Table 4
The estimated serum lipid levels of smokers and nonsmokers from age of 30 years through 70 years at 10 years intervals

Age groups (years)	30		40		50		60		70	
	Nonsmokers	Smokers	Nonsmokers	Smokers	Nonsmokers	Smokers	Nonsmokers	Smokers	Nonsmokers	Smokers
Male										
Total cholesterol (mM)										
Mean	4.83	4.76	5.10	5.02	5.25	5.14	5.28	5.14	5.19	5.00
95%CI	4.80–4.85	4.74–4.78†	5.09–5.11	5.01–5.03†	5.24–5.26	5.13–5.15†	5.26–5.29	5.12–5.15†	5.16–5.22	4.96–5.04†
LDL-cholesterol (mM)										
Mean	2.78	2.73	2.98	2.91	3.10	3.02	3.14	3.04	3.10	2.99
95%CI	2.76–2.80	2.71–2.75*	2.97–2.99	2.90–2.92†	3.09–3.11	3.01–3.03†	3.13–3.15	3.02–3.06†	3.07–3.13	2.95–3.03†
HDL-cholesterol (mM)										
Mean	1.51	1.43	1.53	1.41	1.54	1.40	1.54	1.40	1.52	1.40
95%CI	1.50–1.52	1.42–1.44†	1.53–1.54	1.41–1.42†	1.54–1.54	1.40–1.41†	1.53–1.54	1.39–1.41†	1.51–1.53	1.39–1.42†
Triglyceride (mM)										
Mean	1.19	1.35	1.32	1.60	1.38	1.68	1.36	1.60	1.26	1.34
95%CI	1.16–1.22	1.32–1.38†	1.31–1.34	1.59–1.62†	1.36–1.39	1.67–1.70†	1.34–1.38	1.58–1.62†	1.22–1.30	1.29–1.40*
Female										
Total cholesterol (mM)										
Mean	4.63	4.60	5.06	4.96	5.47	5.35	5.86	5.79	6.23	6.28
95%CI	4.61–4.65	4.56–4.65	5.05–5.07	4.93–4.98†	5.46–5.48	5.32–5.38†	5.85–5.88	5.74–5.85*	6.20–6.27	6.15–6.40
LDL-cholesterol (mM)										
Mean	2.53	2.51	2.88	2.82	3.23	3.16	3.56	3.54	3.88	3.96
95%CI	2.51–2.54	2.47–2.55	2.88–2.89	2.79–2.84†	3.22–3.24	3.13–3.19†	3.55–3.58	3.49–3.59	3.85–3.92	3.85–4.08
HDL-cholesterol (mM)										
Mean	1.72	1.66	1.75	1.67	1.76	1.65	1.75	1.61	1.71	1.55
95%CI	1.71–1.73	1.64–1.68†	1.75–1.76	1.65–1.68†	1.76–1.77	1.64–1.67†	1.74–1.75	1.59–1.64†	1.69–1.72	1.50–1.61†
Triglyceride (mM)										
Mean	0.83	0.97	0.93	1.04	1.06	1.19	1.22	1.41	1.42	1.70
95%CI	0.82–0.84	0.94–1.00†	0.92–0.93	1.03–1.06†	1.05–1.06	1.17–1.21†	1.21–1.23	1.37–1.44†	1.40–1.44	1.62–1.78†

The values were estimated for each age using the least square means methods in the mixed effects model, and were adjusted for the examination year in 1996 and BMI = 22.

* $p < 0.05$ (nonsmoker vs. smoker).

† $p < 0.0001$ (nonsmoker vs. smoker).

from Craig et al. [4], serum cholesterol concentrations were higher in smokers in all (22 studies) but one study. In addition, LDL cholesterol levels were higher in the smoking group by 1.7% from six studies compared with nonsmokers. Although the reason for this discrepancy of the effect of smoking in total and LDL cholesterol is not clear, some ethnic differences including dietary habits, physical activities, or life style as well as differences in public health awareness may have contributed to the inconsistency in observations between us and others. In fact, Halfon et al. found smoking to be associated positively with LDL cholesterol in males of European, but not of African descent [15]. Freedman et al. also reported in their longitudinal observation of early adulthood that although white male and female smokers had a larger increase in LDL cholesterol compared with nonsmokers, in black females smoking was inversely associated with LDL cholesterol [6].

We demonstrated in cross-sectional observation that HDL cholesterol levels were lower and triglyceride levels were higher in female as well as male smokers than in nonsmokers at most of the age groups examined, which was in agreement with other published results [4].

