Table 2. Hazard ratios for breast cancer associated with BMI in the JACC Study

BMI	Cases	Person-years	Age-adjusted		Multivariate[a]	
			Hazard ratio	95% CI	Hazard ratio	95% CI
Premenopausal women						
<18.5	3	4799	0.89	(0.28–2.89)	0.82	(0.25–2.68)
18.5–19.9	6	10327	0.83	(0.35–1.97)	0.78	(0.33–1.84)
20–23.9	39	55363	1.00	Reference	1.00	Reference
24–28.9	13	25975	0.71	(0.38–1.33)	0.76	(0.40–1.43)
≥29	1	2453	0.54	(0.07–3.97)	0.62	(0.08–4.58)
P for trend			0.97		0.82	
Postmenopausal women						
<18.5	7	19412	0.71	(0.33–1.55)	0.64	(0.30–1.40)
18.5–19.9	7	28831	0.47	(0.22–1.02)	0.46	(0.21–1.00)
20–23.9	77	146684	1.00	Reference	1.00	Reference
24–28.9	71	93372	1.47	(1.06–2.03)	1.50	(1.09–2.08)
≥29	10	10427	2.00	(1.03–3.89)	2.13	(1.09–4.16)
P for trend			<0.0001		<0.0001	

BMI, body mass index.
[a]Adjusted for age, height, age at menarche, age at menopause (among postmenopausal women only), years of education, parity, marital status, use of exogenous female hormone, first-degree family history of breast cancer, smoking status, alcohol drinking, physical activity, and study area.

Table 3. Multivariate hazard ratios for breast cancer associated with baseline BMI and weight change among postmenopausal women in the JACC Study

Weight change from age 20 years	Baseline BMI <24		Baseline BMI ≥24	
	Hazard ratio	95% CI	Hazard ratio	95% CI
Premenopausal women				
Loss, unchanged, or gain of <10 kg	1.00	Reference	0.94	(0.35–2.55)
Gain of ≥10 kg	0.53	(0.07–3.96)	1.88	(0.85–4.16)
Postmenopausal women				
Loss, unchanged, or gain of <10 kg	1.00	Reference	1.34	(0.69–2.58)
Gain of ≥10 kg	0.99	(0.24–4.19)	2.55	(1.47–4.42)

BMI, body mass index.
Adjusted for age, height, age at menarche, years of education, parity, marital status, use of exogenous female hormone, first-degree family history of breast cancer, smoking status, alcohol drinking, physical activity, and study area.

5-kg/m^2 increment of BMI, after adjustment for potential confounders.

An effect of weight gain between age 20 years and baseline on breast cancer risk was observed only among postmenopausal women. The HR (95% CI) for 1 increment of weight gain was 1.04 (1.01–1.07). Among premenopausal women it was 0.99 (0.94–1.04) and not significant.

The combinatorial effect of baseline BMI and weight change between age 20 years and baseline was examined to evaluate the effect of these factors separately (Table 3). In premenopausal women, no significant HR or association was found. Conversely, in postmenopausal women, only those with a baseline BMI of 24 or higher and weight gain of at least 10 kg from age 20 years to baseline had a significant HR (2.55, 95% CI: 1.47–4.42), as compared with those with a baseline BMI of less than 24 and a weight gain of less than 10 kg from age 20 years to baseline. These findings indicate that weight gain after age 20 years and consequent overweight/obesity are combined risk factors for breast cancer among postmenopausal women. This combined effect was particularly strong in older women (HR: 4.08, 95% CI: 1.88–8.88). In addition, weight at age 20 years was not a significant predictor of breast cancer after adjustment for height at baseline and other potential confounders among premenopausal and postmenopausal women in this study. Furthermore, similar results were obtained after excluding the 33 breast cancer cases that occurred during the first 2 years of follow-up (data not shown).

DISCUSSION

To our knowledge, this is the first prospective report from Japan on the association between obesity/weight gain and breast cancer risk by age group. Our findings revealed a significant association between BMI/weight gain and postmenopausal breast cancer risk, particularly among older women. For postmenopausal women, especially those aged 60 years or older, weight gain after age 20 years and consequent

overweight/obesity were identified as combined risk factors for breast cancer, after adjusting for potential confounders. In other words, being overweight or obese at baseline was a much greater risk factor among women who were postmenopausal, were aged 60 years or older, and had gained at least 10 kg from age 20 years to baseline.

Our results for postmenopausal women are consistent with those obtained in a number of studies worldwide. The adjusted HR per 5-kg/m^2 increment in BMI in the present study (1.68) was slightly higher than the summary risk ratios from a meta-analysis[4] of studies conducted in the Asia-Pacific (1.31), North America (1.15), and Europe and Australia (1.09). Breast cancer prevention via weight control is expected to be more effective among postmenopausal women in the Asia-Pacific region. With regard to cancer pathogenesis, the increased risk in overweight/obese postmenopausal women is due to the fact that adipose tissue is the major source of estrogenic hormones after menopause.[33,34] Furthermore, our results conform with those of an earlier report showing that adult weight gain might be better than cross-sectional BMI as an adiposity index.[35]

In contrast, we did not observe any significant association between BMI/weight change and breast cancer risk among premenopausal women. In our cohort, age at baseline was 40 years or older; thus, follow-up did not completely cover the premenopausal period. A previous study reported an inverse association between BMI and breast cancer risk among white women. One hypothesis is that young overweight women are more likely to have anovulatory cycles with less cumulative exposure to endogenous estrogen.[36,37] Another hypothesis is that there is greater clearance of estrogen by the liver in young overweight women.[38] These hypotheses are strengthened by results from studies suggesting that the inverse associations are limited to women with tumors that are estrogen receptor- and progesterone receptor-positive.[25–28] Thus, the heterogeneity of pathologic types among premenopausal breast cancer weakens the association and possibly explains the inconsistent results among non-white racial/ethnic groups. This heterogeneity of cancer etiology in relation to BMI and receptor type makes cancer prevention in premenopausal women difficult and of less practical importance. Further investigations of cancer pathogenesis are needed among non-white racial/ethnic groups.

A major advantage of the present study was its prospective design, which may avoid the possibility of recall bias inherent to case-control studies. Moreover, information on other breast cancer risk factors was included, and potential confounding factors were controlled in analyses of the association.

This study has some limitations that should be considered when interpreting our results. First, because we did not have updated information on menopausal status, which would modify the association between BMI/weight change and breast cancer, the possibility of misclassification of menopausal status at breast cancer onset should be considered. Such misclassification would be problematic in premenopausal women, since recently menopausal women would be misclassified as premenopausal during the follow-up period. Such misclassification could partly explain the inconsistent results from several studies of the association between body size and breast cancer among premenopausal women. Studies of younger women with updated information on menopausal status should be initiated among premenopausal women. However, this limitation is a minor concern for postmenopausal women. Changes during follow-up, especially those related to lifestyle, might alter the results. However, many risk factors, such as marriage status, number of children, and family history of breast cancer, would be unlikely to change after age 40. To our knowledge, substantial changes in risk factors for breast cancer related to BMI have not been reported.

Second, because we used simple questionnaires at baseline only, we have data at only 2 time points, ie, age 20 years and baseline. We did not have data on the time period of weight gain, which would provide useful information for recommendations. Lack of information on weight gain around menopause would also weaken the association among premenopausal women. Furthermore, weight at age 20 years is retrospective information and may be systematically biased among women at extremes of body size. However, these data were obtained before breast cancer diagnosis, and therefore any misclassification is not likely to be differential.

The accuracy of cancer identification in the present study was not ideal. We estimated that 36.5 cases of incident breast cancer were not included in our follow-up, and this number is not inconsiderable. However, these cases would be independent of body size; thus, estimated HRs would tend toward the null.

