while cardiac work falls dramatically, and is increasingly being perceived as a potential key lesion in the failing heart.

On the other hand, there is the possibility that UA itself may induce LVH. Previous reports have shown that UA impairs NO generation and induces endothelial dysfunction and smooth muscle cell proliferation[9] Moreover, UA is able to induce inflammatory mediators, such as tumor necrosis factor, in vitro and potentially stimulates mitogen-activated protein kinases, which are known to induce cardiac hypertrophy![10] Indeed, accumulating data support the idea that UA possesses specific toxic or other properties that could contribute to cardiac hypertrophy and heart failure pathophysiology. These findings reveal that UA may be the cause of cardiac hypertrophy in part, attributable to an increase in its serum level, via stimulation of endothelial dysfunction, smooth muscle cell proliferation, and inflammation.

So far there is strong evidence that increased UA is associated with atherosclerosis and an increased risk of cardiovascular events. The findings of Mitsuhashi et al. also suggest that the serum level of UA affects cardiac hypertrophy in men! However, whether UA per se is a cause of cardiovascular disease, especially cardiac hypertrophy, remains to be settled. Prospective randomized studies targeting UA reduction are necessary to finish this discussion.

This finding by Mitsuhashi et al[1] is not only potentially of value in preventing cardiac hypertrophy but also raises interesting questions regarding the pathophysiological action of UA on the cardiovascular system.

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Original Article

A Promoter Polymorphism of Lamin A/C Gene is an Independent Genetic Predisposition to Arterial Stiffness in a Japanese General Population (The Tanno and Sobetsu Study)

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Aim: We examined the hypothesis that there is a positive, independent association between polymorphisms of lamin A/C gene (*LMNA*) and arterial stiffness in Japanese.
Methods: The subjects were 261 men (mean age, 64.4±0.7 years) selected from inhabitants of the towns of Tanno and Sobetsu in a rural area of Japan who underwent medical check-ups. We conducted clinical examinations, including measurement of bilateral brachial-ankle pulse wave velocity (baPWV) as a marker of arterial stiffness, and genetic analysis. Subjects with atrial fibrillation, subjects with ankle-brachial index <0.9, and subjects taking any medication were excluded. We selected two single nucleotide polymorphisms (SNPs) as markers of *LMNA*, 1908C/T in exon 10 and -1030C/T in the promoter region, which we have recently identified. All genotypes were clearly determined by the TaqMan PCR method.
Results: Genotype frequencies of the two polymorphisms satisfied the Hardy-Weinberg equilibrium. The baPWV of -1030C/T polymorphism was significantly greater in subjects with CC genotype than in subjects with CT+TT genotype (1,652±22.1 cm/s vs. 1,552±43.0 cm/s, $p=0.039$); however, no significant difference was found for 1908C/T polymorphism. The baPWV was found to be significantly associated with age, body height, systolic blood pressure, and smoking habit; therefore, we next performed multiple regression analysis including these parameters, and found an independent, significant association between baPWV and -1030C/T polymorphism.
Conclusion: Promoter -1030C/T polymorphism of *LMNA* is a possible genetic predisposition to arterial stiffness in the Japanese population.

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Key words; Arterial stiffness, Nuclear lamina, Genetics, Single nucleotide polymorphism (SNP)

Introduction

Hutchinson-Gilford progeria syndrome (HGPS; Online Mendelian Inheritance in Man #176670) is a rare sporadic disorder with premature aging, and patients with this syndrome are likely to have coronary artery disease, stroke, or other cardiovascular diseases[1-3]. HGPS induces severe systemic arterial stiffness, which leads to fatal myocardial infarction or stroke before an average age of 13 years. Approximately 80% of HGPS cases are caused by a single base change of C to T in position 1824 on exon 11 of a gene encoding nuclear lamins A and C[4,5]. Lamins are structural protein components of nuclear lamina, a protein network underlying the inner nuclear membrane that determines nuclear shape and size, and

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constitute a class of intermediate filaments. The gene encoding lamins A and C is named lamin A/C gene (*LMNA*; Gene ID 4000), and it spans approximately 24 kb and contains 12 exons on chromosome 1q21. Alternative splicing within exon 10 of *LMNA* gives rise to 2 different mRNAs that code for prelamin A and lamin C.

HGPS is associated with premature arterial stiffness, and it is therefore thought that *LMNA* is involved in the pathophysiology and genesis of arterial stiffness that occurs concurrently with the accelerated aging process. It has been reported that *LMNA* has several single nucleotide polymorphisms (SNPs). The 1908C/T polymorphism (rs #4641), one of the SNPs on *LMNA*, in exon 10 is associated with a risk for developing metabolic traits, including insulin resistance. A positive association between 1908C/T polymorphism and metabolic abnormalities has been reported in Inuit[6], Japanese[7], Pima Indians and Armish[8].

However, there is no data on the association between *LMNA* and arterial stiffness in a general population. Arterial stiffness is mainly determined by measuring pulse wave velocity (PWV)[9]. PWV reflects systemic arteriosclerosis as well as relating to cardiovascular risk factors[10] and ischemic heart disease in type 2 diabetes mellitus[11]. PWV is also a predictor of cardiovascular mortality in patients with end-stage renal disease[12] or hypertension[13] and in elderly individuals[14], independently of age, blood pressure, and cardiac mass.

The purpose of this study was to examine the relationship between polymorphisms of *LMNA* and arterial stiffness in a cross-sectional epidemiological study of a Japanese general population, the Tanno and Sobetsu study.

Materials and Methods

We recruited 586 male inhabitants of Tanno Town and Sobetsu Town who had undergone medical check-ups in 2003. Tanno and Sobetsu are located in Hokkaido, the northernmost island of Japan. The Tanno and Sobetsu study was started in 1977 with a population-based prospective cohort design. Detailed epidemiological findings have already been reported[15-18].

The subjects completed a standard questionnaire regarding their medical history and their smoking and drinking habits. We measured anthropometric parameters, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, plasma glucose, immunoreactive insulin (IRI), highly sensitive C-reactive protein (hs-CRP), and adiponectin in all subjects. Brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI) were measured using Form® PWV/ABI (Omron Colin Co., Ltd., Tokyo, Japan) and the average of right baPWV and left baPWV was adopted[19]. Insulin sensitivity was determined by homeostasis model assessment of the insulin resistance (HOMA-IR) index, which was calculated as plasma glucose (mg/dL) × immunoreactive insulin (μU/L)/405. Blood samples were collected in the early morning after fasting for 8–11 hours. Blood pressure was measured twice after 5 minutes of rest, with the subjects seated.

Exclusion criteria were atrial fibrillation, suspected arteriosclerosis obliterans (ASO) defined as ABI on any side lower than 0.9, and taking any medication, in order to rule out drug effects. After excluding 219 of the 586 male subjects according to the above criteria, we conducted genetic analysis. Finally, 261 male subjects were successfully genotyped. All subjects gave written informed consent to participate in the genetic analysis and in all other procedures associated with the study. The Institutional Review Board (IRB) of Osaka University and the IRB of Sapporo Medical University both approved the study protocol.

Genomic DNA was extracted from 200 μL buffy coat using a QIAamp DNA Blood Kit (QIAGEN K. K., Tokyo, Japan). C-to-T transversion at nucleotide position 1908 in exon 10 of the lamin A/C gene (*LMNA* 1908C/T; rs #4641) and C-to-T transversion at nucleotide position -1030 in the promoter region of *LMNA* (*LMNA* -1030C/T; no rs#) were determined by the TaqMan-polymerase chain reaction (PCR) method. The *LMNA* 1908C/T polymorphism was detected using the following primers and probes: forward, 5'-CGA GGA TGA GGA TGG AGA TGA C-3'; reverse, 5'-CCT CAG CGG CGG CTA C-3'; cytosine base (C)-specific probe, 5'-VIC-CAC TCA CGT GGT GGT G-MGB-3'; and thymine base (T)-specific probe, 5'-FAM-CAC TCA CAT GGT GGT G-MGB-3'. The *LMNA* -1030C/T polymorphism was detected using the following primers and probes: forward, 5'-CCA CTA CCT TCT TTC TGG CTG AA-3'; reverse, 5'-ACT AGG TCC CAG ATT TCT GTG GTT-3'; cytosine base (C)-specific probe, 5'-VIC-CAG CCA ATG TTG GGT C-MGB-3'; and thymine base (T)-specific probe, 5'-FAM-ACA GCC AAT ATT GGG TC-MGB-3'. PCR was carried out using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: initial denaturation at 95℃ for 10 min, followed by 40 cycles of 92℃ for 15 sec and 60℃ for 60 sec. The fluorescence level of PCR

Table 1. Baseline characteristics of study subjects (n=261)

	Male (n=261)
Age (years)	64.6 ± 0.7
BMI (kg/m²)	23.5 ± 0.2
SBP (mmHg)	133 ± 1.3
DBP (mmHg)	75 ± 0.7
Total cholesterol (mg/dL)	193 ± 2.0
Triglyceride (mg/dL)	112 ± 4.6
HDL cholesterol (mg/dL)	52 ± 0.8
Current smoker (%)	33.0
HOMA-IR	1.0 ± 0.07
hsCRP (mg/dL)	0.107 ± 0.008
Adiponectin (ng/mL)	6.1 ± 0.2
baPWV (cm/s)	1,631 ± 19.7

Values are expressed as the mean ± SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

Table 2. Comparison of parameters between CC genotype and CT + TT genotype of 1908C/T polymorphism

	CC (n=157)	CT + TT (n=104)	p
Age (years)	65.3 ± 0.9	63.5 ± 1.2	0.24
BMI (kg/m²)	23.5 ± 0.3	23.7 ± 0.3	0.58
SBP (mmHg)	133 ± 1.7	136 ± 2.1	0.30
DBP (mmHg)	75 ± 0.9	77 ± 1.1	0.07
Total cholesterol (mg/dL)	194 ± 2.7	190 ± 3.4	0.09
Triglyceride (mg/dL)	112 ± 5.1	113 ± 10.0	0.91
HDL cholesterol (mg/dL)	52 ± 0.9	52 ± 1.7	0.96
Current smoker (%)	32.4	5.2	0.75
HOMA-IR	1.1 ± 0.1	1.0 ± 0.2	0.56
hsCRP (mg/dL)	0.11 ± 0.008	0.082 ± 0.02	0.10
Adiponectin (ng/mL)	6.1 ± 0.2	6.0 ± 0.4	0.80

Values are expressed as the mean ± SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

products measured using an ABI PRISM 7900HT Sequence Detector (Applied Biosystems) differentiated the three genotypes of these two polymorphisms.