In longitudinal study, we observed apparent differences of smoking effect on serum lipid levels with age, except for HDL cholesterol levels, in which the effect of smoking is rather constant with age. The effect of smoking on the estimated total and LDL cholesterol in both genders is similar to the cross-sectional observation that total and LDL cholesterol decreased in male and female smoker up to elderly age and up to middle age, respectively. However, as shown in Fig. 3, the differences of the estimates of total and LDL cholesterol levels between smokers and nonsmokers based on the longitudinal observation suggest that there is an age effect on the influence of smoking on serum cholesterol concentrations. In addition, this analysis illustrated a gender difference with regard to this effect. In men, smoking is associated with lower total and LDL cholesterol at any given age, although there is an age effect in that the difference becomes larger with age. In women, the effect of smoking is not constant; an inverted influence on total and LDL cholesterol is detected, as in women younger than 60 years, the smoking is associated with lower cholesterol, but after 65 years smoking is associated with higher cholesterol levels. The reason for this remains unknown, although the life style changes or hor-

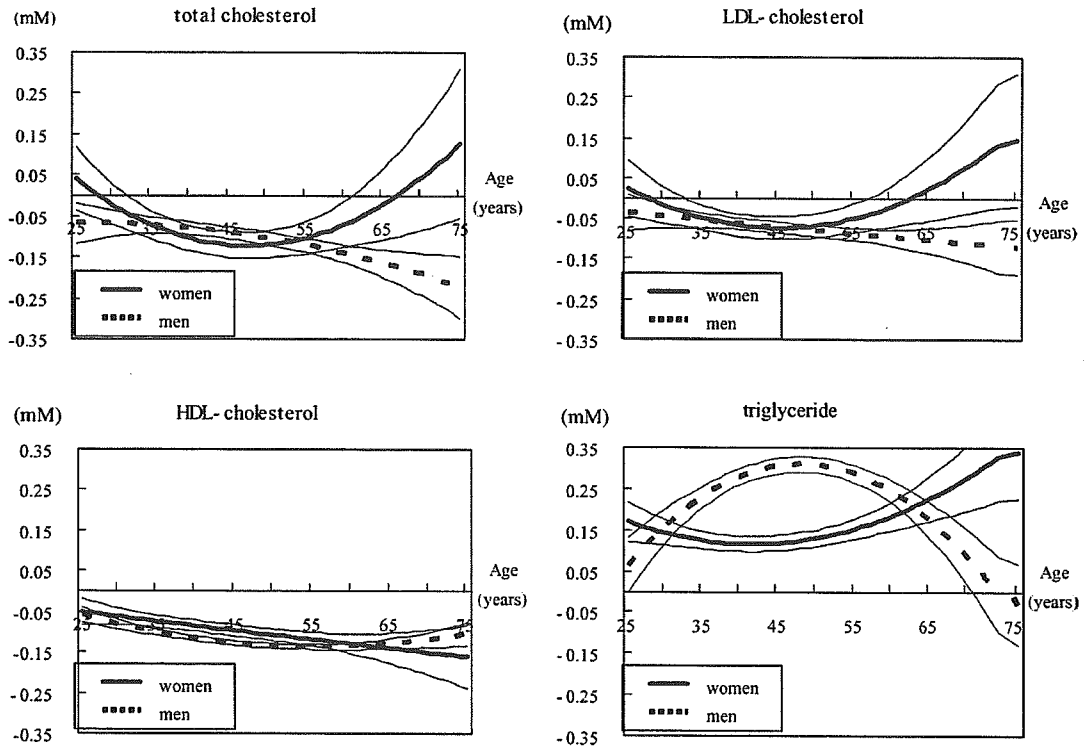

Fig. 3. The difference of estimated lipid levels (the lipid levels of smoker–those of nonsmokers) between current smokers and nonsmokers at individual age from 25 years through 75 years based on the longitudinal analysis. The curves show the average of the difference of estimated lipid levels based on the longitudinal analysis of mixed effect model between smokers and nonsmokers at each age. Thin curves indicate 95% CI.

monal changes in females after menopause might be involved in this inverted effect of smoking.

The effect of smoking on triglyceride levels also exhibits dynamic changes with age and gender difference. Based on longitudinal observation, smoking is associated with higher triglyceride levels at any age examined in both genders. In men, the strongest difference in triglyceride levels between smokers and nonsmokers is seen in middle age, and in women the stronger difference is seen after middle age. The reason for this gender difference and age-dependent effect of smoking on triglyceride levels remains unknown.

It seems that plasma enzymes involved in the metabolism of triglycerides and HDL cholesterol are potentially affected by smoking. However, there are conflicting observations. Some laboratories demonstrated that hepatic lipase is increased in smokers [16], and others demonstrated no difference between smokers and nonsmokers [17], or decreased hepatic lipase in smokers [18]. The hepatic lipase has been shown to be activated in smokers, and lectin:cholesterol acyl transferase activity has been shown to be unchanged [19] or decreased [17] compared with nonsmokers. Plasma cholesterol ester transfer protein activity has been shown to be marginally decreased in smokers in one study [17] and increased in another [19]. Plasma post-heparin lipoprotein lipase activity has been shown not to differ between smokers and nonsmokers in some studies [18,20] and to be increased in smokers in another study [17]. The reasons for these conflicting results on the effect of smoking on plasma enzymes regulating serum lipids and lipoproteins levels are not clear, but it is possible that the effect of smoking on these enzymes is dependent on the gender, age, genetic background, or ethnicity of the subjects.

It should be noted that some selection bias such as healthy worker bias may exist in our study, since most of the subjects were healthy office workers. In addition, the subjects may be aware of their lipid levels, since they had received annual examinations at a health examination center. There is another limitation of this study. Previous observations suggest that the effect of smoking on serum lipid levels is dose-dependent [4,6]. In this study, the data of smoking level in individuals were not available. In addition, alcohol consumption has an effect on serum lipid levels [21]. However, in the present study, the serum lipid levels were not adjusted to account for variations of alcohol consumption.

