·2	66·7
TC (mg/dL)	197·6 ± 37·1	199·5 ± 36·5
TG (mg/dL)	121·4 ± 37·1	163·1 ± 83·3**
HDL-C (mg/dL)	49·5 ± 13·3	43·8 ± 13·2*
LDL-C (mg/dL)	125·1 ± 34·3	125·5 ± 34·3
Prevalence of Hypertension[§] (%)	64·1	78·2
Systolic BP (mmHg)	130·0 ± 17·2	130·0 ± 22·9
Diastolic BP (mmHg)	74·6 ± 10·6	75·4 ± 12·2
Prevalence of drug-treated patients (%)	53·1	68·3
Prevalence of High fasting glucose[¶] (%)	19·3	40·0
FPG (mg/dL)	100·5 ± 25·1	116·7 ± 42·4*
HbA1c (%)	5·4 ± 1·0	6·3 ± 1·7*
Fasting apo B-48 μg/mL	3·9 ± 2·4	6·9 ± 2·6**
Adiponectin μg/mL	7·8 ± 4·3	6·4 ± 4·2*
Prevalence of the metabolic syndrome		
In Japanese criteria (%)	17·2	29·2*
In NCEP-ATPIII criteria (%)	22·6	53·1*

BMI, body mass index; BP, blood pressure; FPG, Fasting plasma glucose; TC, total cholesterol; TG, triglyceride; CAD, coronary artery disease.
[†]Number of men vs. women.
[‡]Ratio of subjects with TG ≥ 150 mg/dL and/or HDL-C < 40 mg/dL.
[§]Ratio of subjects with systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg.
[¶]Ratio of subjects with FPG ≥ 126 mg/dL). The statistical significance of differences in TC, TG, HDL-C, LDL-C, systolic BP, diastolic BP, FPG, HbA1c, fasting apo B-48 and adiponectin were determined by Mann–Whitney's U-test, those in the prevalence of smoking, dyslipidaemia, hypertension, high FPG and the Mets were determined by the chi-square test. Significance was established at $P < 0·05$. *$P < 0·01$, **$P < 0·0001$.

atherogenicity of CM-R might be prolonged throughout the day in CAD patients with a pathophysiological background of MetS. We also found that a small number of CAD patients and non-CAD subjects had high fasting apo B-48 levels (> 10 μg/mL) (Fig. 1). In our former study, we found subjects with high apo B-48 levels (> 10 μg/mL) among patients with any type of hyperlipidaemia [8]. In that study, high fasting apo B-48 levels (> 10 μg/mL) were mainly observed in patients with type I, III, IV and V hyperlipidaemia [8], probably because of the characteristics of a genetic polymorphism (impaired lipoprotein lipase (LPL) activity, the existence of apo E2/E2 phenotype or apo A5) or the existence of PH. Although we did not diagnose the type of hyperlipidaemia in subjects with high fasting apo B-48 levels (> 10 μg/mL), high levels of fasting apo B-48 in CAD patients might be partly a result of impaired lipoprotein metabolism caused by a genetic disorder of apoproteins, enzymes and receptors, or the existence of MetS.

High fasting apo B-48 levels and atherosclerosis

A number of studies have suggested that CM-R had highly atherogenic properties, but there is still no consensus as to whether CM-R accumulation correlates with the development of atherosclerotic cardiovascular diseases. By multiple regression analysis, it was determined that log-apo B-48 was the only significant determinant of the existence of CAD ($P < 0·0001$) among other metabolic parameters related to coronary risk (Table 2).

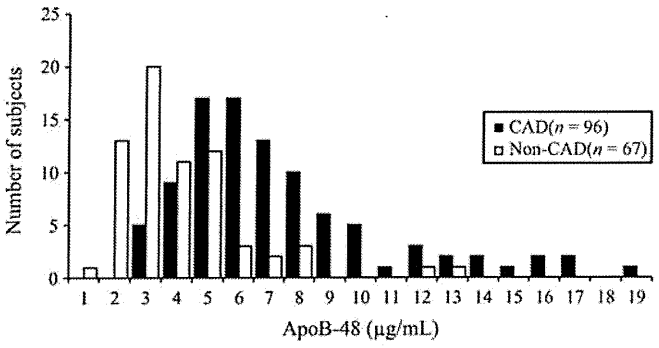

Figure 1 Distribution of Fasting Serum Apo B-48 Levels in non coronary artery disease (Non-CAD) Subjects and Patients with CAD. Fasting serum concentrations of apo B-48 in non-CAD subjects (open squares, n = 96) and patients with CAD (closed squares, n = 67). Serum apo B-48 level=1 represents concentrations between 0·0 and 1·0 μg/mL.