In summary, our findings support the hypothesis that a weight gain of 10 kg or more and consequent overweight/obesity (BMI $\geq$24) are combined risk factors for breast cancer among Japanese postmenopausal women, particularly those aged 60 years or older. Thus, to prevent breast cancer, weight gain after age 20 years should be avoided and weight control should be increasingly emphasized with increasing age. The association between body size and premenopausal breast cancer was not clear in the present study and varies across studies; thus, optimal weight for breast cancer prevention cannot be specified at this time.

ONLINE ONLY MATERIALS

Abstract in Japanese.

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RESEARCH ARTICLE Open Access

Japan Diabetes Outcome Intervention Trial-1 (J-DOIT1), a nationwide cluster randomized trial of type 2 diabetes prevention by telephone-delivered lifestyle support for high-risk subjects detected at health checkups: rationale, design, and recruitment

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Abstract

Background: Lifestyle modifications are considered the most effective means of delaying or preventing the development of type 2 diabetes (T2DM). To contain the growing population of T2DM, it is critical to clarify effective and efficient settings for intervention and modalities for intervention delivery with a wide population reach. The Japan Diabetes Outcome Intervention Trial-1 (J-DOIT1) is a cluster randomized controlled trial to test whether goal-focused lifestyle coaching delivered by telephone can prevent the development of T2DM in high-risk individuals in a real-world setting. This paper describes the study design and recruitment of the study subjects.

Methods: For the recruitment of study subjects and their follow-up annually over 3 years, we employed health checkups conducted annually at communities and worksites. Health care divisions recruited from communities and companies across Japan formed groups as a cluster randomization unit. Candidates for the study, aged 20-65 years with fasting plasma glucose (FPG) of 5.6-6.9 mmol/l, were recruited from each group using health checkups results in 2006. Goal-focused lifestyle support is delivered by healthcare providers via telephone over a one-year period. Study subjects will be followed-up for three years by annual health checkups. Primary outcome is the development of diabetes defined as FPG≥7.0 mmol/l on annual health checkup or based on self-report, which is confirmed by referring to medical cards.

Results: Forty-three groups (clusters), formed from 17 health care divisions, were randomly assigned to an intervention arm (22 groups) or control arm (21 clusters) between March 2007 and February 2008. A total of 2840 participants, 1336 from the intervention and 1504 from the control arm, were recruited. Consent rate was about 20%, with no difference between the intervention and control arms. There were no differences in cluster size and characteristics of cluster between the groups. There were no differences in individual characteristics between the study arms.

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Conclusion: We have launched J-DOIT1, a nation-wide trial to prevent the development of T2DM in high-risk individuals using telephone-delivered intervention. This trial is expected to contribute to evidence-based real-world preventive practices.

Trial registration : UMIN000000662.

Background

Type 2 diabetes mellitus (T2DM) is rapidly becoming one of the major health issues of the 21st century [1,2]. A recent survey performed by the Ministry of Health, Labour and Welfare has projected that approximately 8.9 million people have diabetes and another 13.2 million people are at high risk for diabetes in Japan [3,4]. There is an urgent need for effective strategies to combat this pandemic. The Finnish Diabetes Prevention Study (DPS) [5] and US Diabetes Prevention Program (DPP) [6] both clearly showed that intensive lifestyle intervention can prevent or delay the development of T2DM in a high-risk population. Thus, lifestyle modifications are considered the most effective means of delaying or preventing the development of T2DM [7,8]. The DPP and DPS interventions have been translated into church [9], weight loss clinic [10], YMCA [11], primary care [12], and community [13] settings. However, translating the findings of clinical research, such as the DPS and DPP, into a real-world practice [14] on a large-scale still remains to be addressed. Japan has adopted the universal medical care insurance system, where all the people are insured by one of the public medical insurance systems [15,16]. In 2003 the Health Promotion Law was enforced aiming at preventing lifestyle-related diseases including T2DM. Now it has become mandatory for all Japanese adults to undergo health checkups provided by public medical care insurance at least once a year. There are two main types of statutory health checkup programs; 1) workplace health checkup programs managed by employers (companies setting), and 2) community health checkup programs managed by municipalities (communities setting) for self-employed, unemployed and retired individuals. People are registered at health care divisions in their workplaces or communities, and through the health care divisions, health checkups are provided. Health checkups are becoming part of routine health care. As a whole about 50% of adults undergo health check-ups annually. A large number of high-risk subjects for diabetes are identified every year through these health checkups. It is questionable, however, to what extent annual health checkups contribute toward overcoming the pandemic of diabetes. There is a big gap between identifying high risk subjects and preventing diabetes in the real world. One of the reasons for this may be a lack of evidence-based effective and efficient prevention programs which are easily accessible. The Japan Diabetes Outcome intervention Trial-1 (J-DOIT1) is a nation-wide, cluster randomized controlled trial [17], aiming to establish effective and efficient programs to prevent the development of T2DM in high-risk individuals through lifestyle modifications. The cluster randomization design has the advantages of administrative convenience, ease of obtaining the cooperation of investigators, enhancement of subject compliance, and avoidance of treatment contamination [18]. Health care divisions recruited from communities and companies across the country formed groups as a cluster randomization unit. The data of annual health checkups obtained from each group are utilized for identifying high-risk individuals and follow-up. This paper presents the study protocol in detail, including the rationale and the recruitment results. As a national project, this information should be widely referred to and shared by researchers and practitioners in preventive medicine.

Methods

This study has been approved by the Ethical Committee of the Japan Foundation for the Promotion of International Medical Research Cooperation (National Center for Global Health and Medicine, Tokyo, Japan).

Study design

The present study is a cluster randomized controlled trial [19,20] aimed at involving Japanese men and women, aged 20-65 years, at high risk for developing T2DM. For the recruitment of study subjects and their follow-up, we employ health checkups conducted annually by health care divisions at communities and worksites. A total of 17 health care divisions were enrolled across the country. Large health care divisions, with a large number of examinees and branches covering different areas, were divided into groups. A total of 43 groups were thus formed from 17 health care divisions, with each group having approximately 1,000-6,000 annual health checkup examinees. For the cluster randomization, these groups were randomly allocated to either an intervention or a control arm. Using the 2006 health checkup data obtained from each cluster, lifestyle support centers sent a program kit to the candidates who met the eligibility criteria and invited them to participate in the study. The kit included an explanation about the study's aims and protocol, a consent form, and a questionnaire regarding lifestyle and health status. Those

who consented to participate and completed the questionnaire were registered as study participants at lifestyle support centers, after their eligibility was checked based on their self-reported health status. Subjects in the intervention arm will receive non-face-to-face intervention via telephone or mobile-phone over the course of one year. Subjects in control arm will receive no such intervention. The progression to diabetes will be monitored by an annual health checkup and questionnaire over three years. All data for the study are collected at the lifestyle support centers and sent to the data management center in a de-identified form.

Recruitment of health care divisions

By advertising on the internet or by direct contact, we invited health care divisions at communities and companies to participate in the study. The inclusion criteria for the participating health care division were; 1) it conducts health checkups according to guidelines by the Health Promotion Law, 2) as a rule it has 2,000 or more examinees annually, 3) it can provide the study group with health checkups data every year starting from 2006, and 4) it can conduct lifestyle survey every year using a questionnaire prepared by the study team. Health care divisions, in which study team members are directly involved as industrial physicians, were excluded. Seventeen health care divisions, widely distributed throughout the country, agreed to participate in the study. Among them 14 health care divisions belonged to companies, 2 to municipalities, and 1 was a mixture of small-sized companies and municipalities. They were all approved by the steering committee. A large health care division, covering many distant areas, was divided into groups. This process was done by the health care division itself mainly based on the area and number of examinees. A total of 43 groups were thus formed from 17 health care divisions. The number of groups formed in each health care division ranged from 1 to 10. Each group included 700 to 6,000 annual examinees. Some groups that were small were pooled with others. Using the results of health checkups in 2006, candidates who met inclusion criteria (described later) were identified in each group.