In the present study, we observed that the effect of smoking on serum lipid levels is age-dependent and that there is a gender difference based on the cross-sectional as well as longitudinal analysis. In men, smoking is associated with lower total and LDL cholesterol at any given age between 25 and 75 years. In women younger than 60 years, smoking is associated with lower cholesterol, but after 60–65 years smoking is associated with higher cholesterol levels. HDL cholesterol levels were lower in male and female smokers than in nonsmokers at most of the age groups examined. Smoking is

associated with higher triglyceride levels in any age examined in both genders except in males above 70 years. In men, the greatest difference in triglyceride levels between smokers and nonsmokers is seen in middle age, and in women, the greatest difference is seen after middle age.

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ORIGINAL ARTICLE

Preproghrelin Leu72Met variant contributes to overweight in middle-aged men of a Japanese large cohort

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Objective: To investigate whether Leu72Met polymorphism of the preproghrelin gene is associated with overweight/obesity in middle-aged and older Japanese.
Design: Cross-sectional analysis.
Subjects: A total of 2238 community-dwelling middle-aged and older Japanese people (age: 40–79 years) who participated in the first wave of examinations in the National Institute for Longevity Sciences – Longitudinal Study of Aging from April 1998 to March 2000.
Measurements: The Leu72Met polymorphism of prepoghrelin gene, anthropometric variables including body weight, body mass index (BMI), waist circumference, waist-to-hip ratio and whole-fat mass and biochemical variables including serum lipid levels, fasting plasma glucose, insulin and homeostasis model assessment for insulin resistance.
Results: The frequencies of the Leu72Leu, Leu72Met and Met72Met alleles were 63.4, 32.7 and 4.0%, respectively. No differences in the genotype distributions of the Leu72Met polymorphism were found between genders or age groups, and no significant associations were observed between polymorphism and anthropometric variables in women and older men. However, middle-aged men who were 72Met allele carriers showed a higher body weight change from body weight at 18 years of age, as well as a higher waist circumference and a tendency to a higher waist-hip-ratio than noncarriers. Although there were no significant differences in the genotype distribution according to BMI in women and older men, a significantly higher frequency of the 72Met allele was found in the higher BMI group (BMI$\geq$25 kg/m^2) of middle-aged men than in the normal-weight group. No significant associations were observed between polymorphism and serum lipid, glucose or insulin levels.
Conclusions: These results suggest that the 72Met allele of the preproghrelin gene is a contributing factor for midlife weight change in men.
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Keywords: ghrelin; polymorphism; preproghrelin; lipid metabolism; glucose metabolism

Introduction

Ghrelin has been shown to be the natural ligand of the previously identified 'orphan' growth hormone secretagogue receptor.[1] Although widely expressed in many tissues, ghrelin is most abundantly produced by the stomach.[1] Ghrelin is much more than a mere natural growth hormone secretagogue, however: it has been found to have profound growth hormone-independent weight- and appetite-increasing effects.[2] Ghrelin stimulates food intake in both rodents and humans,[2,3] and is strongly involved in the regulation of energy homeostasis.[4] This suggests that derangement in the ghrelin system could play a role in obesity. In addition, ghrelin may affect carbohydrate and lipid metabolisms.[5,6]

Recently, three major polymorphisms in the human ghrelin gene were described.[7] One of these nucleotide changes, a single-base substitution C214A with Met replacing Leu at codon 72 in the preproghrelin amino-acid sequence, seems to be associated with an earlier onset of obesity,[7–9] but it has also been proposed that 72Met could provide protection against the accumulation of fat.[10] Thus, previous studies on preproghrelin genetic variants have arrived at contradictory findings as to their role in obesity. Additionally, most studies have had only child and adoles-

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cent subjects, whereas few studies have targeted the middle aged or elderly, or randomly sampled community-dwelling individuals.

The aim of the present study was to test whether genetic variants in the preproghrelin gene (Leu72Met) could play a role in predisposing carriers to overweight/obesity or be associated with anthropometric data, serum lipid levels and values related with glucose metabolisms in a middle-aged to elderly Japanese population.

Materials and methods

Subjects

The present study consisted of a cross-sectional analysis of 1110 women and 1128 men who participated in the first wave of examinations in the National Institute for Longevity Sciences – Longitudinal Study of Aging (NILS-LSA) from April 1998 to March 2000. The subjects of the NILS-LSA were male and female residents 40–79 years old. The population of Obu city and Higashiura town in the Aichi prefecture in central Japan was stratified by both age and gender, and randomly selected from resident registrations in cooperation with the local governments. The number of male and female participants was to be the same to test gender difference. Age at the base line is to be 40–79 years and the number of participants in each decade (1940s, 1950s, 1960s, 1970s) is to be the same. The examinations include various areas of gerontology and geriatrics such as medical examinations, anthropometry, body composition, physical functions, physical activities, psychological assessments, nutritional analysis and molecular epidemiology. The subjects will be followed up every 2 years. The details of the NILS-LSA have been described elsewhere.[11] Randomly selected men and women were invited by mail to attend an explanatory meeting. At that meeting, the procedures for each examination and the follow-up schedule were fully explained. Written, informed consent to the entire procedure was obtained from each participant. The study was approved by the Ethics Committee of the National Institute for Longevity Sciences.