Associations between the polymorphisms and clinical variables were analyzed using one-way analysis of variance (ANOVA). Differences in genotype or allele distribution were examined by χ^2 analysis. Multiple regression analysis was used to assess the contribution of confounding factors. All numerical values are expressed as the means ± SEM. Significance was defined as $p<0.05$. All statistical analyses were conducted using JMP software version 5.1.2J for Windows (SAS Institute Inc., Cary, NC, USA).

Results

The 261 male subjects had a mean age of 64.6 ± 0.7 years, mean body mass index (BMI) of 23.5 ± 0.2 kg/m², and mean brachial-ankle pulse wave velocity (baPWV) of 1,631 ± 19.7 cm/sec. **Table 1** shows the baseline characteristics of all study subjects. The genotype frequencies of the two polymorphisms of *LMNA* examined did not significantly differ from the values predicted by the Hardy-Weinberg equilibrium. The frequencies of CC, CT and TT genotypes of exon 10 1908C/T polymorphism were 60%, 32% and 8%, respectively, and the frequencies of CC, CT and TT genotypes of promoter -1030C/T polymorphism were 79%, 16% and 5%, respectively. Since the number of subjects with TT genotype of these two polymorphisms was small, we adopted a recessive model of the

Table 3. Comparison of parameters between CC genotype and CT + TT genotype of -1030C/T polymorphism

	CC (n=207)	CT + TT (n=54)	p
Age (years)	64.8 ± 0.8	63.9 ± 1.5	0.61
BMI (kg/m²)	23.6 ± 0.2	23.2 ± 0.4	0.37
SBP (mmHg)	134 ± 1.4	131 ± 2.8	0.27
DBP (mmHg)	76 ± 0.8	74 ± 1.6	0.24
Total cholesterol (mg/dL)	191 ± 2.2	199 ± 4.4	0.38
Triglyceride (mg/dL)	112 ± 5.9	106 ± 7.3	0.56
HDL cholesterol (mg/dL)	53 ± 1.0	52 ± 1.3	0.88
Current smoker (%)	35.4	28.1	0.26
HOMA-IR	1.1 ± 0.1	1.0 ± 0.1	0.27
hsCRP (mg/dL)	0.11 ± 0.01	0.10 ± 0.01	0.43
Adiponectin (ng/mL)	6.0 ± 0.3	6.2 ± 0.3	0.52

Values are expressed as the mean ± SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

C allele (CC vs. CT + TT) for the two polymorphisms. **Tables 2** and **3** show the clinical parameters of each genotype of the 1908C/T polymorphism and -1030C/T polymorphism of *LMNA*, respectively. Despite previous findings[6-8], there was no significant relationship between the T allele of 1908C/T polymorphism and metabolic traits in our study cohort. BaPWV of subjects with CC genotype of -1030C/T polymorphism was significantly greater than that of

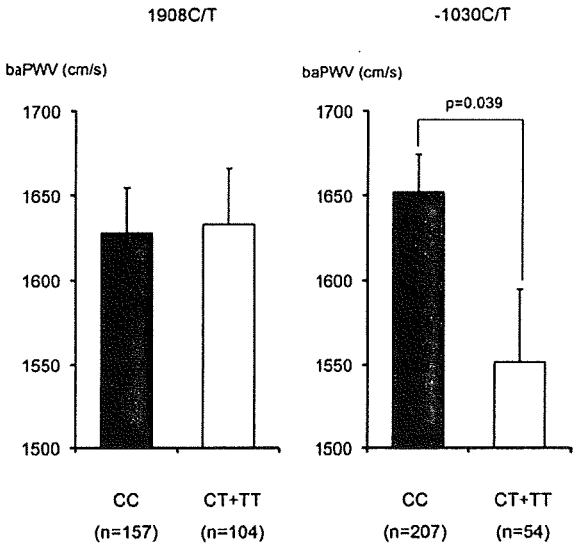

Fig. 1. Comparison of brachial-ankle pulse wave velocity (baPWV) according to genotypes of *LMNA* 1908C/T and -1030C/T polymorphisms.

subjects with CT+TT genotype of -1030C/T polymorphism (1,652±22.1 cm/s vs. 1,552±43.0 cm/s, $p=0.039$), while there was no significant difference in the genotype of 1908C/T polymorphism (**Fig. 1**). BaPWV showed significant positive correlations with age, systolic blood pressure and smoking habit and a significant negative correlation with body height. We therefore selected these factors as covariates of multiple regression analysis for baPWV and *LMNA* -1030C/T polymorphism (**Table 4**). There was an independent relationship between the genotype of -1030C/T polymorphism and baPWV after adjusting covariates. Mean baPWVs of -1030/CC and -1030/CT +TT genotypes were 1,650±17.1 cm/s and 1,571± 32.0 cm/s, respectively, after adjusting covariates.

Since there was a strong linear correlation between age and baPWV, we analyzed the genotypic difference of -1030C/T in this correlation. The gradient of the regression line of subjects with CC genotype was significantly high compared to that of subjects with CT+TT genotype (data not shown), indicating that subjects with CC genotype of -1030C/T polymorphism might be susceptible to the progression of arterial stiffness by aging.

Discussion

We examined the hypothesis that there is a positive, independent association between polymorphisms

Table 4. Multiple regression analysis for brachial-ankle pulse wave velocity (baPWV)

Term	Estimate	SE	t	p
Age	12.5	1.58	7.9	<0.0001
Body height	0.684	2.55	0.3	0.79
Systolic blood pressure	7.69	1.15	6.7	<0.0001
Smoking habit	−6.34	15.9	−0.40	0.69
-1030C/T CC vs. CT+TT	−39.4	17.8	−2.2	0.028

$R^2=0.48$ ($n=261$)

of *LMNA* and arterial stiffness in a cross-sectional study of a Japanese population. Genotype frequencies of the two polymorphisms satisfied the Hardy-Weinberg equilibrium. The mean baPWV of -1030C/T polymorphism was significantly greater in subjects with CC genotype than in subjects with CT+TT genotype; however, no significant difference was found for 1908C/T polymorphism. On the other hand, age, body height, systolic blood pressure, and smoking habit were significantly associated with baPWV. Multiple regression analysis including covariates revealed that subjects with the -1030T allele had a significantly lower level of baPWV.

The 1908C/T polymorphism of *LMNA* has been reported to be related to metabolic abnormalities or insulin resistance in Japanese[7] and Armish[8], but a relationship between this polymorphism and arterial stiffness was not found in the present study. In addition, no relationship was found between 1908C/T polymorphism and metabolic traits in our population. This may due to the characteristics of our subjects. Subjects taking any medication were excluded from this study, and the number of subjects with type 2 diabetes mellitus or dyslipidemia was therefore small; however, the promoter -1030C/T polymorphism of *LMNA*, which we have recently identified by direct sequencing in the subjects, was independently associated with baPWV as a marker of arterial stiffness. To our knowledge, this is the first report of a relationship between *LMNA* polymorphism and arterial stiffness.

In addition, we investigated the relation between the prevalence of cardiovascular diseases and SNPs in *LMNA* using a cross-sectional method; however, there was no significant relation between cardiovascular diseases and SNPs. Because the subjects of our study were relatively healthy and had a low prevalence of cardiovascular diseases, our investigation might lack statistical power. A prospective study to elucidate this relation is now ongoing. Because of the shortness of the follow-up period, we do not have valuable results

at present, but we will report the obtained results in the future.

Mutations in *LMNA* have been discovered in a staggering variety of inherited diseases called "laminopathies[20]". To date, several laminopathies are more familiar than Hutchinson-Gilford progeria syndrome (HGPS), such as Dunnigan-type familial partial lipodystrophy (FPLD), Emery-Dreifuss muscular dystrophy, Charcot-Marie-Tooth disease, limb-girdle muscular dystrophy, mandibuloacral dysplasia, dilated cardiomyopathy with conduction abnormality and early onset of atrial fibrillation. Laminopathies are caused by a mutation of *LMNA*, and are likely to include cardiovascular diseases[21, 22]. The precise mechanisms of this relationship are unknown, but it is speculated that *LMNA* regulates metabolic traits as well as arterial stiffness and thus results in the aging process. HGPS is a laminopathy that is mainly caused by C-to-T mutation in position 1824 of *LMNA* and results in systemic arteriosclerosis. Recent studies have shown that this mutation causes nuclear blebbing induced by anchoring the mutant lamin A (called "progerin", which lacks 50 amino acids near the carboxy terminus) to the inner nuclear membrane, resulting in dysregulated gene transcription, heterochromatin disorganization[23, 24], and increased vulnerability of the nuclear membrane. Numerous abnormalities present in HGPS are common phenomena that occur in cells not only of HGPS patients but also aged individuals in the general population, such as nuclear blebbing, epigenetic changes and increased levels of DNA damage[25]; therefore, *LMNA* seems to be one of the key genes regulating aging as well as arterial stiffness.