Randomization

For cluster randomization, the groups were randomly allocated to either an intervention (n=22) or a control (n=21) arm. Randomization was performed 3 times according to 3 recruitment periods (March to April, May to June, and July to August in 2007). When two or more groups were made from one health care division, they were allocated to each of the arms within the health care division. Some small groups were pooled with others. Allocation was carried out using stratified randomization with seven strata of companies or communities in the first period, five strata in the second period, and three strata in the third period. A randomization list was prepared by an independent statistician using the SAS PLAN procedure with seed = 4989. This procedure was conducted using SAS version 9.1 (SAS Inc., Cary, NC, USA). Simple randomization was performed with 2 levels of treatment. The groups were notified of their allocation status before study subjects were recruited. The subjects were notified of their allocation status when they were recruited.

Health checkups

Guidelines for health check implementation were announced in 2004 based on the Health Promotion Law. In 2006 mandatory items to be checked included 1) anamnesis of past history including history of medication and smoking, 2) subjective and objective symptoms, 3) body height and weight, 4) Body Mass Index (BMI), calculated as body weight (kg) divided by square of body height (m^2), 5) blood pressure, 6) serum alanine aminotransferase, asparate aminotransferase and gamma glutamyltranspeptidase, 7) serum triglycerides, HDL cholesterol and LDL cholesterol, 8) fasting plasma glucose, and 9) urinalysis. At health checkup sites anthropometric measurements were done by public health nurses or industrial nurses. Height was measured in the standing position by public health nurses or industrial nurses. Weight was measured without shoes or heavy clothes to the nearest 0.1 kg using standard calibrated scales. Systolic and diastolic blood pressure values were measured in the sitting position [21]. Blood was withdrawn after 8 hours of fasting and analyzed with standard methods in clinical laboratories under the nationally certified laboratory management system. If blood was withdrawn from people who had not fasted, plasma glucose data was treated as casual plasma glucose and triglycerides values were omitted from the analysis. We did not perform any additional tests for this study.

Inclusion and exclusion criteria for study subjects

Using the 2006 year health checkups data, candidates who met the inclusion criteria were identified in each cluster. Inclusion criteria included an age of 20-65 years and impaired fasting glucose (IFG) defined as a fasting plasma glucose concentration (FPG) of 100-125 mg/dL (5.6-6.9 mmol/L). In the 2006 year health checkups, however, blood sampling was not always done in the fasting state. In those individuals where the FPG was not available, plasma glucose concentrations (casual plasma glucose, CPG) of 118-143 mg/dL (6.6-7.9 mmol/L) [22,23] were considered eligible. A CPG ≥11.1 mmol/l (200 mg/dl) indicates diabetic type of glucose tolerance according to the report of the committee on the classification and diagnostic criteria of diabetes mellitus

[24,25]. A CPG is also used as the risk assessment for cardiovascular disease in Japan [26]. Exclusion criteria included diagnosed diabetes, a previous history of diabetes taking anti-diabetic agents, a HbA1c of ≥ 6.5% [27]. Women with a history of gestational diabetes could be enrolled. Physical or medical conditions that do not allow exercise, pregnancy or possible pregnancy, evidence for of type 1 diabetes mellitus, liver cirrhosis or chronic viral hepatitis (type B or type C), and use of a cardiac pacemaker were also included as exclusion criteria. We also excluded those who had already participated in other lifestyle modification programs and those who could not obtain the approval from their doctors.

Enrollment of the study subject

We outsourced some parts of the study works to three existing private companies (Tokio Marine & Nichido Medical Service Co., Ltd., National Education Association, INC. VISIT HEALTH Co., Ltd., and Meiji Yasuda System Technology Co., Ltd., Japan). They were all practicing healthcare services. They participate in this study as a lifestyle support center, which managed the recruitment and enrollment of study subjects and the lifestyle intervention. The lifestyle support center sent a program kit by mail to the eligible subjects in each cluster, inviting them to participate in the study. The kit included an explanation about the study's aims and protocol, a consent form, and a questionnaire regarding lifestyle and health status. Those who consented to participate and completed the questionnaire were enrolled as study participants at the lifestyle support center, after their eligibility was checked based on their self-reported present and past health conditions and, when available, based on information from physicians in the health care divisions.

Characteristics of study subjects

As mentioned above, using a questionnaire, subjects in both the intervention and control arms were asked about their lifestyle (diet, exercise habits, and smoking history) and present and past health conditions. They were categorized into following groups by their BMI, based on the WHO Western Pacific Regional Office (WPRO) criteria; <18.5 as "Underweight", 18.5 to 22.9 as "Normal", 23.0 to 24.9 as "Overweight", 25.0 to 29.9 as "Obese I", and ≥30.0 as "Obese II" [28,29]. To define the Metabolic Syndrome in this study we used the modified criteria of the third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Cholesterol in Adults (NCEP/ATPIII) [30,31]. When three or more of the following components were present in an individual, the individual was judged to have the Metabolic Syndrome: 1) serum triglycerides ≥150 mg/dL [≥ 1.69 mmol/L];, 2) HDL-cholesterol <40 mg/dL [< 1.04 mmol/L] for men and <50 mg/dL [< 1.29 mmol/L] for women, 3) fasting plasma glucose ≥100 mg/dL [≥ 5.6 mmol/L], 4) blood pressure ≥130/85 mmHg, or use of blood pressure lowering agents, and 5) a BMI of ≥25 kg/m^2 [32]. In 2006, when the baseline data were obtained, waist size was not measured in the majority of the health checkup sites. Therefore, BMI was substituted for waist circumference.

Goals for lifestyle changes

The goals for lifestyle change are set for each subject from the following four points; 1) habitual exercise (10,000 steps or more per day or 60 min or more per week of accumulated moderate levels of exercise), 2) achievement and maintenance of an appropriate body weight (a 5% reduction in body weight in subjects with a BMI of ≥25 kg/m^2 or a 3% reduction in subjects with a BMI of 23.0-24.9 kg/m^2), 3) habitual intake of dietary fiber (five or more dishes of vegetables per day or 350 g or more of vegetables per day), and 4) restrictions on alcohol intake (1 "go" (180 ml) or less per day in terms of Japanese sake. 1 "go" of Japanese sake contains 23 g of ethanol [33]).

Lifestyle intervention

After setting goals, the intervention and control arms will receive different treatments. For subjects in the control arm, a weight scale (HBF-354 IT-2; Omron Healthcare Co., Ltd., Japan) and a pedometer (HJ-710 IT; Omron Healthcare Co., Ltd., Japan) with a storage function are provided. They will periodically receive newsletters from the lifestyle support center, which run health-related information and messages to encourage them to undergo a health checkup regularly. These are done to minimize the potential for greater attrition from subjects in the control arm. For the subjects in the intervention arm, in addition to the services provided to the control arm, telephone-delivered lifestyle support will be provided over a one-year period through one of the three lifestyle support centers. In addition to phone calls, written information delivered by mail is also used. Subjects monitor achievement of their own personal action plan. They are encouraged to measure body weight and the number of footsteps every day and send the accumulated data to the lifestyle support center monthly via a transmitter (DC-100; JMS Co., Ltd., Japan). The staff will monitor the achievement of subject's goals regularly and give advice by phone or mail (Figure 1). As mentioned before the intervention is outsourced to private companies. Because the sample size is large, we use three companies. The National Education Association, INC. VISIT HEALTH Co., Ltd., Ltd., Meiji Yasuda System Technology Co., Ltd., and Tokio Marine & Nichido Medical Service Co., Japan will manage 16, 18 and 9 groups, respectively. All study subjects in each group will be