Anthropometric variables

Body weight was measured to the nearest 0.01 kg using a digital scale, height was measured to the nearest 0.1 cm using a wall-mounted stadiometer and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference and waist-to-hip ratio (WHR) were used as the indices for body fat distribution in this study. Waist-to-hip ratio was calculated as the ratio of waist circumference measured at the mid-point between the anterior superior iliac crest and the lowest rib-to-hip circumference. Whole-body fat mass, assessed by dual-energy X-ray absorptiometry (QDR-4500; Hologic, Madison, OH, USA), was used as an index for determining body composition. The subjects' weight at 18 years of age was obtained by questionnaire. Weight change was defined as the current weight minus the weight at 18 years of age.

Biochemical assays of blood

An antecubital blood sample was drawn from each subject after an overnight fast. Serum total cholesterol, triglycerides and low-density lipoprotein cholesterol were determined enzymatically, serum high-density lipoprotein cholesterol was measured by the heparin–manganese precipitation method and fasting plasma glucose was assayed by the glucose oxidase method. Lipoprotein (a) was measured in plasma using a commercially available ELISA. Plasma insulin was measured by radioimmunoassay. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5.[12]

Determination of preproghrelin genotypes

Genotypes were determined using a fluorescence-based allele-specific DNA primer assay system (Toyobo Tsuruga Gene Analysis, Tsuruga, Japan). The polymorphic regions (Leu72Met (C214A)) of preproghrelin were amplified by polymerase chain reaction with allele-specific sense primers labeled at the 5′-end with either fluorescein isothiocyanate (5′-CCG ACC CGG ACT TCC XTT-3′) or Texas red (5′-GTA CCG ACC CGG ACT TCC XG-3′) and with an antisense primer labeled at the 5′-end with biotin (5′-GGC TCC GCC CGG AAG ATG-3′). The reaction mixtures (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 mmol/l $MgCl_2$ and 1 U of rTaq DNA polymerase (Toyobo Co., Ltd, Osaká, Japan) in polymerase buffer. The amplification protocol consisted of initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 68°C for 30 s; a final extension was conducted at 68°C for 2 min. Further details are provided elsewhere.[13]

Data analysis

Quantitative data were compared among three groups by one-way analysis of variance and the Tukey–Kramer *post hoc* test, and between two groups by the unpaired Student's *t*-test. Allele frequencies were estimated by the gene-counting method, and the χ^2 test was used to identify any significant departure from Hardy–Weinberg equilibrium. In the analyses to examine the association between genotypes and lipid or glucose metabolisms, participants who were being treated with lipid-lowering medications or oral hypoglycemic agents or insulin were excluded. Unless indicated otherwise, a *P*-value of <0.05 was considered to be statistically significant. The data were analyzed with the Statistical Analysis System (SAS), release 8.2.

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Results

Genotype frequencies for the preproghrelin Leu72Met polymorphism were CC (Leu72Leu) 0.634, CA (Leu72Met) 0.327 and AA (Met72Met) 0.04. These frequencies are consistent with those expected under Hardy–Weinberg equilibrium. There were no significant differences in the genotype distributions of preproghrelin Leu72Met polymorphism between men and women, or among the different age groups (Table 1).

As shown in Table 2, although there were no differences in current body weight and body weight at 18 years of age between genotypes, middle-aged men who were 72Met allele carriers showed both a higher body weight change from body weight at 18 years of age ($P=0.013$, CC vs CA/AA) and higher waist circumference ($P=0.038$, CC vs CA/AA) than noncarriers. Among the middle-aged men in the present study, the Leu72Leu genotype was associated with the lowest BMI (trend, $P=0.049$), and the 72Met allele carriers tended to have a higher WHR ($P=0.062$, CC vs CA/AA) than subjects with the Leu72Leu genotype. However, no differences in anthropometric measurements among Leu72Met genotypes were observed in older men, or in female cohorts (Table 3).

In order to assess the association of the Leu72Met polymorphism with overweight or obesity, genotype and allele frequencies were compared among normal-weight ($BMI<25\,kg/m^2$) and overweight/obese ($BMI\geqslant25\,kg/m^2$) groups (Table 4). Although there were no significant differences in the genotype distribution according to BMI in women and older men, a significantly higher frequency of CA, AA or CA/AA was found in the higher BMI group than in the normal-weight group among middle-aged men.

No significant association was observed between the three genotypes and serum lipid, fasting glucose, insulin, HbA1c or HOMA-IR levels in men and women (Table 5). The preproghrelin Leu72Met genotypes showed similar allele frequencies in diabetic individuals and in non-diabetic controls (data not shown).