In order to elucidate the function of -1030C/T promoter polymorphism of *LMNA*, we searched for transcription factors likely to bind around -1030C/T, using online databases of TRANSFAC, JASPAR, IMD, and CBIL/GibbsMat (http://www.cbil.upenn.edu/cgi-bin/tess/tess/). Motif analysis revealed one transcription factor, CREB-binding protein/CCAAT recognition factor (CBP/CRF), which has homology with the sequence including the -1030T allele. This may result in a difference in *LMNA* expression. Subjects with the -1030T allele are likely to have a strong expression of lamin A and C matrix and might have a stable nuclear membrane against environmental insult, represented by reactive oxygen species.

Our study has several limitations. First, our study was conducted using a small number of subjects in a Japanese population. Second, this study was is designed as a cross-sectional method. Although arteriosclerosis occurs mostly in aged individuals, the genotype-phenotype relationship showd also be analyzed in a time-considered, longitudinal study. Third, the mechanisms by which transcriptional activities of *LMNA* are regulated by promoter -1030C/T polymorphism are unclear. Further study is required to clarify the function of promoter -1030C/T polymorphism.

In conclusion, promoter -1030C/T polymorphism of *LMNA* might be associated with arterial stiffness in Japanese independent of metabolic traits. The mechanism of the influence of *LMNA* on arterial stiffness may reveal part of the mechanism of a common condition susceptible to arteriosclerosis, aging.

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Original Article

Increased Serum Apolipoprotein B48 Concentration in Patients with Metabolic Syndrome

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Aim: Postprandial hyperlipidemia is characterized by an increase of chylomicron remnants (CM-R), and is a risk factor for atherosclerosis. Apolipoprotein (apo) B48 exists exclusively in chylomicroms and CM-R, and fasting plasma levels of apo B48 may reflect high postprandial levels of chylomicrons and/or CM-R. We hypothesized that fasting apo B48 levels may be increased in metabolic syndrome.
Methods: We investigated 1,349 inhabitants (528 men and 821 women aged 62.4 ± 12.8 y; mean ± S.D.) of two towns in rural Hokkaido, who underwent health checks in 2005.
Results: The fasting apo B48 level was significantly higher in males than females (geometric mean 1.92; 95% CI 1.80–2.04 μg/mL, vs. 1.69; 95% CI 1.61–1.76 μg/mL; $p < 0.001$). Ln (apo B48) showed a significant positive correlation with total cholesterol and ln (triglycerides), and a negative correlation with HDL-cholesterol. The correlation between ln (apo B48) and ln (triglycerides) was strong. Apo B48 was significantly higher in men and women with than without metabolic syndrome. Regression analysis revealed that ln (apo B48) was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and ln (triglyceride).
Conclusion: Fasting apo B48 levels are raised in individuals with metabolic syndrome.

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Key words; Chylomicrons, Hypertriglyceridemia, Apolipoprotein B48, Metabolic syndrome

Introduction

Postprandial hyperlipidemia, which is characterized by increased levels of chylomicron remnants (CM-R), is considered to be a risk factor for atherosclerosis[1, 2]. Chylomicrons are assembled in the small intestine and undergo lipolysis by lipoprotein lipase in the plasma to generate CM-R. Because CM-R are then rapidly taken up by the liver, it has been assumed that fasting plasma levels of these particles are very low[3].

Postprandial hyperlipidemia is related to metabolic syndrome[4-6]. This syndrome is characterized by insulin resistance, hypertension, dyslipidemia, and hyperglycemia, and is an important risk factor for atherosclerosis. In metabolic syndrome, dyslipidemia is characterized by hypertriglyceridemia and a low high density lipoprotein (HDL)-cholesterol level. Recent studies have shown that postprandial hyperlipidemia is a major cause of hypertriglyceridemia associated with metabolic syndrome[6, 7].

Though CM-R are thought to play an important role in dyslipidemia associated with metabolic syndrome, CM-R concentrations in patients with this syndrome have not yet been reported. Apo B48 exists exclusively in chylomicroms and CM-R. Several methods of measuring the apo B48 concentration in plasma or in triglyceride-rich lipoproteins have been reported[8-15]. These methods include sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[8-10], SDS-PAGE coupled with Western blotting[11, 12], and competitive enzyme-linked immunosorbent assay (ELISA) with polyclonal antibodies[13, 14].

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Sakai *et al.* reported an ELISA for measuring apo B48 in fasting serum that employed a monoclonal antibody against apo B48[15]. We also recently developed an ELISA to measure the serum level of apo B48 using another monoclonal antibody[16].

We hypothesized that the fasting plasma level of apo B48 may be increased in patients with metabolic syndrome; therefore, we measured fasting plasma levels of apo B48 in order to evaluate the relationship between CM-R and metabolic syndrome. We also investigated the factors regulating apo B48 levels in fasting plasma.

Subjects and Methods

The subjects were 1,349 inhabitants (528 men and 821 women) of two towns in a rural area of Hokkaido, Japan, who underwent routine health checks in 2005. Blood samples were collected from all subjects after an overnight fast. Systolic and diastolic blood pressures were measured at rest in the sitting position. The fasting plasma glucose (FPG) level and plasma levels of total cholesterol, triglycerides, HDL-cholesterol, and low density lipoprotein (LDL)-cholesterol were measured by enzymatic methods. Immunoreactive insulin (IRI) was measured by an enzyme immunoassay (EIA). Waist circumference was measured at the level of the umbilicus in the standing position. The HOMA-IR (homeostasis model assessment insulin resistance index) was calculated as FPGxIRI/405, after excluding individuals who had an FPG above 126 mg/dL and/or were on treatment for diabetes[17].

Apolipoprotein B48 was measured by EIA[16]. Briefly, a 96-well microtiter plate (Nalge Nunc International, Japan) was coated with an anti-apoB-48 monoclonal antibody (4C8) by overnight incubation at 4℃. After washing the microtiter plate with phosphate-buffered saline, 50-μL aliquots of 100-fold-diluted serum or plasma (diluted with 0.05 mol/L Tris-HCl buffer, pH 7.5, 0.15 mol/L NaCl, and 0.1% Triton X-100) were added in duplicate to the wells and the plate was incubated at room temperature (20–25℃) for 1 hr. Aliquots (50 μL) of the apoB-48 standard (2.5 ng/mL to 160 ng/mL: 7-point calibration curve) were incubated in the same way. After the plate was washed three times, 50 μL biotin-conjugated anti-apoB-48/B-100 (ICN Pharmaceuticals Inc., USA) diluted in 0.01 mol/L phosphate buffer (pH 7.2) with 0.15 mol/L NaCl and 0.1% bovine serum albumin was added to each well and incubated with gentle shaking at room temperature for 1 hr. After the plate was washed, 50 μL horseradish peroxidase-conjugated avidin solution was added followed by incubation at room temperature for 30 min. After the plate was washed, 50 μL chromogenic substrate solution was added to each well and incubated with shaking at room temperature for 20 min until the color developed. Then 50 μL of stop solution was added to each well and plate was read at 450 nm using a Spectra-Fluor-Plus plate reader (Tecan, USA).

Metabolic syndrome was defined according to Japanese criteria[18]. Briefly, a waist circumference of more than 85 cm in men and 90 cm in women combined with more than one of the following factors led to a diagnosis of metabolic syndrome: plasma triglycerides >150 mg/dL and/or HDL cholesterol <40 mg/dL, systolic blood pressure >130 mmHg and/or diastolic blood pressure >85 mmHg, and FPG >110 mg/dL. Some subjects were taking medications, but subjects with or without medications were grouped together for this study.

The mean ± SD or median with interquartile range is shown to summarize the characteristics of the study subjects by sex. Between-group comparisons of the means and median were performed by unpaired *t*-test and the Wilcoxon rank-sum test, respectively. The relationship of serum lipids and lipoproteins with apo B48 was examined by correlation and multiple regression analysis. Pearson's correlation coefficients were calculated for the correlation. Stepwise multiple regression analysis was used to determine independent predictors of apo B48, with p-to-enter and p-to-retain set at 0.10 each. Statistical significance was declared if the two-sided p value was less than 0.05. Statistical analyses were performed using JMP software (SAS Institute, Cary, NC).

Results

The age of all subjects, men and women was 62.4 ± 12.8 years, 64.2 ± 12.8 years and 61.2 ± 12.7 years (mean ± S.D.), respectively. The mean body mass index (BMI) did not differ significantly between men and women (23.9 ± 3.1 vs. 23.5 ± 3.6, respectively). Plasma levels of total cholesterol, HDL-cholesterol and LDL-cholesterol were significantly lower ($p<0.001$) in men than in women, whereas the values of apo B48, triglycerides, and FPG were significantly higher in men ($p<0.001$) (**Table 1**). **Fig. 1** shows the distribution of apo B48 in men and women. The mean apo B48 level was 1.92 μg/mL in men and 1.69 μg/mL in women.