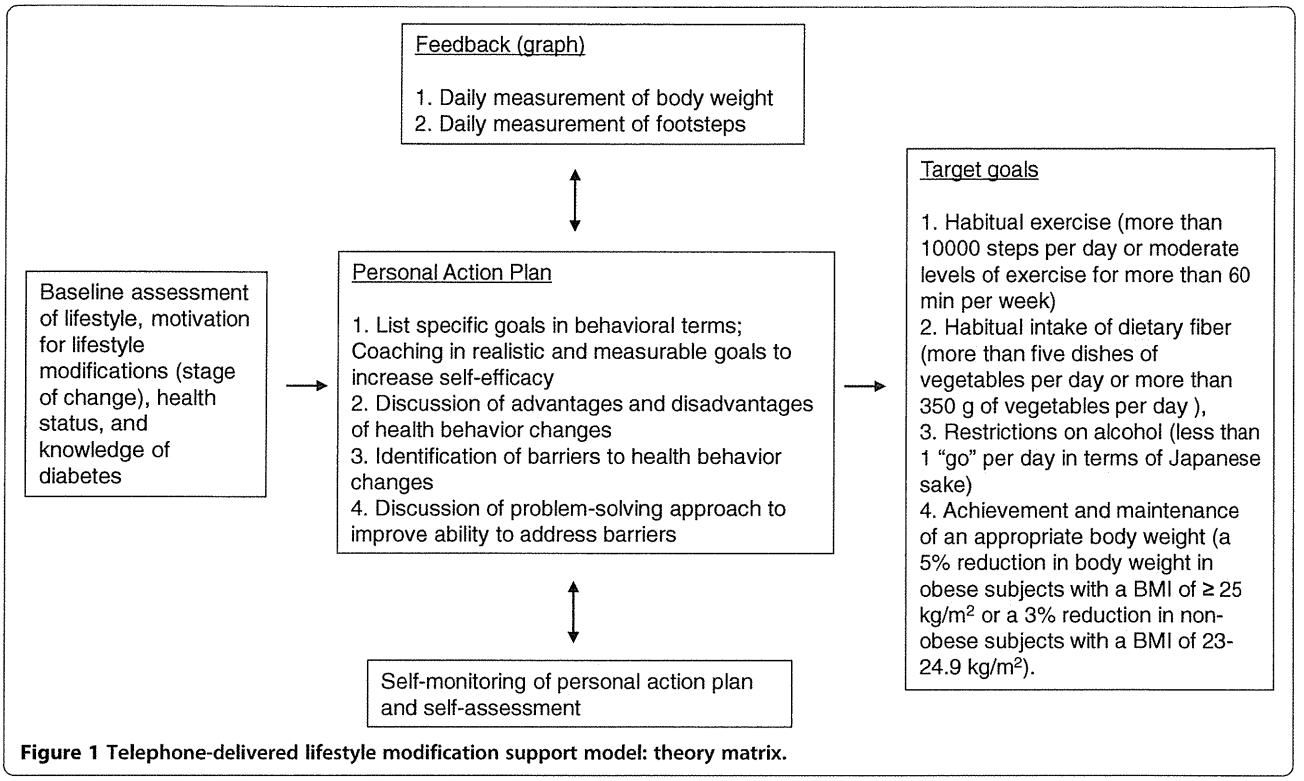

Figure 1 Telephone-delivered lifestyle modification support model: theory matrix.

managed by the same company. We do not standardize the intervention program. Each company uses its own intervention schedule approved by the study group (Table 1). The intervention is standardized within each company. Public health nurses and registered dieticians employed by the lifestyle support centers have college degree and at least 5 years work experience of the intervention. In addition, we will hold educational sessions on diabetes and its prevention for them and training sessions to improve their skills of telephone counseling with motivational interviewing. As shown in the Table 1, there are considerable differences in the quantity of services among the companies. Participants will receive phone calls at least 3 times, and at most 10 times, over one year with the length of each call being between 15-30 minutes.

Follow-up and outcome

Participants will be followed up over a three- year period using data from an annual health checkup and a questionnaire regarding health and lifestyle. The questionnaires are mailed out to the participants from the lifestyle support center with self-addressed envelopes. If a completed questionnaire is not sent back to the lifestyle support center within two weeks, the lifestyle support center will contact the participant first by mail and then by telephone. We made a manual for this process. The primary outcome is the development of diabetes in

Table 1 Schedules of telephone counseling of the three lifestyle support centers

	National Education Association INC. VISIT HEALTH	Meiji Yasuda System Technology	Tokio Marine & Nichido Medical Service
Introduction and welcome call	In Week 1	In Month 2	In Month 3
Support calls	In Months 2, 3, 4, 7, and 10.	In Months 3, 4, 5, 6, 7, 8, 9, 10, and 11.	In Months 7 and 12
Advice sheets by mail	No	Monthly, during Month 2-12	Monthly, during Month 2-12
Feedback by graph (body weight and footsteps)	Monthly	Monthly	Monthly
The number of groups in the control/ intervention arm	8/8	8/7	4/5
The number of subjects in the control	595/722	413/484	328/298

Data are n.

participants whose FPG concentration is 100-125 mg/dL (5.6-6.9 mmol/L) at baseline. The development of diabetes is defined as; #1) a rise in FPG to a level equal to or greater than 126 mg/dL (7.0 mmol/L) as revealed in the follow-up annual health checkup, and #2) a diagnosis of diabetes or use of anti-diabetic drugs as reported in the annual questionnaire with confirmation by referring to medical records. Other outcomes are changes in body weight, BMI, plasma glucose, blood pressure, serum lipids, HbA1c, the percentage of subjects with the Metabolic Syndrome, lifestyle, and the development of cardiovascular diseases.

Dropout and discontinuance

Dropout cases in the present study include; 1) participants who have not undergone an annual health checkup after enrollment, and 2) participants who have lost contact with the study team. Discontinuous cases are defined as; 1) participants who have developed adverse events that make continuance impossible, 2) participants who request to discontinue, 3) participants who are judged inappropriate for continuing the study by the project leader for various reasons.

Data management

Data management is outsourced to CIMIC Co., Ltd., Japan, a contract research organization offering clinical research management services. All data obtained in the study will be stored in de-identified forms in the data management center and used in conformity with the study aims only. The project leader (HK) has overall responsibility for management of the study data.

Blinding

Study participants and the staff members are not blinded to the study arm status. Analysts who perform final data analysis will be blinded.

Sample size

The present study is likely to observe a significantly longer diabetes-free period in the intervention than in the control arm. Thus, the null hypothesis is that the diabetes-free period in the intervention arm is the same as that in the control arm. The sample size(S) needed is calculated using the formula [34,35]; $S = (1 + [\text{cluster size} - 1] \times \text{ICC}) \times N$, where N represents the sample size required when study subjects are randomized individually, and ICC represents an intra-cluster coefficient [36]. Based on the available prospective data from Japanese population the yearly incidence of diabetes among high-risk group varies between 2 and 7% [37,38]. When calculated on the assumption that the annual incidence of diabetes is 4% in the control arm and the intervention reduces the incidence by 50%, N will be 1100 with an alpha of 5% and a power of 90% according to Shoenfeld & Richter [34]. When the ICC and the cluster size (number of individuals in each cluster) are assumed to be 0.02 and 60, S and the number of clusters will be 2398 and 40, respectively. Assuming that the dropout rate is 30%, 3426 subjects are needed. On the assumption that 1) the prevalence of high-risk individuals in each cluster is 10%, and 2) 30% of eligible subjects consent to participate in the study, the total number of health checkup examinees required would be approximately 114,200, and the number of health checkup examinees in each cluster will be approximately 2900. For descriptive analyses of the diabetes-free duration, the Kaplan-Meier method is used.