Table 1 Distribution of Leu72Met genotype of preproghrelin gene of the subjects

	n	CC		CA		AA		CA/AA	
		n	%	n	%	n	%	n	%
Total	2228	1412	63.4	728	32.7	88	4.0	816	36.6
Men*†	1121	709	63.3	371	33.1	41	3.7	412	36.8
Women	1107	703	63.5	357	32.3	47	4.3	404	36.5
Age (years)†§									
40–49	562	364	64.8	177	31.5	21	3.7	198	35.2
50–59	556	357	64.2	177	31.8	22	4.0	199	35.8
60–69	560	359	64.1	180	32.1	21	3.8	201	35.9
70–79	550	332	60.4	194	35.3	24	4.4	218	39.6

*CC, CA, AA, men vs women, $\chi^2=0.6159$, $P=0.7350$; †CC, CA/AA, men vs women, $\chi^2=0.0160$, $P=0.8995$; †CC, CA, AA, age groups, $\chi^2=2.9716$, $P=0.8124$; §CC, CA/AA, age groups, $\chi^2=2.9149$, $P=0.4049$.

Table 2 Anthropometric variable of men according to age group and Leu72Met polymorphism of preproghrelin gene

	Middle aged (n = 563)								*Older (n = 556)*							
	CC		*CA*		*AA*		*CA/AA*		*CC*		*CA*		*AA*		*CA/AA*	
	Mean	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*
Weight (kg)	64.6	0.5	65.6	0.7	67.5	2.0	65.8	0.6	59.7	0.5	59.1	0.6	57.2	1.9	58.9	0.6
Weight at 18 years (kg)	56.9	0.4	56.4	0.5	57.2	1.5	56.5	0.5	55.3	0.4	54.5	0.5	55.4	1.5	54.6	0.5
Weight change from 18 years (kg)	7.7	0.4	9.2	0.6	10.3	1.7	9.3	0.5*	4.6	0.5	4.9	0.7	1.8	2.1	4.6	0.7
Height (cm)	167.0	0.3	167.2	0.4	166.3	1.4	167.2	0.4	162.0	0.3	161.9	0.4	161.7	1.2	161.9	0.4
BMI (kg/m^2)	23.1	0.1	23.4	0.2	24.4	0.6	23.5	0.2†	22.7	0.2	22.5	0.2	21.9	0.6	22.4	0.2
Waist circumference (cm)	82.2	0.4	83.4	0.6	84.9	1.8	83.6	0.6‡	82.4	0.5	82.1	0.6	80.5	1.9	81.9	0.6
Hip circumference (cm)	92.2	0.3	92.7	0.4	93.4	1.1	92.8	0.3	90.0	0.3	89.6	0.4	88.7	1.1	89.5	0.3
Waist-hip-ratio	0.891	0.003	0.899	0.004	0.907	0.012	0.899	0.004§	0.913	0.003	0.914	0.005	0.904	0.014	0.913	0.004
Fat mass (kg)	20.6	0.2	21.2	0.3	20.8	1.0	21.2	0.3	21.9	0.2	22.0	0.3	22.1	0.9	22.0	0.3

Except for *, †, ‡ and §, no significant trends and differences were detected among three groups (CC, CA and AA) and between two groups (CC and CA/AA). *$P=0.013$ (CC vs CA/AA); †$P=0.049$ (trend); ‡$P=0.038$ (CC vs CA/AA); §$P=0.062$ (CC vs CA/AA). Analysis of variance and the Tukey–Kramer *post hoc* test or the unpaired Student's *t*-test between two groups. BMI = Body mass index.

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Table 3 Anthropometric variable of women according to age group and Leu72Met polymorphism of preproghrelin gene

	Middle aged (n = 553)								*Older (n = 552)*							
	CC		*CA*		*AA*		*CA/AA*		*CC*		*CA*		*AA*		*CA/AA*	
	Mean	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*
Weight (kg)	53.9	0.4	53.9	0.6	54.4	1.6	54.0	0.6	50.8	0.4	50.9	0.6	52.4	1.7	51.1	0.6
Weight at 18 years (kg)	48.8	0.3	49.1	0.4	49.2	1.2	49.1	0.4	47.9	0.4	47.7	0.5	49.2	1.4	47.8	0.5
Weight change from 18 years (kg)	5.2	0.4	4.8	0.6	5.2	1.6	4.9	0.5	3.0	0.5	3.2	0.7	3.1	1.9	3.2	0.7
Height (cm)	154.0	0.3	154.1	0.4	154.4	1.0	154.1	0.3	148.5	0.3	148.6	0.4	147.9	1.2	148.5	0.4
BMI (kg/m^2)	22.7	0.2	22.7	0.2	22.8	0.7	22.7	0.2	23.0	0.2	23.0	0.2	24.1	0.7	23.2	0.2
Waist circumference (cm)	73.5	0.5	73.4	0.6	73.4	1.7	73.4	0.6	76.4	0.5	77.5	0.7	77.8	2.0	77.5	0.7
Hip circumference (cm)	91.5	0.3	91.5	0.4	90.9	1.1	91.4	0.4	89.8	0.3	89.9	0.4	90.8	1.2	90.0	0.4
Waist-hip-ratio	0.802	0.003	0.801	0.005	0.806	0.012	0.802	0.004	0.849	0.004	0.860	0.005	0.855	0.014	0.860	0.005
Fat mass (kg)	30.7	0.3	30.3	0.4	30.5	1.0	30.3	0.3	32.3	0.3	32.7	0.4	33.3	1.1	32.7	0.4

No significant trends and differences were detected among three groups (CC, CA and AA) and between two groups (CC and CA/AA). Analysis of variance and the Tukey–Kramer *post hoc* test or the unpaired Student's *t*-test between two groups. BMI = Body mass index.