As shown in **Fig. 1**, the distribution of apo B48 was skewed to the left. Data were therefore normalized by logarithmic transformation for further statistical analysis. The triglyceride and HOMA-IR data were

Table 1. Characteristics of the subjects

	Men (n=524)	Women (n=819)	p-value
Age (years)[a]	64.2±12.8	61.2±12.7	<0.0001
BMI (kg/m²)[a]	23.9±3.1	23.5±3.6	0.01
Waist (cm)[a]	85.9±8.7	83.1±10.7	0.04
MS with/without	397/125	741/78	<0.0001
T. chol (mg/dL)[a]	192.4±31.1	204.4±30.9	<0.0001
TG (mg/dL)[b]	99 (74-140)	83 (63-117)	<0.0001
HDL-C (mg/dL)[a]	53.9±13.2	62.1±14.3	<0.0001
LDL-C (mg/dL)[a]	106.2±27.6	115.8±27.3	<0.0001
SBP (mmHg)[b]	138 (124-153)	135 (117-151)	<0.001
DBP (mmHg)[b]	78 (70-86)	74.5 (66-83)	<0.0001
FPG (mg/dL)[a]	102.9±23.4	95.0±18.9	<0.0001
IRI (μU/mL)[b]	3.9 (2.6-5.8)	4.1 (2.8-5.8)	0.38
HOMA-IR (mg/dL×μU/mL)[b]	0.924 (0.602-1.387)	0.909 (0.636-1.341)	0.02
ApoB 48 (μg/mL)[b]	1.80 (1.20-3.10)	1.61 (1.14-2.45)	<0.001

[a]Mean±SD
[b]Median (25th and 75th interquartile range)
p values were based on paired t-test and Wilcoxon rank-sum test for mean and median, respectively.

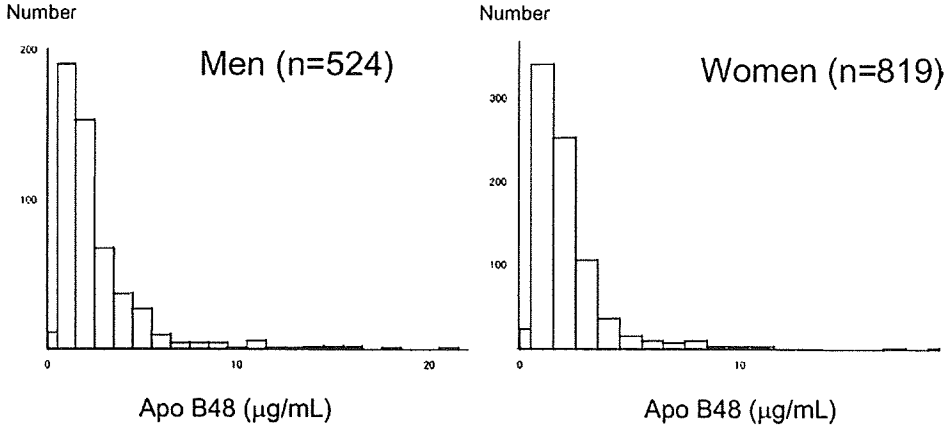

Fig. 1. Distribution of ln (apo B48) in men and women.
Geometric means and 95% central limits are 1.92 μg/mL (1.80-2.04) in men and 1.69 μg/mL (1.61-1.76) in women.

also normalized because of their skewed distribution (data not shown).

The ln (apo B48) showed a weak positive correlation with total cholesterol, and a weak negative correlation with HDL-cholesterol (**Fig. 2**). In addition, ln (apo B48) and ln (triglycerides) showed a strong positive correlation (r=0.53 in men and r=0.48 in women).

Ln (apo B48) also showed a strong positive correlation with ln (HOMA-IR) (**Fig. 2**). The fasting apo B48 level was significantly higher in both men and women with metabolic syndrome than without (**Table 2**).

Ln (apo B48) was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and ln (triglyceride) by multiple regression analysis (**Table 3**).

Discussion

In this study, we measured plasma apo B48 levels with a novel ELISA. According to previous reports, the fasting plasma apo B48 concentration ranges between

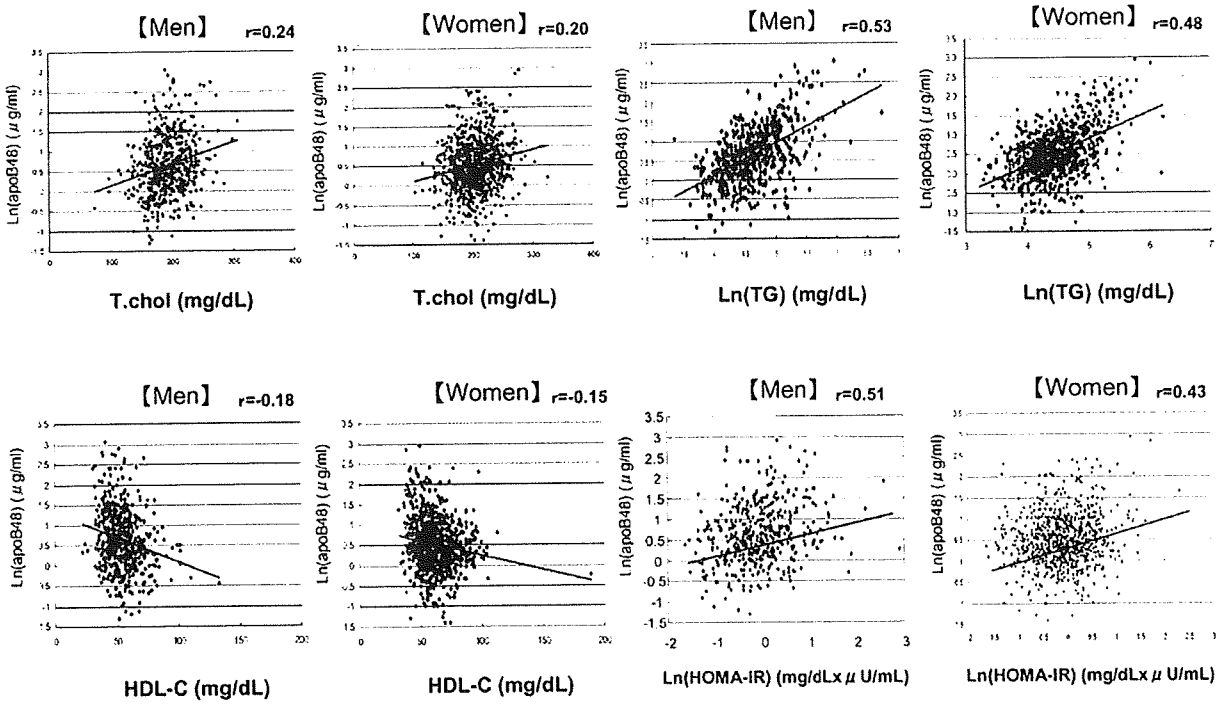

Fig. 2. Correlation of ln (apo B48) with total cholesterol, ln (triglycerides), HDL-cholesterol, and ln (HOMA-IR) in men and women.

Table 2. Apo B48 levels according to metabolic syndrome in men and women

Sex	Metabolic syndrome	N	Geometric mean (95% CI)
Men	(−)	397	1.76 (1.65–1.89)
	(+)	125	2.50 (2.17–2.88)
			$p<0.0001$
Women	(−)	741	1.64 (1.57–1.72)
	(+)	78	2.19 (1.86–2.59)
			$p<0.0001$

CI, confidence interval
p-value was based on unpaired *t*-test.

0.08 μg/mL and 60 μg/mL[8-15]. Our data (men: 1.92 μg/mL; women: 1.69 μg/mL (mean value)) were also in this range, and were similar to the level reported by Sakai *et al.* for normolipidemic subjects (5.2 ± 3.8 mg/mL) using a similar ELISA with another monoclonal antibody against apo B48[15]. Among the methods available to measure apo B48, ELISA systems based on monoclonal antibodies are valuable because they are simple and quantitative methods.

In this study, we measured fasting plasma levels of apo B48. It has been suggested that a high fasting apo B48 level reflects high postprandial concentrations of chylomicrons and/or CM-R[12]; therefore, we assumed that a high fasting plasma level of apo B48 indicated the existence of postprandial hyperlipidemia.

The B48 concentration was higher among men than women (**Fig. 1**). Sakai *et al.* previously found that men also had higher apo B48 levels than women among normolipidemic subjects[15]. These results may indicate that women show more rapid catabolism of chylomicrons and/or CM-R, or less intestinal fat absorption, or both.

Apo B48 showed a significant and strong correlation with triglycerides (**Fig. 2**). Cortner *et al.* reported that delayed catabolism of CM-R leads to hypertriglyceridemia[3]. Since very low density lipoprotein (VLDL) and VLDL remnants are considered the main contributors to plasma triglyceride concentration, the close relationship between apo B48 and triglyceride levels indicates that delayed catabolism of CM-R leads to the accumulation of VLDL or VLDL remnants. It is interesting that the plasma concentration of apo B48, which is far lower than that of apo B100 (0.15–0.2 vs.