Statistical analyses

The analyses are done using Statistical Package for Social Science software version 19.0 (SPSS Inc., Chicago, IL, USA) or SAS version 9.3 (SAS Inc., Cary, NC, USA). The analysis will be done on an intention to treat basis. Survival curves for the development of diabetes will be estimated by the Kaplan–Meier method. The log rank test will be also conducted. We will take into account the clustering effect in the main outcome analysis and sub-analysis using the LWA model (Lee, Wei and Amato) [39-41]. Cox regression analysis will be used to calculate the unadjusted and adjusted HRs and 95% CIs for arm and risk factors. In multivariable Cox analysis, all significant variables selected for the univariate analysis will be used with the criterion of $p<0.1$. Student's t-test (or Mann-Whitney U-test according to the frequency distribution of the variable) will be used to compare the means (or the distribution) of the two study arms for continuous variables. Chi-square test or chi-square for trend will be used to compare proportions for categorical variables. We do not adjust for the clustering effect for analysis of the secondary outcome. Those cases with missing data will be simply omitted in the relevant analysis. A p value less than 0.05 is considered significant.

Results

Forty-three groups, formed from 17 health care divisions at companies or communities across the country, were randomly assigned to a control arm (21 groups) or an intervention arm (22 groups) between March 2007 and February 2008. Figure 2 shows the flow of recruitment of study subjects through annual health checkups. Approximately 230, 000 individuals (male 85%) underwent health checkups by those 43 groups in 2006. Among them, 14,473 subjects (7494 in the control and 6979 in the intervention arm) met the inclusion criteria and received an invitation letter to participate in the study. As a result 1643 subjects from the control and 1491 subjects from the intervention arm consented to participate.

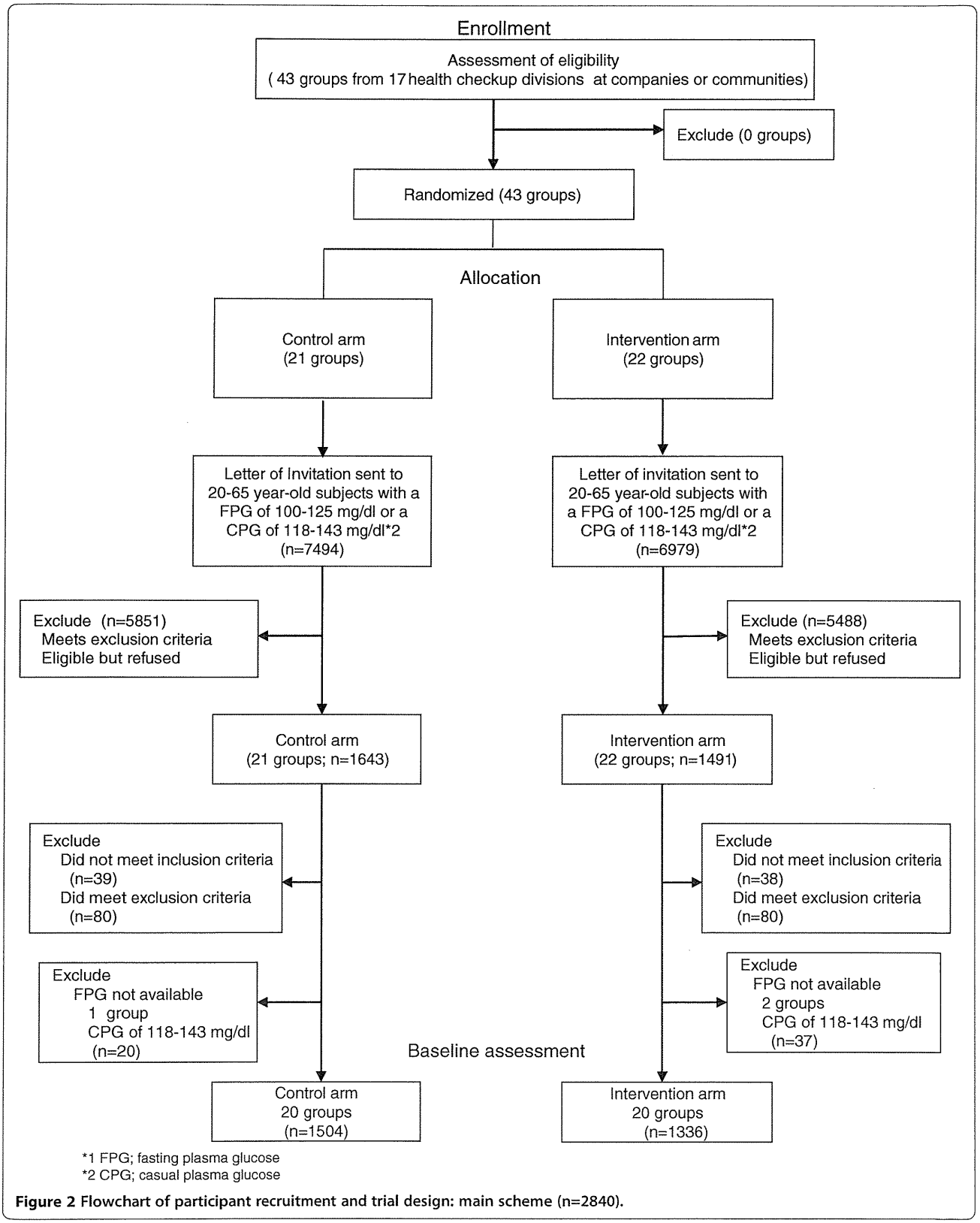

Figure 2 Flowchart of participant recruitment and trial design: main scheme (n=2840).

Finally, 2897 subjects were enrolled, with 1524 in the control and 1336 in the intervention arm. The overall consent rate of the study was approximately 20% with no difference between the study arms. Among the 2897 subjects, 57 subjects (20 in the control and 37 in the intervention arm) were enrolled with CPG of 118-143 mg/dL 6.6-7.9 mmol/L). As shown in Figure 2, those subjects are not included for the main outcome analysis. The remaining 1504 in the control and 1336 in the intervention arm will be followed up for the development of diabetes (primary outcome). The median (interquartile range) of the group size in the control arm before and after screening for eligibility was 301 (200-442) and 61 (35-88), respectively and those in the intervention arm was 313 (158-587) and 60 (41-94), respectively. There is no difference in group size between arms. In one group in the control and two groups in the intervention arm no participants were enrolled with FPG. Those three groups were not included in the calculation of cluster size. The number of company settings, community settings, and mixed settings in the intervention arm were 16, 3, and 1, respectively. The number of company settings, community settings, and mixed settings in the control arm were 15, 3, and 2, respectively. There were no differences between the arms in the characteristic of the participants in terms of age, sex ratio, FPG levels, BMI, and the prevalence of obesity (Table 2). No difference was found in the prevalence of the Metabolic Syndrome, either (Table 3). All follow-up data will be collected by winter 2012.

Discussion

We have launched this J-DOIT1 trial to test whether goal-focused lifestyle support delivered by healthcare providers via the telephone is feasible and effective for preventing or delaying the development of T2DM in high-risk individuals. Statutory health checkup programs, provided annually by public medical care insurance, would offer significant advantages for carrying out this study. Thus, for the recruitment of study subjects and their follow-up, biochemical and anthropometric data are all obtained from health checkup sites.