Table 4 Distribution of Let72Met genotype of preproghrelin gene

	n	*CC*		*CA*		*AA*		*CA/AA*		P	P*
		n	%	n	%	n	%	n	%		
All age groups											
Men											
BMI < 25 kg/m^2	854	546	63.9	280	32.8	28	3.3	308	36.1	0.411	0.393
BMI > 25 kg/m^2	267	163	61.1	91	34.1	13	4.9	104	39.0		
Women											
BMI < 25 kg/m^2	866	558	64.4	273	31.5	35	4.0	308	35.6	0.454	0.224
BMI > 25 kg/m^2	241	145	60.2	84	34.9	12	5.0	96	39.8		
Middle ages (younger than 60 years)											
Men											
BMI < 25 kg/m^2	413	280	67.8	123	29.8	10	2.4	133	32.2	0.032	0.036
BMI > 25 kg/m^2	151	88	58.3	54	35.8	9	6.0	63	41.7		
Women											
BMI < 25 kg/m^2	446	288	64.6	139	31.2	19	4.3	158	35.4	0.694	0.395
BMI > 25 kg/m^2	108	65	60.2	38	35.2	5	4.6	43	39.8		
Older (60 years or older)											
Men											
BMI < 25 kg/m^2	441	266	60.3	157	35.6	18	4.1	175	39.7	0.692	0.394
BMI > 25 kg/m^2	116	75	64.7	37	31.9	4	3.5	41	35.3		
Women											
BMI < 25 kg/m^2	420	270	64.3	134	31.9	16	3.8	150	35.7	0.604	0.389
BMI > 25 kg/m^2	133	80	60.2	46	34.6	7	5.3	53	39.9		

P-value by the χ^2 analysis among groups CC, CA and AA. *P**-value by the χ^2 analysis between groups CC and CA/AA. BMI = Body mass index.

Discussion

We observed that the frequency of the 72Met allele of the present cohort was 36.6%. It has been demonstrated that the frequency of the 72Met allele of the preproghrelin gene is approximately 8% in the Caucasian population and approximately 2% in the black population in three different cohorts.[10] Compared with these previous studies, the frequency of the 72Met allele in our Japanese cohort was much higher than that observed in Caucasian or African populations, probably reflecting genetic/ethnic heterogeneity.

The Leu72Met polymorphism of preproghrelin was previously found in a group of obese French children and adolescents.[9] In this case, a significant association was observed between the 72Met allele and earlier age of onset of obesity. Additionally, obese Italian children and adoles-

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Table 5 Metabolic variables and Leu72Met polymorphism of preproghrelin gene

	Men									*Women*								
	n	*CC*		*CA*		*AA*		*CA/AA*		n	*CC*		*CA*		*AA*		*CA/AA*	
		Mean	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*		*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*
Total cholesterol (mM)[a]	1044	5.48	0.03	5.49	0.05	5.42	0.14	5.48	0.04	996	5.83	0.03	5.92	0.05	5.83	0.14	5.91	0.05
Triglyceride (mM)[a]	1027	1.48	0.04	1.53	0.06	1.32	0.16	1.51	0.10	977	1.20	0.03	1.23	0.04	1.21	0.10	1.23	0.04
HDL-C (mM)[a]	1044	1.49	0.01	1.48	0.02	1.49	0.06	1.48	0.02	996	1.71	0.02	1.71	0.02	1.71	0.06	1.71	0.02
LDL-C (mM)[a]	1035	3.40	0.03	3.42	0.05	3.36	0.13	3.42	0.04	980	3.57	0.03	3.63	0.05	3.62	0.14	3.63	0.05
Lipoprotein (a) (mM)[a]	1034	0.39	0.02	0.37	0.03	0.35	0.07	0.37	0.02	980	0.40	0.02	0.46	0.03	0.33	0.07	0.44	0.02
Glucose (mM)[b]	1049	5.71	0.04	5.74	0.05	5.91	0.15	5.75	0.05	1051	5.51	0.03	5.52	0.05	5.20	0.13	5.49	0.04
Insulin (μU/ml)[b]	1048	8.28	0.22	8.21	0.31	7.63	0.91	8.15	0.29	1050	8.23	0.19	8.57	0.27	8.02	0.74	8.51	0.25
HbA1c (%)[b]	1064	5.21	0.02	5.26	0.03	5.41	0.10	5.28	0.03	1071	5.16	0.02	5.15	0.03	5.06	0.07	5.14	0.02
HOMA-IR[b]	1048	2.20	0.08	2.13	0.11	2.06	0.33	2.13	0.11	1050	2.07	0.06	2.20	0.09	1.88	0.26	2.16	0.09

[a]Analysis of subjects who were not under lipid treatment. Adjusted for age. [b]Analysis of subjects who were not on oral hypoglycemic agents or insulin. Adjusted for age. No significant differences were observed in any metabolic values among three different genotypes (CC, CA and AA) or between CC and CA/AA. Analysis of variance and the Tukey–Kramer *post hoc* test or the unpaired Student's *t*-test between two groups. Abbreviations: HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; LDL, low-density lipoprotein.