Table 3. Stepwise multiple regression analysis of ln (apoB48) in relation to serum lipids, lipoproteins, and glucose-related parameters ($n = 1,089$)

	Regression coefficient	S.E.	t value	p value
Age	−0.0069	0.0013	−5.12	<0.0001
BMI	−0.0213	0.0599	−3.55	0.0004
Total cholesterol	0.0066	0.0021	3.13	0.0018
HDL cholesterol	−0.0068	0.0022	−3.15	0.0017
LDL cholesterol	−0.0047	0.0020	−2.28	0.0227
ln (Triglycerides)	0.5520	0.0715	7.72	<0.0001
ln (HOMA-IR)	0.0583	0.0337	1.73	0.0939

Sex, SBP, DBP, total cholesterol, HDL cholesterol, LDL cholesterol, apo E, ln (triglyceride), and ln (IRI) were also included as explanatory variables in the model, but they did not remain in the final model.

100–120 mg/dL), has such a significant relationship with the VLDL or VLDL remnant level. This may because the triglyceride content of CM-R is very high when compared to VLDL or VLDL remnants.

Apo B48 was also positively correlated with HOMA-IR (**Fig. 2**), which is a marker of insulin resistance, and the apo B48 level was significantly higher in subjects with metabolic syndrome than without (**Table 2**). These results indicate that apo B48 increases in the presence of insulin resistance and/or metabolic syndrome. Since insulin resistance is considered to be involved in the development of metabolic syndrome[19, 20], insulin sensitivity might influence the level of apo B48. It has been reported that insulin resistance shows a negative correlation with lipoprotein lipase mRNA expression and activity in adipose tissue[21]. Thus, defects of lipoprotein lipase may cause the accumulation of apo B48 particles. In fact, it has been reported that insulin resistance might lead to postprandial hyperlipidemia[22, 23].

Because our subjects with metabolic syndrome showed higher fasting plasma concentrations of apo B48, there is a possibility that CM-R may play a role in the increased risk of atherosclerosis related to this syndrome. Vine *et al.* reported that impaired postprandial metabolism of apo B48 led to atherosclerosis in rats with metabolic syndrome[24]. On the other hand, Velero *et al.* reported that the fasting apo B48 level does not predict the risk of coronary heart disease[25]. Thus, whether the fasting apo B48 level influences the risk of atherosclerosis remains to be determined.

Multiple regression analysis revealed that apo B48 was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride. Gender does not affect apo B48 by this method, which may be due to an other factor related to gender (i.e., LDL cholesterol or HDL cholesterol) having a strong association with apo B48.

In conclusion, the fasting plasma level of apo B48 was correlated with the serum triglyceride concentration, and apo B48 levels were higher in rural Japanese subjects with metabolic syndrome than those without; however, further studies of other populations are needed to confirm these results.

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Impact of weight change on specific-cause mortality among middle-aged Japanese individuals

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ABSTRACT

Background: The aim of this study was to investigate the associations between long-term weight change after age 20 and overall mortality and cause-specific mortality in the general Asian population.

Methods: From 1990 to 2005, the Japan Public Health Center (JPHC)-based prospective study conducted a follow-up of 42 242 men and 46 177 women aged 40–69 years with no history of cardiovascular disease (CVD) or cancer. Sex-specific multivariable-adjusted hazard ratios for cause-specific mortality were computed in accordance with weight change categories from age 20, as assessed by a self-administered questionnaire, and clustered by the JPHC communities and age groups, using Cox's proportional hazard model.

Results: During the 12.9-year follow-up, there were 6494 deaths, including 2888 from cancer, 1011 from CVD and 2595 from other causes. In all, weight loss ≥5 kg since age 20 increased hazard ratios for all-cause mortality in men (1.44, 95% CI 1.32 to 1.56) and women (1.33, 95% CI 1.17 to 1.52) compared with maintenance of a stable weight, and elevated risk was also found within each age group. The risk of weight loss was higher for individuals in the younger age group. Weight loss predicted cancer and CVD mortality only for men ≥50 years of age. The increased risk was observed regardless of whether the individual was ill, a smoker or overweight at baseline or at age 20. There was an inverse association between weight gain and mortality risk.

Conclusion: Weight loss strongly predicted all-cause, cancer and CVD mortality, primarily for men. An unfavourable effect of weight gain was small at the population level.

Weight change and weight fluctuation are strongly associated with all-cause and cause-specific mortality. The Nurses' Health Study cohort found that obesity and weight gain since early adulthood were closely related to mortality from all causes.[1] Several US and European prospective studies documented an increased risk of mortality associated with weight loss[2–5] and weight fluctuation[6 7] in elderly or middle-aged people. However, it is controversial whether or not weight gain is more hazardous for life expectancy considering covariates related to body weight such as smoking and illness.[4 5] Recently, health policy has given much attention to obesity and weight gain linked to the metabolic syndrome.[8–10] However, relatively little is known about possible associations between long-term weight changes, especially weight loss, and mortality in the general Asian population.

A national survey in Japan documented that mean body mass index (BMI) was 23.2 kg/m^2 in men and 22.5 kg/m^2 in women aged 15 years or over, and the percentages of obesity (≥30.0 kg/m^2) in men and women were very low: 2.6% and 3.6%, respectively.[11] Observational studies have shown a U-shaped, L-shaped or J-shaped association between BMI and mortality in Japan and other Asian countries.[12–14] Nevertheless, there is little evidence regarding the influence of either high or low BMI and weight change.

To better understand weight change for Japanese individuals with low BMI and its association with specific-cause mortality, we conducted a large prospective study that included 88 419 men and women across Japan with a median 12.9 years of follow-up.

METHODS

Study population

Our subjects were 42 242 men and 46 177 women aged 40–69 years who had no history of ischaemic heart disease, stroke or cancer and who were available for reports on weight change in the Japan Public Health Center (JPHC)-based prospective study. The JPHC study consisted of cohorts I and II, which began in 1990 and in 1993, respectively, as described elsewhere.[12 15] In brief, the cohort I and II populations were residents aged 40–59 years in five public health centre (PHC) areas (Ninohe PHC of Iwate Prefecture, Yokote PHC of Akita Prefecture, Saku PHC of Nagano Prefecture, Chubu PHC of Okinawa Prefecture and Katsushika PHC of Tokyo) and residents aged 40–69 years in six PHC areas (Mito PHC of Ibaraki Prefecture, Nagaoka PHC of Niigata Prefecture, Suita PHC of Osaka Prefecture, Chuo-higashi PHC of Kochi Prefecture, Kamigoto PHC of Nagasaki Prefecture and Miyako PHC of Okinawa Prefecture), respectively. The entire population included 140 420 men and women. Of them, 113 461 individuals answered the self-report questionnaire. From that group, 88 419 were available for our analysis based on the inclusion criteria mentioned above (response rate 78%). The present study was approved by the Ethics Committee of the National Cancer Center.

Measurements

We assessed demographic characteristics, including height, weight, medical history, smoking habits and regular alcohol drinking, using a self-administered questionnaire at baseline. The amount of ethanol consumed per week was evaluated by measuring the weekly frequency and the type of alcoholic beverage (beer, sake, whiskey, shochu and wine). Histories of hypertension and diabetes were ascertained by the question, "Have the following conditions been diagnosed by physicians?", with a

list of hypertension, diabetes and other chronic diseases as potential responses.

Weight change in cohort I was determined by the question, "Any changes of your weight (more than 5 kg) since the age of 20?", with potential responses of loss, no changes or gain. In cohort II, participants were asked, "What was your weight when you were 20 years old?" We computed the weight change from the difference between the weight reported at age 20 and at baseline. A total of 47 856 individuals (22 520 men and 25 336 women) from cohort I and 40 563 individuals (19 722 men and 20 841 women) from cohort II who reported that their weight had changed since age 20 were included in the analysis of weight change.

The following variables were used as covariates with dummy variables: non-smoker, light smoker (<20 cigarettes/day) and heavy smoker (≥20 cigarettes/day); sports and physical exercise (≥1 day/week, other); those who took drugs (hypertension, hyperlipidaemia, diabetes, gout); and those who had been diagnosed by a doctor (hypertension, diabetes, gastroduodenal ulcer, liver disease and kidney disease). Alcohol intake per week was estimated from the frequency and amount of alcohol consumed as defined by the ethanol concentration of major alcoholic beverages. These values were classified into categorical variables using a traditional portion in Japan: non-drinker, 1–23 g/day, 23–46 g/day, 46–69 g/day and ≥69 g/day.[16] These groups correspond to the categories related to incident cardiovascular disease (CVD) among Japanese. All variables were assessed with a self-administered questionnaire.

The underlying cause of death was determined based on death certificates and coded by the International Classification of Diseases, ninth revision (ICD-9) until 1995, and translated into the corresponding ICD-10 codes or coded by the ICD-10 after that. Deaths from cancer were defined as C00–C97, and deaths from CVD were defined as I20–25 and I60–69 (ICD-10).

Statistical analysis

With a median 12.9 years of follow-up from 1990 (cohort I) and 1993 (cohort II) to the end of 2005, person-years were calculated as the period from the date of the baseline to that of the first endpoint (death, emigration or loss) or to 31 December 2005. Among the participants, 25 moved out of Japan, one withdrew participation and 248 (0.3%) were lost to follow-up.

Weight change was classified into three comparable categories between cohorts I and II: loss (≥5 kg), stable (change <5 kg) and gain (≥5 kg). Furthermore, in the cohort II subjects, we reclassified weight change into five categories to analyse a dose–response relationship between weight change and mortality risk: loss ≥10 kg, loss 5–9 kg, stable (change <5 kg), gain 5–9 kg and gain ≥10 kg.