Cluster randomization

In recently reported lifestyle intervention studies, both individual randomization and cluster randomization [42,43] have been used. The cluster randomization design has the advantages of administrative convenience, ease of obtaining the cooperation of investigators, enhancement of subject compliance, and avoidance of treatment contamination. Since study subjects in this trial are employees of the same workplaces or inhabitants of the same communities, we chose cluster randomization to avoid diluting the effect of the intervention. The contamination could occur with individual randomization e.g. by control subjects receiving part of the intervention in a shared environment. Generally, cluster randomized trials, are susceptible to a range of methodological problems including selection bias [18]. Selection bias can be avoided by recruiting and enrolling the study subjects into the study before the groups are allocated to the study arms [44]. In our study design,

Table 2 Participant characteristics by randomized intervention assignment

Variables	Control arm	Intervention arm
	(n=1504)	(n=1336)
Age, years	49 (44–54)	49 (44 – 55)
Male,%	85.0	83.8
Body mass index, kg/m^2	24.0 (22.3 – 25.8)	24.2 (22.3 – 26.3)
WRPO criteria*		
Underweight (less than 18.5 BMI),%	2.1	1.8
Normal (18.5-22.9 BMI),%	33.2	31.1
Overweight (23.0-24.9 BMI),%	28.4	28.1
Obesity I (25.0-29.9 BMI),%	32.0	33.7
Obesity II (Over 30.0 BMI),%	4.3	5.4
Systolic blood pressure, mmHg	125 (114 – 136)	125 (116 – 135)
Diastolic blood pressure, mmHg	80 (71 – 87)	79 (72 – 87)
Total cholesterol, mmol/l	5.4 (4.9 – 6.0)	5.5 (4.8 – 6.1)
HDL-cholesterol, mmol/l	1.5 (1.3 – 1.8)	1.5 (1.2 – 1.8)
Triglyceride, mmol/l	1.3 (0.9 – 1.9)	1.3 (0.9 – 1.8

Values are median (interquartile range or percentage. * The subjects were categorized into following groups based on the WHO West Pacific Regional Office (WPRO) criteria; less than 18.5 BMI, as "Underweight", 18.5 to 22.9 as "Normal", 23.0 to 24.9 as "Overweight", 25.0 to 29.9 as "Obese I" and over 30.0 BMI as "Obese II".

Table 3 Components of metabolic syndrome by randomized intervention assignment and sex

Variables	Control arm				Intervention arm			
	Men		Women		Men		Women	
	(n=1279		(n=225)		(n=1119)		(n=217)	
1. BMI ≥ 25 kg/m^2	479	37.5%	67	29.8%	456	40.8%	66	30.4%
2. Hypertension	580	45.3%	75	33.3%	514	45.9%	66	30.4%
3. TG ≥ 150 mg/dl	429	33.5%	30	13.3%	360	32.2%	29	13.4%
4. HDL<40 mg/dl in men, <50 mg/dl in women	77	6.0%	27	12.0%	76	6.8%	24	11.1%
5. Hyperglycemia	1279	100.0%	225	100.0%	1119	100.0%	217	100.0%
Risk factors of metabolic syndrome								
1 factor	365	28.5%	99	44.0%	299	26.7%	97	44.7%
2 factors	416	32.5%	71	31.6%	359	32.1%	66	30.4%
≥3 factors	496	38.8%	55	24.4%	459	41.0%	53	24.4%

Data are number or percentage. Five subjects were excluded from the analyses because of missing data except for fasting plasma glucose.

however, the study subjects were recruited after the clusters were randomly allocated to the intervention or control arm. The reason for nor recruiting and enrolling subjects before randomization was that it was not practical due to the nature of the intervention, in which it takes too long to recruit individuals first. The individuals or the recruiters were not blinded to the allocation status. Careful attention should be paid to the likelihood of selection bias in our sample based on the cluster sizes between the two arms and comparison of the participants.

Telephone-delivered interventions

Structured intensive lifestyle modification can prevent T2DM in hospital and clinic settings [45-47], and primary healthcare settings [48]. To target young and middle-aged people, who are busy with work, this study employs a non face-to-face intervention using the telephone. Telephone-delivered intervention has a greater accessibility and potential availability of participants for the interview than face-to-face provided support. They facilitate, in a cost-effective manner [37], repeated contact and support for the participant necessary to promote maintenance of physical activity and diet. Thus telephone counseling would make it possible to deliver lifestyle intervention widely, at a low cost, but in a personalized way. There has been increasing interest in lifestyle support using the telephone [49-53]. However, it is unknown whether telephone-delivered support for lifestyle modification by healthcare providers is a feasible and effective way to prevent or delay the development of T2DM. If it is proved effective, lifestyle coaching by healthcare providers using telephone would be a promising tool for reducing the incidence of diabetes.

Retention

The final sample size (2840 participants) would provide >80% power to detect a 50% reduction in the rate of development of T2DM among participants assigned to the lifestyle intervention with a 5% level of significance (two-sided), after no adjustment for losses in follow-up. The follow-up of participants is scheduled to finish in March 2012. Retention of participating health checkup facilities and subjects are critical for the success of this study. Drop-out rates are generally high in lifestyle programs conducted in primary healthcare clinical settings. To secure enough samples for analysis, participants are encouraged to attend an annual health checkup through a letter from the lifestyle support center. The lifestyle support center gives safety advice to prevent sport injuries which could lead to dropping out of the study.

BMI and the Metabolic Syndrome

We included not only overweight and obese subjects, but also subjects with a BMI of < 23 kg/m^2. Therefore, the BMI ranged widely from <18.5 to >30 in our study subjects with an average value of 24.3. Only 39.0% of men and 30.1% of women had a BMI of ≥25 kg/m^2. Compared with western populations, obesity is less common in our general population [54]. It has also been reported that about 25% of subjects with impaired glucose tolerance have normal or even underweight categories of BMI [36]. It seems that the relationship between BMI and the risk of diabetes is not so straightforward in our population. Thus, we did not set eligibility criteria in terms of BMI. It would be of interest to study the incidence of diabetes and see what strategies are effective to prevent the development of diabetes in those with a lower BMI. In 2008, the concept of the Metabolic Syndrome was introduced in the health checkup program in our country [55]. Mukai et al. suggested that the Metabolic Syndrome significantly increased the risk of incident T2DM, irrespective of the presence or absence of impaired fasting glucose(IFG), and is therefore a valuable tool to identify individuals at high risk of T2DM in the general population in Japan

[56]. In this study, we found 39.8% of men and 24.8% of women have ≥3 risk factors for cardiovascular diseases, suggesting they have the Metabolic Syndrome. The present study would allow us to compare the incidence of T2DM in IFG subjects with or without the Metabolic Syndrome in a subanalysis.

Limitations

This study has several potential limitations. One is that we identified high risk subjects using fasting plasma glucose. We will follow them as to the development of diabetes using fasting plasma glucose determined at annual health checkups and a questionnaire. We do not add any other biochemical examinations such as the oral glucose tolerance test. Therefore, we may miss diabetic subjects having normal fasting but elevated 2 h plasma glucose levels [57-59]. We may also miss subjects with IGT, IFG and IGT, both associated with a substantially increased risk of developing diabetes, are considered to be of a different entity. In the majority of populations thus far studied, IGT is more prevalent than IFG. Thus, we must be careful in interpreting results. It is possible that the efficiency of identifying high-risk subjects will be increased by combining FPG and HbA1c data [60]. This study used results obtained in 2006 annual health checkups as baseline data. At that time, only 58.5% of participating checkup sites included the measurement of HbA1c as a health checkup item. Second, the present study lacks information on the use of drugs, such as fibrate, nicotinic acid, and fish oil, which affect the metabolism of HDL-cholesterol and triglycerides. This may have led us to underestimate the prevalence of the Metabolic Syndrome. Third, participants were predominantly from workplaces. We did not succeed in recruiting more participants from communities. Since men outnumber women in many workplaces in Japan, the study population was predominantly male. This bias may limit the generalizability of our results.