cents with the preproghrelin 72Met allele have also been reported to become obese earlier than homozygous patients for the wild Leu72 allele, even though 72Met allelic frequency was similar between obese and control groups.[8] These findings were not confirmed, however, in a group of extremely obese German children.[14] In addition, one report suggests that preproghrelin 72Met carrier status may be protective against fat accumulation.[10] A limited number of observations have been made on the relationship between preproghrelin Leu72Met polymorphism and overweight/obesity in middle-aged subjects, and no report has been published to date on older subjects. In a Swedish middle-aged female obese cohort, no difference of 72Met allele frequency was observed between obese subjects and controls.[7] However, the self-reported age of onset of weight problems tended to be lower among 72Met allele carrier obese subjects than among those without this allele.

In the present study, we observed a significant effect of the preproghrelin variant on overweight/obesity only in middle-aged men, as the 72Met allele was more commonly observed among overweight/obese middle-aged men. We also demonstrated that body weight change from weight at 18 years of age is associated with Leu72Met variants, given that middle-aged men with the 72Met allele had a greater body weight change than Leu72 homologous subjects. Similar trends were also observed for BMI, waist circumference and WHR in middle-aged men, but not in older men or in women when our population was subdivided into three subgroups according to preproghrelin genotype. Consequently, the 72Met allele may contribute to body weight change from adolescence to middle age in men but not in women. We observed the absence of the effect of Leu72Met genotypes on the anthropometric measurements in older man. Although we do not know the exact reasons, the effects of aging or environmental influences may overcome the genetic influence on the anthropometric measurements. The limitation of our study is that the weight at 18 years was recalled by the participants in the present study, as the documented measurements of weight at 18 years of age were not available. Although several studies have observed that adults are able to recall their earlier weights fairly accurately,[15] it is possible that the reported weight might not be accurate or under-reported. In fact, it has been reported that overweight women underestimated their earlier weights and that lean men overestimated their earlier weight.[16]

Based on recent studies, it appears that ghrelin may play a role in the glucose and lipid metabolisms. However, only limited data are currently available with regard to the effect of ghrelin polymorphism on these metabolisms. It has been reported that Leu72Met polymorphism is associated with triglyceride or lipoprotein (a) levels.[10,17] In the present study, however, we observed no association between serum lipid levels, fasting glucose, insulin, HbA1c or HOMA-IR levels and preproghrelin Leu72Met genotypes.

In the present study of a community-dwelling Japanese middle-aged to elderly cohort, we demonstrated that the 72Met allele of the preproghrelin gene is a contributing factor for midlife weight change in men but not in women or elderly men. However, Leu72Met polymorphism was not found to be associated with the metabolic variables studied.

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Underuse of Medications for Chronic Diseases in the Oldest of Community-Dwelling Older Frail Japanese

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OBJECTIVES: To test the following hypotheses: (1) the rate of polypharmacy, defined as six or more prescribing medications, is lower in the oldest old (≥85) than in younger older people (65–84); (2) beneficial medication use is lower in the oldest old; (3) the underuse of these medications in the oldest old is associated with physical or cognitive impairment or comorbid conditions.

DESIGN: A cross-sectional study of the baseline data from the Nagoya Longitudinal Study for Frail Elderly.

SETTING: Community-based.

PARTICIPANTS: One thousand eight hundred seventy-five community-dwelling older people (632 men, 1,243 women).

MEASUREMENTS: The data, which were collected at the patients' homes or from care-managing center records, included the clients' demographic characteristics, depression status as assessed using the short version of the Geriatric Depression Scale, a rating for basic activities of daily living (ADLs), prescribed medications, and physician-diagnosed chronic diseases.

RESULTS: The oldest old had less polypharmacy even after controlling for ADLs and comorbid conditions. The underuse of beneficial medications for the oldest old was observed after adjusting for ADLs, cognitive impairment, comorbid conditions, antithrombotic agents for subjects with a history of cardiovascular diseases, acetylcholinesterase inhibitors for those with dementia, and antidepressants for those with depression. However, being aged 85 and older was not associated with the underuse of hypoglycemic and antihypertensive agents by those with diabetes mellitus and hypertension, respectively.

CONCLUSION: Among community-dwelling frail older people, the rate of polypharmacy is lower in the oldest members than in the younger ones. The underuse of prescribed medications for chronic diseases/conditions of frail older people is common but not for all conditions. J Am Geriatr Soc 2006.

Key words: polypharmacy; undertreatment; elderly

It has been reported that the underuse of medications, defined as the omission of drug therapy that is indicated for the treatment or prevention of a disease or condition, is an important and increasingly recognized problem in older people.[1,2] The underprescribing of drugs seems to have a negative effect on health outcomes for older people,[3,4] but apart from concern about the risks of the excess prescribing of inappropriate or unnecessary drug therapy for older people,[5,6] there is still insufficient knowledge about the adverse consequences associated with the underprescribing of beneficial drug therapies. It is not known whether all kinds of medications are underused in older people or whether specific medications for specific chronic diseases or conditions are selectively underused in older people. In addition, knowledge about the factors that influence the underuse of medications for the common chronic diseases of older people is sparse. There is also a lack of knowledge about how functional and psychological factors influence the use of medication by physicians or how frailty and comorbidity affect drug use by older people.