Sex-specific hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated after adjusting for age (continuous); current BMI; smoking status (non-smoker, <20 cigarettes/day and ≥20 cigarettes/day); ethanol intake (non-drinker, 1–23 g/day, 23–46 g/day, 46–69 g/day and ≥69 g/day); sports and physical exercise; medications or past history of hypertension and diabetes; and past history of liver disease and kidney disease as dummy variables, stratified by the JPHC communities and age groups of 40–49 years, 50–59 years and 60–69 years to adjust for an exposure period of weight change since age 20, using Cox's proportional hazard models. A test for linear trends was also carried out using a weight change variable (continuous) adjusted for the same covariates. Statistical significance was assumed at $p<0.05$. SAS software, V.9.1 (SAS Institute, Inc., Cary, North Carolina, USA), was used for all analyses.

RESULTS

During a median 12.9 years of follow-up, we documented 6494 deaths among 42 242 men and 46 177 women from combined cohorts I and II, including 2888 deaths from cancer, 1011 from CVD and 2595 from other causes. Figure 1 shows sex-specific mortality rates from all causes, cancer, CVD and other causes among individuals according to the category since age 20 by age group. It clearly illustrates high mortality rates from any cause for subjects with weight loss in each age group.

Table 1 shows population characteristics by weight change category and p values for differences among them. Those who reported weight loss ≥5 kg since age 20 indicated lower BMIs at baseline and higher BMIs at age 20; these data were available only for cohort II subjects. The percentages of smokers or those who had a past history of and had taken medication for diabetes were higher in men and women with weight loss. Significant differences between groups were recorded for most of the variables investigated, except for alcohol intake and gout in women.

Table 2 shows sex-specific multivariable-adjusted HRs for the cause of death comparing respondents with those with stable weight as a reference group. In men, an inverse association between weight gain and mortality was found for all-cause mortality, cancer mortality and other causes of mortality. HRs for all-cause mortality in the multivariable model were 1.44 (95% CI 1.32 to 1.56) and 0.89 (95% CI 0.82 to 0.97) for men with weight loss and weight gain, respectively. Those for all-cause mortality and other causes of mortality were likely to be high in the younger age group, and those for cancer mortality increased in older men. There was no increased risk of death among men with weight gain. In women, the multivariable model indicated an L-shaped association and an elevated risk of death for those with weight loss (1.33; 95% CI 1.17 to 1.52); however, risks of cancer and CVD were not increased significantly. Similar to men, the HRs for other causes of death were increased in younger women with weight loss. The association of weight gain with mortality was not clear. We analysed results after deletion of the first 5 years of follow-up and computed similar HRs for weight loss and gain (data not shown). Although each risk was somewhat attenuated, the elevated mortality risks for men and women with weight loss were almost the same.

In the subgroup analyses (table 3), as for the relationship of illnesses and smoking status with mortality, men and women with weight loss who were not ill and men and women with weight loss who smoked were at risk of mortality from all causes, cancer (men only) and other causes. Men who smoked or had illnesses were also at increased mortality risk.

Table 4 shows multivariable-adjusted HRs of weight change categories for death from all causes, cancer, CVD and other causes, stratified by baseline BMI (<18.5 kg/m^2, 18.5–24.9 kg/m^2 and ≥25 kg/m^2). The reference group was the 18.5–24.9 kg/m^2 baseline BMI group and stable weight change. In any group, HRs seemed to be increased significantly among individuals with weight loss. The highest HRs for all causes were found in persons with both baseline BMI <18.5 kg/m^2 and weight loss (≥5 kg).

Since we obtained self-reported weights at study entry and at age 20 from individuals in cohort II, we calculated multivariable-adjusted HRs for all-cause mortality classified into five weight change categories by BMI at age 20 (fig 2). Regardless of whether an individual was overweight at age 20, weight loss ≥10 kg was a risk factor for mortality. Among non-overweight

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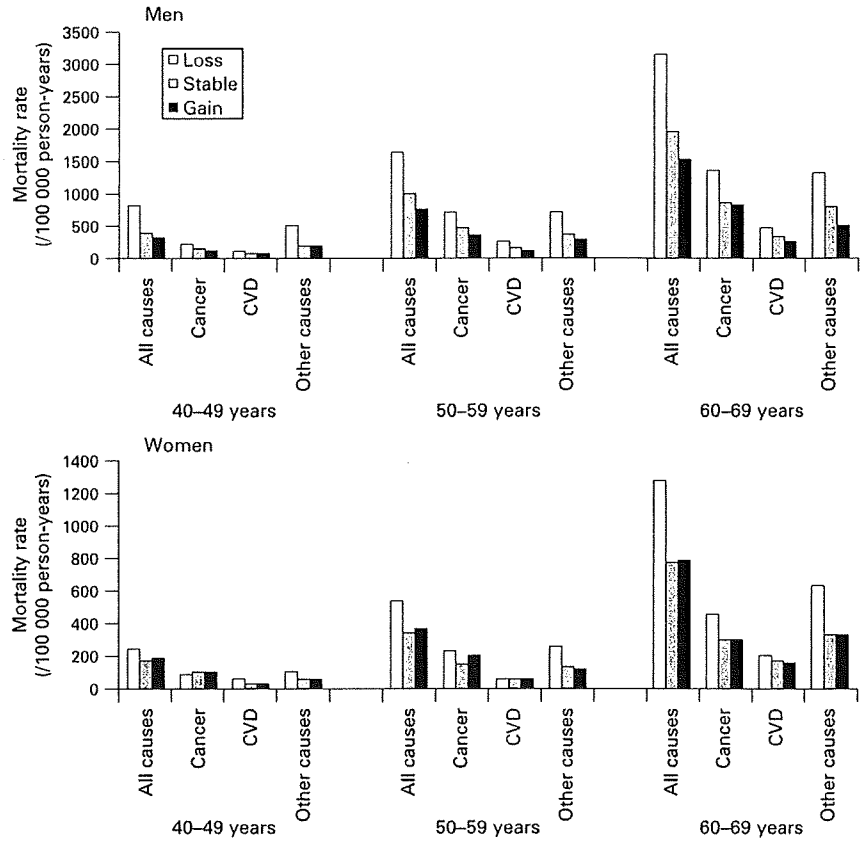

Figure 1 Sex-specific mortality rates from all causes, cancer, CVD and other causes among individuals with weight loss ≥5 kg, stable weight (change, <5 kg) and weight gain ≥5 kg since age 20 by age group.

Table 1 Population characteristics by sex and weight change categories since age 20

	Men				Women			
	Weight change since age 20				Weight change since age 20			
	Loss ≥5 kg	Stable, change <5 kg	Gain ≥5 kg	p Value	Loss ≥5 kg	Stable, change <5 kg	Gain ≥5 kg	p Value
Number	5159	14 338	22 745		5852	14 522	25 803	
Age, years	55.1	50.6	49.1	<0.001	52.1	49.4	49.9	<0.001
Baseline body mass index, kg/m²	21.5	22.0	25.0	<0.001	21.0	21.6	24.8	<0.001
Body mass index at 20,* kg/m²	24.4	21.8	21.0	<0.001	24.1	21.3	20.3	<0.001
Smoking status, %								
Non-smoker	35.5	41.0	52.4	<0.001	89.3	91.7	92.8	<0.001
<20 cigarettes/day	19.0	15.0	11.8		6.9	5.8	4.4	
≥20 cigarettes/day	45.5	43.9	35.9		3.8	2.5	2.8	
Alcohol intake, g/week	212.0	209.3	202.5	<0.05	18.0	18.1	19.7	0.189
Sports and physical exercise, %								
≥1 day/week	16.6	19.1	20.5	<0.001	15.7	19.7	18.8	<0.001
Persons who took drugs for, %								
Hypertension	13.8	8.8	12.3	<0.001	9.9	7.4	13.6	<0.001
Hyperlipidaemia	1.0	1.0	2.0	<0.001	1.7	1.5	2.4	<0.001
Diabetes	5.4	1.6	1.6	<0.001	2.1	0.8	1.2	<0.001
Gout	1.2	0.9	2.0	<0.001	0.4	0.2	0.3	0.054
Persons who had been diagnosed by a doctor with, %								
Hypertension	17.0	12.4	18.0	<0.001	10.6	9.2	16.5	<0.001
Diabetes	11.0	4.8	5.6	<0.001	3.6	1.7	2.8	<0.001
Liver disease	3.3	2.2	2.4	<0.001	1.1	0.8	0.9	<0.05
Kidney disease	2.8	2.1	1.9	<0.001	2.6	1.9	2.2	<0.001
Other illnesses†	23.8	18.6	17.3	<0.001	14.8	12.6	12.6	<0.001

*Data were available for 19 677 men and 20 786 women in cohort II.
†Includes any of asthma, allergy, stomach ulcer and gallstone.