Table 2 Univariate and multivariate analyses of correlations between the existence of coronary artery disease and various metabolic parameters

	Univariate *P* value	Multivariate *P* value
Age	0·1581	–
Sex	0·3698	–
Log-BMI	0·4645	–
Smoking	0·0492	–
TC	0·7440	–
LDL-C	0·8508	–
HDL-C	0·0085	0·3721
Triglyceride	0·0017	0·1098
Systolic BP	0·9747	–
Diastolic BP	0·6757	–
FPG	0·0081	0·6110
HbA1c	0·0008	0·3036
Log-apo B-48	< 0·0001	< 0·0001
Log-APN	0·0239	0·6039

BMI, body mass index; TC, total cholesterol; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c. APN, adiponectin. Univariate analysis was assessed using Pearson's correlation analysis. Multivariate analysis was assessed using stepwise multiple regression analysis.

Moreover, as shown in Fig. 2, the prevalence of CAD was significantly higher in subjects with high levels of apo B-48 than in subjects with low levels of apo B-48, irrespective of other coronary risk factors such as high TG, low HDL-C, high HbA1c or low plasma adiponectin levels. These results clearly show that the frequency of coronary stenosis was significantly and strongly correlated with fasting apo B-48 level, suggesting that the accumulation of CM-R was the strongest risk factor for CAD in these study subjects. Whereas the cut-off value of apo B-48 was 4·34 as determined by ROC curve analysis, the specificity was unfortunately low (1-specificity; 0·33) perhaps because of some degree of coronary stenosis in subjects who were diagnosed as non-CHD subjects. Therefore, high levels of apo B-48 might correlate with patients with more severe CAD than with patients with less severe CAD or non-CAD subjects.

Clustering of high apo B-48 level and other coronary risk factors

The prevalence of CAD gradually increased with higher levels of apo B-48 in association with high TG, low HDL-C, high HbA1c or low plasma adiponectin levels (Fig. 2). Whereas high TG, low HDL-C, high HbA1c and low plasma adiponectin levels are independent coronary risk factors, these metabolic disorders correlated with the accumulation of CM-R. The accumulation of CM-R was associated with insulin resistance and prevalence of type II diabetes mellitus [23]. Plasma adiponectin and leptin concentrations were inversely and directly associated with plasma apo B-48, whereas plasma apo B-48 level was significantly and positively associated with plasma insulin, HOMA and visceral fat areas [25]. The combination of high levels of apo B-48 and other metabolic disorders related to coronary risk may synergistically increase atherogenicity. However, high levels of fasting apo B-48 independently enhanced the prevalence of CAD, irrespective of TG, HDL-C, HbA1c or plasma adiponectin levels. This indicates that a high level of apo B-48, namely the accumulation of CM-R, was the strongest risk status for the prevalence of CAD of all metabolic disorders, as shown in Table 2. These results suggest that without the measurement of fasting apo B-48 level, we may underestimate the CAD risk by the assessment of metabolic disorders using TG, HDL-C, HbA1c or plasma adiponectin levels. For the assessment of CAD risk in subjects with MetS or subjects with little coronary risk, measuring apo B-48 levels remains useful. Subjects with high apo B-48 levels should be assessed carefully by a variety of pharmacological and physiological approaches [26]. Both atorvastatin and fenofibrate have been shown to improve the postprandial increase of CM-R markedly [27,28]. We have also recently reported that ezetimibe, an intestinal cholesterol transporter inhibitor, improves PH in patients with type IIb hyperlipidaemia [29] by reducing the intestinal production of CMs [30]. As fasting and postprandial levels of apo B-48 decrease by these physiological and pharmaceutical interventions [26–29], the