Conclusions

We have launched J-DOIT1, a nation-wide cluster randomized controlled trial to prevent development of T2DM in high-risk individuals using telephone-delivered intervention. Using annual health checkup data, a large cohort has been developed and successfully randomized. This trial is expected to contribute to evidence-based real-world preventive practices.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HK, the project leader, is involved in all aspects of the study. KI, MK, NI, and MN designed the study, and prepared the protocol. NS, KK, YS, KT, and KO were involved in drafting the manuscript. KT, JS, SS and SM participated in statistical analysis. All authors have read and approved the final version of the manuscript.

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RAPID COMMUNICATION

Significant interaction between *RETN* -420 *G/G* genotype and lower BMI on decreased risk of type 2 diabetes mellitus (T2DM) in Japanese – the J-MICC Study

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Abstract. We examined the association of the *RETN* (*resistin*) -420 C>G polymorphism (rs1862513) with risk of diabetes mellitus (DM), considering lifestyle factors, in Japanese. Subjects were participants of J-MICC Study, where 2,651 participants aged 35-69 years provided their blood for genotyping and lifestyle data after informed consent. Odds ratio (OR) of DM for *RETN*-420 *G/G* genotype was estimated using unconditional logistic regression model. Statistically significant interaction on risk of DM was observed between *RETN*-420 *G/G* genotype and BMI<25 (OR for interaction = 0.12; P = 0.046), and when subjects with *RETN*-420 *C/C+C/G* and BMI ≥ 25 (n = 69 for DM and 544 for non-DM) were defined as the reference, the adjusted ORs for subjects with *RETN*-420 *G/G* genotype and BMI≥25 (n = 10 for DM and 111 for non-DM), *RETN*-420 *C/C+C/G* and BMI < 25 (n = 81 for DM and 1,605 for non-DM), and *RETN*-420 *G/G* and BMI < 25 (n = 1 for DM and 230 for non-DM) were demonstrated to be 0.72 (95% confidence interval: 0.36-1.46), 0.40 (0.28-0.56) and 0.03 (0.005-0.25), respectively. The present study revealed the significant interaction of *RETN*-420 *G/G* genotype with lower BMI on the decreased risk of DM, but the direction was opposite to the reported ones in Japanese. We should be careful in interpretation of the present study results because of the limited sample sizes. Further investigation of this association as well as of the actual biological roles of RETN in the genesis of human metabolic disorders including DM will be required.

Key words: Resistin, Single nucleotide polymorphisms, Diabetes mellitus, BMI

WITH THE INCREASING prevalence of sedentary lifestyle in the recent years, lifestyle-related diseases such as coronary heart disease (CHD) or cerebrovascular diseases is placing an increasing burden on populations both in developed and developing countries. Numbers of studies established that disruptions in the controls of blood glucose levels increase the risk of these diseases [1]. Resistin (RETN), a protein hormone produced both by adipocytes and immunocompetent cells including those residing in adipose tissue, has been implicated in the pathogenesis of obesity-mediated insulin resistance and type 2 diabetes mellitus (T2DM) [2]. In contrast, some human studies of late

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years demonstrated that resistin is mainly expressed in monocytes and macrophages, but not in adipocytes in humans [3]. One polymorphism of *RETN* in its 5' flanking region, *RETN* -420 C>G polymorphism (rs1862513) was reportedly associated with the risk of diabetes mellitus (DM) in several ethnicities [4, 5]. One recent study in Japanese demonstrated that the *G/G* genotype of a *RETN* single-nucleotide polymorphism at -420 increases T2DM susceptibility by inducing promoter activity through specific binding of Sp1/3 [6], while another study in Japanese suggested the role of this *RETN* -420C>G polymorphism as a determinant of serum resistin levels as well as a possible risk marker of stroke in patients with T2DM, although the genotype frequencies of *RETN* -420 C>G polymorphism were not significantly different between T2DM patients and controls in this study [7]. The gene-environment interactions between this *RETN* polymorphism and lifestyle factors on the risk of DM is yet to be investigated.

Considering that the DM is a significant contributor towards the increasing burden of chronic diseases as a result of its complications such as CHD or renal failures, finding effective ways to prevent this disease is in pressing need. This study aims to examine the association of the *G/G* genotype of the *RETN* -420 C>G polymorphism with the risk of DM diagnosed by blood glucose levels, considering lifestyle factors to elucidate gene-environment interaction in a Japanese sample using the cross-sectional data of the J-MICC Study.

Subjects and Methods

Study subjects

We leveraged the data repository of Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study), launched in 2005 in 10 areas of Japan, in which about 70,000 voluntarily enrolled participants aged 35-69 years provided their blood and their lifestyle data based on the questionnaire after informed consent [8].

The subjects for this analysis were 4,519 randomly selected J-MICC study participants (about 500 subjects from each of the 10 areas) for whom genotyping of 108 selected polymorphisms were conducted by multiplex polymerase chain reaction-based Invader assay [9]. From these 4,519 subjects, 1,089 were excluded due to fasting blood sugar (FBS) data (1,084 were from the 2 areas without FBS measurement, 5 were from the others), 1 excluded due to genotyping error, 727 excluded due to taking meals within 3 hours before the blood drawings, and 50 excluded due to the implausible values of estimated energy intake (< 1,000 kcal/day or > 4,000 kcal/day) and 1 excluded due to missing information on smoking status, leaving 2,651 subjects eligible for the analyses. Genotype call rate was 99.97% for those with FBS data (n = 3,430). Missing data were considered independent of the exposure-outcome (E-O) relationship. Intakes of energy, carbohydrate and fat were estimated using a food-frequency questionnaire [10, 11], and the proportions of energy from carbohydrate and fat were calculated as energy from each nutrient divided by total energy intake (shown in percentage: % energy). Smoking status, alcohol consumption and BMI were assessed based on the questionnaire. Informed consent was obtained from all the subjects and the study protocol was approved by the Ethics Committees of Nagoya University School of Medicine and the other participating institutions.

Samples and diagnostic criteria

The FBS levels were measured routinely for health check-ups or for research in participating institutions. The diagnostic criterion for DM was FBS $\geq$ 126 mg/dL and/or taking DM medications.

Model building

We selected the logistic regression model in the present study because its parameter estimates have a convenient interpretation in standard study designs. We adopted the strategy of backwards elimination to pair-down model with the goal of getting the most unbiased estimate of the relationship between *RETN* -420 *G/G* genotype and risk of DM using the most parsimonious model. First of all, we extracted all possible variables for consideration using the directed acyclic graph (DAG) [12]. Age, gender, smoking status, alcohol consumption, total energy intake, carbohydrate intake, fat intake and BMI were extracted based on the DAG. We next evaluated the interaction term for effect measure modification by using the Breslow-Day test of homogeneity with $\alpha = 0.05$. Only BMI fulfilled this criterion of all the covariates. Then all the covariates as potential confounders were evaluated. For each covariate, the results of likelihood ratio test (LRT) for each covariate are considered to extract the covariates that can contribute significantly to the improvement of predictability of E-O relationship. The criteria for the signifi-

cance of LRT is $\alpha = 0.10$. As a result of this, age (as continuous variable), BMI (binary variable: BMI < 25 or BMI ≥ 25) and smoking status (recorded as the indicator variables: never smokers, current smokers & former smokers) remained in the final model. All the calculations were done using the STATA version 10 (Stata Corp, College Station, TX).