Table 2 Sex- and age-specific number of deaths and multivariable-adjusted HRs of individuals with weight loss and weight gain since age 20 for death from all causes, cancer, CVD and other causes

		Men						Women					
		Loss ≥5 kg		Stable, change <5 kg		Gain ≥5 kg		Loss ≥5 kg		Stable, change <5 kg		Gain ≥5 kg	
Cause of death	Age group	Deaths	HR* (95% CI)	Deaths	HR	Deaths	HR* (95% CI)	Deaths	HR* (95% CI)	Deaths	HR	Deaths	HR* (95% CI)
All causes	All	1148	1.44 (1.32 to 1.56)	1530	1.0	1747	0.89 (0.82 to 0.97)	418	1.33 (1.17 to 1.52)	563	1.0	1088	0.98 (0.87 to 1.10)
	40–49	125	1.61 (1.31 to 1.98)	355	1.0	527	0.89 (0.76 to 1.05)	72	1.21 (0.91 to 1.61)	164	1.0	302	0.84 (0.68 to 1.05)
	50–59	573	1.41 (1.26 to 1.58)	788	1.0	941	0.88 (0.79 to 0.98)	209	1.33 (1.10 to 1.61)	265	1.0	599	1.01 (0.86 to 1.19)
	60–69	450	1.35 (1.16 to 1.58)	387	1.0	279	0.92 (0.77 to 1.11)	137	1.36 (1.04 to 1.74)	134	1.0	187	1.11 (0.86 to 1.44)
Cancer	All	471	1.27 (1.12 to 1.44)	678	1.0	769	0.90 (0.80 to 1.02)	162	1.17 (0.96 to 1.44)	262	1.00	546	1.04 (0.88 to 1.23)
	40–49	33	1.20 (0.82 to 1.77)	129	1.0	188	0.92 (0.70 to 1.20)	25	0.80 (0.50 to 1.26)	89	1.0	154	0.97 (0.71 to 1.31)
	50–59	244	1.27 (1.07 to 1.50)	383	1.0	434	0.84 (0.72 to 0.98)	89	1.26 (0.95 to 1.67)	123	1.0	320	1.11 (0.88 to 1.40)
	60–69	194	1.34 (1.06 to 1.68)	166	1.0	147	1.13 (0.87 to 1.46)	48	1.44 (0.95 to 2.18)	50	1.0	72	0.95 (0.62 to 1.44)
CVD	All	169	1.34 (1.09 to 1.66)	233	1.0	281	0.81 (0.66 to 0.99)	63	1.22 (0.87 to 1.71)	92	1.0	173	0.82 (0.62 to 1.10)
	40–49	16	1.19 (0.68 to 2.09)	59	1.0	89	0.82 (0.55 to 1.21)	18	1.74 (0.92 to 3.27)	25	1.0	46	0.56 (0.33 to 0.96)
	50–59	86	1.37 (1.03 to 1.84)	111	1.0	148	0.82 (0.62 to 1.10)	24	1.02 (0.60 to 1.72)	39	1.0	90	0.93 (0.61 to 1.42)
	60–69	67	1.30 (0.89 to 1.90)	63	1.0	44	0.72 (0.46 to 1.13)	21	1.12 (0.60 to 2.07)	28	1.0	37	1.18 (0.66 to 2.12)
Other causes	All	508	1.66 (1.47 to 1.89)	619	1.0	697	0.92 (0.81 to 1.05)	193	1.56 (1.27 to 1.91)	209	1.0	369	0.97 (0.80 to 1.18)
	40–49	76	2.06 (1.56 to 2.72)	167	1.0	250	0.90 (0.72 to 1.14)	29	1.60 (1.00 to 2.56)	50	1.0	102	0.91 (0.62 to 1.33)
	50–59	243	1.60 (1.34 to 1.91)	294	1.0	359	0.97 (0.81 to 1.16)	96	1.53 (1.14 to 2.04)	103	1.0	189	0.91 (0.69 to 1.20)
	60–69	189	1.39 (1.10 to 1.76)	158	1.0	88	0.78 (0.57 to 1.06)	68	1.43 (0.98 to 2.10)	56	1.0	78	1.23 (0.83 to 1.85)

*HR was adjusted for age; current body mass index; smoking status (non-smoker, <20 cigarettes/day and ≥20 cigarettes/day); alcohol intake (non-drinker, 1–23 g/day, 23–46 g/day, 46–69 g/day and ≥69 g/day); sports and physical exercise; medications or past history of hypertension and diabetes; and past history of liver disease and kidney disease stratified by JPHC communities and age groups of 40–49 years, 50–59 years and 60–69 years (only for all age groups).
CVD, cardiovascular disease.

Table 3 Sex-specific multivariable-adjusted HRs of death from all causes, cancer, CVD and other causes among subgroups of people with and without illnesses; and smokers and non-smokers

	Men						Women					
	Loss ⩾5 kg		Stable, change <5 kg		Gain ⩾5 kg		Loss ⩾5 kg		Stable, change <5 kg		Gain ⩾5 kg	
Cause of death	Deaths	HR* (95% CI)	Deaths	HR	Deaths	HR* (95% CI)	Deaths	HR* (95% CI)	Deaths	HR	Deaths	HR* (95% CI)
Without illnesses†												
All causes	468	1.46 (1.29 to 1.64)	804	1.0	856	0.96 (0.86 to 1.08)	196	1.22 (1.02 to 1.47)	335	1.0	592	0.99 (0.85 to 1.16)
Cancer	206	1.34 (1.13 to 1.62)	361	1.0	401	1.00 (0.84 to 1.18)	85	1.01 (0.77 to 1.33)	177	1.0	320	1.00 (0.81 to 1.24)
CVD	58	1.18 (0.84 to 1.65)	118	1.0	107	0.72 (0.53 to 0.99)	22	1.20 (0.71 to 2.04)	43	1.0	83	0.91 (0.60 to 1.38)
Other causes	204	1.70 (1.41 to 2.05)	325	1.0	348	1.02 (0.85 to 1.22)	89	1.51 (1.13 to 2.02)	115	1.0	189	1.04 (0.80 to 1.36)
With illnesses†												
All causes	680	1.44 (1.29 to 1.61)	726	1.0	891	0.83 (0.74 to 0.94)	222	1.50 (1.24 to 1.82)	228	1.0	496	0.97 (0.81 to 1.16)
Cancer	265	1.20 (1.01 to 1.43)	317	1.0	368	0.82 (0.69 to 0.97)	77	1.46 (1.06 to 2.00)	85	1.0	226	1.10 (0.83 to 1.45)
CVD	111	1.52 (1.15 to 2.00)	115	1.0	174	0.93 (0.71 to 1.21)	41	1.21 (0.78 to 1.88)	49	1.0	90	0.81 (0.54 to 1.21)
Other causes	304	1.69 (1.43 to 2.00)	294	1.0	349	0.83 (0.69 to 0.99)	104	1.69 (1.26 to 2.26)	94	1.0	180	0.94 (0.70 to 1.25)
Non-smokers												
All causes	347	1.46 (1.27 to 1.69)	529	1.0	815	0.95 (0.84 to 1.08)	356	1.36 (1.18 to 1.57)	493	1.0	975	0.99 (0.87 to 1.12)
Cancer	128	1.31 (1.05 to 1.65)	224	1.0	371	1.00 (0.82 to 1.21)	141	1.20 (0.97 to 1.49)	233	1.0	503	1.06 (0.89 to 1.26)
CVD	50	1.37 (0.94 to 1.98)	79	1.0	133	0.86 (0.62 to 1.19)	46	1.14 (0.77 to 1.67)	76	1.0	148	0.86 (0.62 to 1.18)
Other causes	169	1.64 (1.32 to 2.03)	226	1.0	311	0.95 (0.77 to 1.16)	169	1.61 (1.29 to 2.01)	184	1.0	324	0.95 (0.78 to 1.17)
Smokers												
All causes	790	1.43 (1.29 to 1.58)	993	1.0	926	0.86 (0.77 to 0.95)	57	1.15 (0.79 to 1.66)	68	1.0	108	0.88 (0.61 to 1.25)
Cancer	337	1.24 (1.07 to 1.44)	452	1.0	395	0.84 (0.72 to 0.98)	19	0.99 (0.54 to 1.81)	27	1.0	40	0.92 (0.51 to 1.65)
CVD	118	1.34 (1.03 to 1.73)	150	1.0	146	0.78 (0.60 to 1.02)	15	1.41(0.68 to 2.90)	16	1.0	24	0.59 (0.28 to 1.23)
Other causes	335	1.71 (1.46 to 1.99)	391	1.0	385	0.91 (0.77 to 1.07)	23	1.15 (0.62 to 2.12)	25	1.0	44	1.10 (0.62 to 1.95)

*HR was adjusted for age; current body mass index; smoking status (non-smoker, <20 cigarettes/day and ⩾20 cigarettes/day); alcohol intake (non-drinker, 1–23 g/day, 23–46 g/day, 46–69 g/day and ⩾69 g/day); sports and physical exercise; medications or past history of hypertension and diabetes; and past history of liver disease and kidney disease stratified by JPHC communities and age groups of 40–49 years, 50–59 years and 60–69 years.
†Includes any of hypertension, diabetes, liver disease, kidney disease, asthma, allergy, stomach ulcer and gallstone.
CVD, cardiovascular disease.