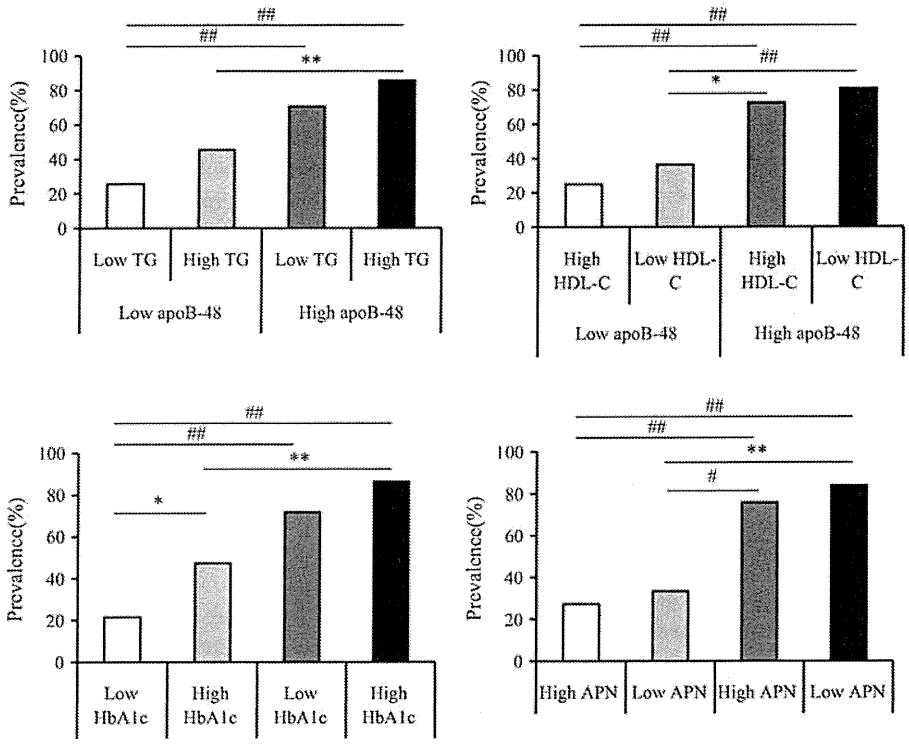

Figure 2 Prevalence Rate of coronary artery disease (CAD) in Subjects with a Combination of High Apo B-48 Levels and Other Coronary Risk Factors. We compared the effect of different metabolic parameters of metabolic syndrome (TG, HDL-C, HbA1c or plasma adiponectin; classified as low and high) on CAD prevalence in patients with low or high apo B-48 levels. We divided all subjects (n = 163) into low (≤ 4·34 μg/mL) and high (> 4·34 μg/mL) apo B-48 levels; these two groups were also divided according to low (< 150 mg/dL) or high (≥ 150 mg/dL) TG levels, high (≥ 40 mg/dL) or low (< 40 mg/dL) HDL-C levels, low (< 5·8%) or high (≥ 5·8%) HbA1c levels or high (≥ 4·0μg/mL) or low (< 4·0 μg/mL) plasma adiponectin levels. The prevalence rates of coronary artery disease were determined in each group, and the statistical significance of differences among these four groups was verified by chi-square test. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{\#}P < 0{\cdot}001$ and $^{\#\#}P < 0{\cdot}0001$

measurement of apo B-48 levels may be useful for managing CAD risk in subjects with MetS or PH.

Limitation of the study

In this study, the subjects were collected from outpatients who came to the cardiovascular department and were susceptible to having CAD. These subjects had already been treated with anti-diabetic drugs or anti-hypertension drugs, and the total number of patients was relatively small compared with that in other related studies.

Conclusion

In conclusion, fasting serum apo B-48 levels are significantly correlated with the existence of CAD and other metabolic disorders. The measurement of fasting apo B-48 is useful for detecting and managing CAD risk in subjects with MetS or low coronary risk.

Acknowledgements

We gratefully acknowledge Fujirebio Inc. and Sekisui Medical Co., Ltd. for measuring our samples with high quality standards. The authors gratefully acknowledge the excellent technical assistance and office work extended by K. Hizu-Shioyama, R. Wada and M. Kato.

Sources of funding

This work was supported by a Grant-in-Aid for Scientific Research (No. 13671191) to S. Yamashita from the Ministry of Education, Science, Sports and Culture in Japan; a grant from Mitsui Life Social Welfare Foundation to S. Yamashita; a Takeda Medical Research Foundation grant to S. Yamashita and in part by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), Foundation for Biomedical Research and Innovation for S. Matsuyama, S. Yamashita, D. Masuda and K. Tsubakio-Yamamoto.

Disclosures

S. Yamashita has received consultancy fees from Fujirebio Inc. Other co-authors have nothing to disclose.

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Received 23 November 2011; accepted 14 April 2012

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