Results

The characteristics of the study subjects are described in Table 1. The genotype frequency for *RETN* -420 C>G polymorphism were 42.6% (1,062/2,490) for *C/C*, 43.7% (1,087/2,490) for *C/G* and 13.7% (341/2,490) for *G/G* among those without DM, while 47.2% (76/161), 46.0% (74/161) and 6.8% (11/161), respectively, among those with DM. The genotype frequencies among the non-DM subjects were significantly different from the Hardy-Weinberg's equilibrium ($\chi^2 = 5.502$, $P = 0.019$); the expected genotype frequency was 42.7% for *C/C*, 45.8% for *C/G*, and 13.7% for *G/G*. Odds ratio (OR) of DM for *RETN*-420 *G/G* genotype was estimated using crude and stratified tabular analysis, and unconditional logistic regression adjusting for age, smoking, and BMI. The crude OR was 0.46 (95% confidence interval [CI]: 0.25-0.86). In stratified analysis with the final model by BMI, the adjusted OR (aOR) was 0.08 (0.01-0.61) among BMI<25 and 0.72 (0.36-1.45) among BMI≥25. Statistically significant interaction on risk of DM was observed between *RETN*-420 *G/G* genotype and BMI < 25 (OR for interaction = 0.12; $P = 0.046$), and when subjects with *RETN*-420 *C/C+C/G* and BMI≥25 (n = 69 for DM and 544 for non-DM) were defined as the reference, the ORs for subjects with *RETN*-420 *G/G* genotype and BMI≥25 (n = 10 for DM and 111 for non-DM), *RETN*-420 *C/C+C/G* and BMI<25 (n = 81 for DM and 1,605 for non-DM), and *RETN*-420 *G/G* and BMI<25 (n = 1 for DM and 230 for non-DM) were demonstrated to be 0.72 (95% CI: 0.36-1.46), 0.40 (0.28-0.56) and 0.03 (0.005-0.25), respectively (Table 2). We also conducted the stratified analysis of the effect of *RETN* -420 C>G polymorphism on DM risk by gender, which revealed the aOR of 0.23 (95% CI: 0.08-0.63) in men and 0.84 (0.37-1.92) in women, the interaction of which didn't reach the statistical significance (likelihood-ratio test: $P = 0.13$).

Discussion

The present study revealed the significant interaction of *RETN*-420 *G/G* genotype with lower BMI on the decreased risk of DM, where the direction of the association was opposite to the reported ones in Japanese. Although *RETN* is known to be a protein hormone produced by adipocytes and has been implicated in the pathogenesis of obesity-mediated insulin resistance and T2DM [2], the actual biological roles of RETN in the genesis of DM and insulin resistance in humans remain still controversial. Some recent studies demonstrated that circulating RETN levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or administration of leptin [13], whose main biological role is an indicator of energy balance as well as a neuromodulator that inhibits appetite by counteracting the effect of neuropeptide Y. The receptor of RETN is yet unknown. The insulin-responsive tissues affected are reported to be highly dependent on the model being studied; the human and mouse promoter sequences are demonstrated to be quite divergent, which makes it no wonder that the adipose-specific gene expression of rodent is not recapitulated in humans [14]. In addition, one research from U.S. demonstrated that serum RETN level is not a significant predictor of insulin resistance in humans [15], and recent meta-analysis did not observe any association between the polymorphism of *RETN* -420 C>G and the risk of type 2 DM [16]. Some of the recent studies also suggest the possibility of racial/ethnic differences in the effect of *RETN* -420 C>G on the risk of type 2 DM in humans, where the association between this *RETN* -420 C>G polymorphism and plasma resistin was found in Japanese, but not in Caucasians [17, 18]. To date, interaction between the *FTO* rs9939609 polymorphism and BMI on metabolic syndrome risk was reported [19], and our finding points towards the novel evidence that variation in the *RETN* gene may also interact with BMI to decrease the risk of metabolic disorder. Recent research also clarified the roles of inflammatory pathways in the induction of RETN in causing insulin resistance in mice [20]. The analysis of the interaction between *RETN* and inflammatory pathways in the causation of T2DM in the actual human epidemiological data might also help provide more detailed insights into the personalized prevention of T2DM in the near future. According to the literature, *RETN* -420*G* allele was shown to have stronger

Table 1 Characteristics of the study subjects and the stratified analyses of the odds ratio of T2DM for *RETN* -420 *G/G vs. C/C+C/G*

Covariates	Non-DM	DM	Total	OR*	95% CI*	Test of Homogeneity (Breslow-Day)	
	N (%)	N (%)	N (%)			χ^2 (df)	*P*-value
RETN -420 C>G genotype							
C/C	1,062 (42.6)	76 (47.2)	1,138 (42.9)				
C/G	1,087 (43.7)	74 (46.0)	1,161 (43.8)				
G/G	341 (13.7)	11 (6.8)	352 (13.3)				
G/G vs. C/C+C/G	- -	- -	- -	0.46	0.25-0.86	-	-
Age							
35-39	121 (4.9)	1 (0.6)	122 (4.6)	0	-		
40-49	511 (20.5)	19 (11.8)	530 (20.0)	0.38	0.01-2.50		
50-59	885 (35.5)	55 (34.2)	940 (35.5)	0.55	0.17-1.40		
60-69	973 (39.1)	86 (53.4)	1,059 (39.9)	0.42	0.13-1.06	0.26 (3)	0.968
Gender							
Male	1,249 (50.2)	107 (66.5)	1,356 (51.2)	0.26	0.07-0.71		
Female	1,241 (49.8)	54 (33.5)	1,295 (48.8)	0.87	0.33-1.98	3.44 (1)	0.064
Smoking							
Never	429 (17.2)	27 (16.8)	456 (17.2)	0.33	0.01-2.10		
Former	567 (22.8)	60 (37.3)	627 (23.7)	0.31	0.06-0.98		
Current	1,494 (60.0)	74 (46.0)	1,568 (59.1)	0.63	0.24-1.39	1.15 (2)	0.563
Alcohol intake							
Non-drinker	1,083 (43.5)	65 (40.4)	1,148 (43.3)	0.72	0.27-1.63		
Drinker (current)							
<23g/day	1,110 (44.6)	64 (39.8)	1,174 (44.3)	0.21	0.02-0.82		
≥23g/day	297 (11.9)	32 (19.9)	329 (12.4)	0.43	0.05-1.81	2.35 (2)	0.309
Energy intake							
<30kcal/kg/day	919 (36.9)	69 (42.9)	988 (37.3)	0.77	0.29-1.73		
≥30kcal/kg/day	1,571 (63.1)	92 (57.1)	1,663 (62.7)	0.27	0.07-0.74	2.52 (1)	0.112
Carbohydrate intake							
<60% energy	1,756 (70.5)	111 (68.9)	1,867 (70.4)	0.47	0.20-0.99		
≥60% energy	734 (29.5)	50 (31.1)	784 (29.6)	0.43	0.08-1.39	0.02 (1)	0.902
Fat intake							
<15% energy	225 (9.0)	18 (11.2)	243 (9.2)	0	0-1.83		
15-20% energy	641 (25.7)	53 (32.9)	694 (26.2)	0.28	0.03-1.08		
20-25% energy	797 (32.0)	50 (31.1)	847 (32.0)	0.69	0.21-1.78		
25-30% energy	531 (21.3)	28 (17.4)	559 (21.1)	0.69	0.13-2.33		
≥30% energy	296 (11.9)	12 (7.5)	308 (11.6)	0.48	0.01-3.47	2.54 (4)	0.638
BMI							
<25	1,835 (73.7)	82 (50.9)	1,917 (72.3)	0.09	0.002-0.50		
≥25	655 (26.3)	79 (49.1)	734 (27.7)	0.71	0.32-1.44	4.81 (1)	0.028

DM, diabetes mellitus; OR, odds ratio; 95%CI, 95% confidence interval.
*OR and 95% CI of T2DM for *RETN* -420 *G/G* stratified by each covariate, unadjusted for any other covariates.