Table 4 Sex-specific multivariable-adjusted* HRs for deaths from all causes, cancer, CVD and other causes, according to weight change category since age 20 stratified by baseline BMI

			Baseline BMI, <18.5 kg/m²			Baseline BMI, 18.5–24.9 kg/m²			Baseline BMI, ≥25 kg/m²		
			Weight change since age 20			Weight change since age 20			Weight change since age 20		
Sex	Cause of death		Loss ≥5 kg	Stable, change <5 kg	Gain ≥5 kg	Loss ≥5 kg	Stable, change <5 kg	Gain ≥5 kg	Loss ≥5 kg	Stable, change <5 kg	Gain ≥5 kg
Men	All causes	Deaths	178	67	6	883	1352	920	77	100	811
		HR* (95% CI)	2.40 (2.04 to 2.82)	1.30 (1.01 to 1.68)	1.59 (0.71 to 3.56)	1.35 (1.23 to 1.47)	1.00	0.83 (0.76 to 0.90)	1.36 (1.08 to 1.72)	0.76 (0.62 to 0.94)	0.80 (0.73 to 0.88)
	Cancer	Deaths	67	25	3	374	597	410	24	48	354
		HR* (95% CI)	1.84 (1.41 to 2.39)	1.14 (0.76 to 1.70)	1.75 (0.56 to 5.45)	1.23 (1.08 to 1.41)	1.00	0.86 (0.76 to 0.98)	0.96 (0.63 to 1.44)	0.83 (0.61 to 1.13)	0.84 (0.74 to 0.97)
	CVD	Deaths	13	11	1	145	211	139	10	11	139
		HR* (95% CI)	1.18 (0.67 to 2.08)	1.32 (0.70 to 2.49)	–†	1.34 (1.07 to 1.68)	1.00	0.75 (0.60 to 0.94)	0.96 (0.51 to 1.83)	0.47 (0.25 to 0.89)	0.78 (0.62 to 0.98)
	Other causes	Deaths	98	31	2	364	544	371	43	41	318
		HR* (95% CI)	3.60 (2.88 to 4.50)	1.47 (1.01 to 2.14)	–†	1.49 (1.29 to 1.71)	1.00	0.83 (0.72 to 0.95)	1.96 (1.43 to 2.68)	0.81 (0.58 to 1.12)	0.77 (0.67 to 0.89)
Women	All causes	Deaths	79	52	9	307	462	517	26	46	554
		HR* (95% CI)	2.12 (1.66 to 2.70)	1.61 (1.21 to 2.16)	2.46 (1.22 to 4.95)	1.26 (1.08 to 1.46)	1.00	0.91 (0.80 to 1.04)	1.29 (0.86 to 1.94)	0.84 (0.62 to 1.14)	1.04 (0.91 to 1.18)
	Cancer	Deaths	29	20	6	117	220	271	13	20	265
		HR* (95% CI)	1.71 (1.15 to 2.55)	1.31 (0.83 to 2.07)	3.15 (1.30 to 7.66)	1.06 (0.84 to 1.33)	1.00	1.00 (0.83 to 1.20)	1.56 (0.89 to 2.75)	0.82 (0.52 to 1.31)	1.11 (0.92 to 1.34)
	CVD	Deaths	7	6	1	50	78	74	6	8	98
		HR* (95% CI)	1.07 (0.49 to 2.34)	0.98 (0.40 to 2.44)	–†	1.16 (0.80 to 1.68)	1.00	0.77 (0.55 to 1.07)	1.36 (0.55 to 3.39)	0.82 (0.39 to 1.71)	1.02 (0.74 to 1.39)
	Other causes	Deaths	43	26	2	140	164	172	7	18	191
		HR* (95% CI)	3.16 (2.24 to 4.46)	2.34 (1.54 to 3.54)	–†	1.56 (1.24 to 1.97)	1.00	0.86 (0.69 to 1.07)	0.96 (0.45 to 2.06)	0.87 (0.53 to 1.42)	0.95 (0.75 to 1.19)

*HR was adjusted for age; current body mass index; smoking status (non-smoker, <20 cigarettes/day and ≥20 cigarettes/day); alcohol intake (non-drinker, 1–23 g/day, 23–46 g/day, 46–69 g/day and ≥69 g/day); sports and physical exercise; medications or past history of hypertension and diabetes; and past history of liver disease and kidney disease stratified by JPHC communities and age groups of 40–49 years, 50–59 years and 60–69 years.
†Not represented because of fewer cases.
BMI, body mass index, CVD, cardiovascular disease.

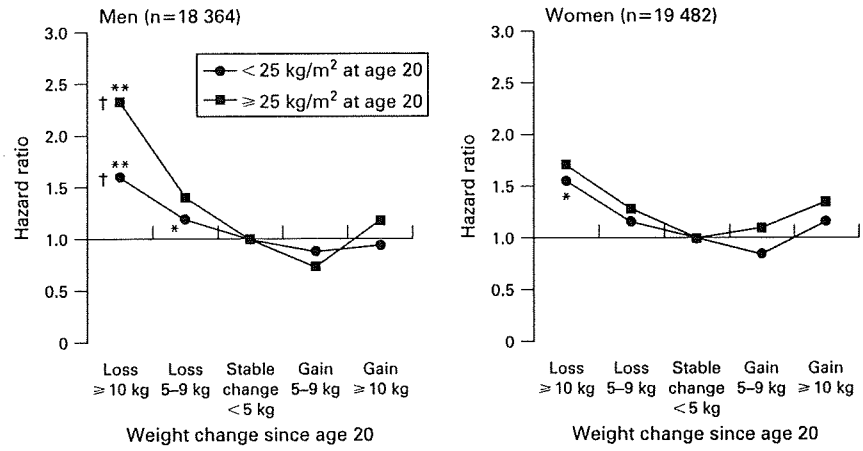

Figure 2 Multivariable-adjusted HRs of all-cause mortality according to weight change category by body mass index (BMI) at age 20 in cohort II. Covariate variables were the same as in table 2. *p<0.05; **p<0.001 for difference versus stable change group. †p<0.001 for linear trends.

and overweight men, an inverse linear association between weight gain and mortality was found (p for trend <0.001).

DISCUSSION

This large prospective cohort study confirmed a strong association between weight loss after early adulthood and all-cause mortality, death from cancer (men only), CVD (men only) and other causes. These findings applied to middle-aged Japanese men and women, regardless of whether they had illnesses, were smokers or were overweight. The HR for all-cause mortality increased with weight loss in each age group and for other causes of death, and was higher in the younger bracket for men and women with weight loss. Further, when subjects were stratified by BMI at baseline or age 20, the association between weight loss and death was the same. On the contrary, weight gain seemed to be protective against mortality in men. These findings remained unchanged after exclusion of the first 5 years of follow-up, which was done to avoid a potential effect of latent diseases. The previous JPHC study reported that both overweight and underweight subjects at baseline had an increased risk of death, representing a U-shaped association. Furthermore, mortality was higher for individuals who were underweight rather than overweight when considered with weight change.[12]

Of interest, weight gain did not predict CVD mortality in the JPHC cohort. Compared with Caucasians, mean BMI is very low in Japanese individuals, leading to a low level of high-sensitivity C-reactive protein,[17] a low grade of atherosclerosis,[18] and one-quarter the mortality from coronary heart disease.[19] Recently, data from this cohort documented that men with high BMIs or with weight gain ≥10 kg who were relatively lean (<21.7 kg/m²) at age 20 were at risk for coronary heart disease,[20] although there were no linear trends between weight gain and risk. Given these previous findings and the significant association between weight loss and death as seen in the present study, it may be hypothesised that obesity-induced atherosclerosis may be rather uncommon in Japan. Instead, we believe that hypertension is an essential factor for atherosclerosis more than other traditional risk factors, as pathological studies have documented.[21 22] These data support our results and suggest two different pathogenic mechanisms of atherosclerosis in Japanese and Caucasians.

The underlying mechanism responsible for the association between weight loss and the risk of death is not fully understood. In general, weight loss is considered to be caused by several physical conditions, such as nutrient deficiency related to liver disease, heavy alcohol drinking, smoking and worsening diabetes. Therefore, even though we adjusted for these confounders in the analysis of multivariable models, the associations still remained. Also, a British study emphasised the effect of smoking on weight change and mortality.[23] In the present study, when subjects were stratified by smoking conditions (current smokers or others), there was an increased risk of death for those with weight loss regardless of smoking habits and no interactions between smoking and weight loss with mortality. People who lost weight may have had underlying health problems or illnesses related to weight loss, although we evaluated several illnesses in this study.

The JPHC study has the advantage of providing large cohorts and assesses the effects of numerous variables on health practices. However, several limitations should be noted. First, we calculated BMIs according to self-reported weight and height values. When analysed in the subgroup in which health check-up data were available, measured BMI almost corresponded to self-reported BMI (r = 0.89 in men and 0.91 in women), as described elsewhere.[12] Second, weight at age 20 was not validated in our study, in spite of the high rank correlation coefficient for self-reported weight; however, a previous study in Japan validated the use of recalled weight for epidemiological studies.[24] Because men with higher BMI tended to underestimate their weights at age 25, a bias for weight change was expected to be large for those individuals. This potential classification bias may weaken the association between weight gain and risk of death among obese people. Third, covariates in our study might not be sufficient to explain the association between weight loss and mortality, because weight change, especially weight loss, is thought to be related to several conditions of illness or an unfavourable lifestyle. Furthermore, we did not exclude people with intentional weight loss in our analysis. Fourth, the specific cause of death was not validated in this study. Validation studies in Japan indicated that diagnoses of death from cancer and stroke were generally correct, but those of death from coronary heart disease were not.[25] Therefore, potential diagnostic bias on death certificates for coronary heart disease was real in the present study.

In conclusion, although there is no doubt that weight gain elevates the risks of atherosclerosis and CVD,[26] we found an inverse relationship in men and an L-shaped association in