The national policy in not only Japan but also Western countries is to enable elderly people to retain their independence as long as possible, to have a high quality of life, and to continue living at home as long as they can. It is essential to prevent frail older people from suffering from recurrent diseases and additional illnesses that would require them to receive care in an acute setting or to be admitted to a nursing home or to cause mortality. Therefore, preventive medication for chronic diseases/conditions is important for frail older people living in a community setting.

In the present study targeting frail, community-dwelling elderly persons (≥65), the following hypotheses were tested: (1) the rate of multiple medication use is lower in the oldest people (≥85) than in younger ones (65–84); (2) beneficial medication use for common chronic conditions such as cardiovascular disease (CVD), dementia, depression,

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diabetes mellitus, and hypertension is lower in the oldest people than in younger ones; and (3) the underuse of these medications in the oldest old is associated with physical impairment, cognitive impairment, or comorbid conditions.

METHODS

Study Design and Subjects

The present study consisted of a cross-sectional analysis of 1,875 elderly persons (632 men, 1,243 women) who participated in the Nagoya Longitudinal Study for Frail Elderly (NLS-FE). The study population was community-dwelling older people (≥65) eligible for long-term care insurance (LTCI) who lived in Nagoya City, Japan, and were provided various home care services from the Nagoya City Health Care Service Foundation for Older People, which has 17 visiting nursing stations associated with care-managing centers. Japan introduced a universal-coverage LTCI program in April 2000[7,8] that covers care for people aged 65 and older and people aged 40 and older with 15 specific diseases such as cerebrovascular disease and presenile dementia. Under the LTCI program, care levels (Level 0 to Level 5) are determined according to eligibility criteria. Older people in the community who are eligible for LTCI are frail and chronically ill, have physical and mental problems, and are easy to admit to an acute hospital or institute setting. During the registration period for the NLS-FE (November 1, 2003, to December 31, 2003), 1,875 of 3,630 elderly clients agreed to participate in this study. The NLS-FE participants were scheduled to undergo comprehensive in-home assessments at baseline and 6, 12, and 24 months by trained nurses. In the present study, the cross-sectional data from the baseline assessment were used. Informed consent for participation was obtained verbally from the patients or, for those with substantial cognitive impairment, from a surrogate (usually the closest relative or legal guardian), as well as from caregivers, according to procedures approved by the institutional review board of Nagoya University Graduate School of Medicine.

Data Collection

Three hundred twenty-eight nurses visited the clients' homes and collected the data using standardized interviews with patients or surrogates and caregivers and from care-managing center records. The data included clients' demographic characteristics, depressive symptoms as assessed using the short version of the Geriatric Depression Scale (GDS-15),[9] and a rating for seven basic activities of daily living (ADLs) (feeding, bathing, grooming, dressing, toileting, walking, and transferring), with summary scores ranging from 0 (total disability) to 20 (no disability).[10]

Information obtained from care-managing center records included the following physician-diagnosed chronic conditions: ischemic heart disease, congestive heart failure, liver diseases, cerebrovascular disease, diabetes mellitus, dementia, chronic obstructive pulmonary disease, renal disease, cancer, hypertension, pressure ulcer, depression, and diseases constituting the Charlson Comorbidity Index,[11] which represent the sum of a weighted index that takes into account the number and seriousness of preexisting comorbid conditions. In the present study, only a limited number of subjects diagnosed for depression by a physician according to the care-managing center records were observed. Therefore, the participants were considered to be depressed if their GDS-15 score was 6 or higher.

The data also included the number of prescribed medications and their corresponding therapeutic classes, including antihypertensive drugs, antiplatelets, anticoagulants, antipsychotic medications (including antidepressants), hypoglycemics, nonsteroidal antiinflammatory drugs and acetaminophen, anti-Alzheimer's disease drugs (acetylcholinesterase inhibitors), gastrointestinal medications, and insulin. The information about regular prescribed medications was recorded in interviews with patients and caregivers and taken from prescription records and classified by nurses using standard instruments. Clients eligible for LTCI have their own primary care physicians, who submit a report on their clinical status every 6 months.

Statistical Analysis

Analysis of variance with a Bonferroni correction for multiple comparisons was used to determine differences between age groups (65–74, 75–84, and ≥85) for continuous variables, and the Kruskal-Wallis test was used to test categorical variables. The chi-square test was used to compare the presence of chronic diseases/conditions or the number of prescription medications used between age groups. Univariate and multivariate logistic regression was used to determine which characteristics of older people predicted multiple medication use or the underuse of beneficial medication. For the logistic regression analysis, the ADL score (range 0–20) was categorized into three groups with approximately equal number of participants in each group: high function (≥18), mid function (12–17), and low function (≤11). The number of prescribed medications was also categorized into four groups (0, 1–2, 3–5, and ≥6). All analyses were performed using SPSS version 11.0 (SPSS, Inc., Chicago, IL).

RESULTS

Table 1 shows the characteristics of the participants according to age group. ADL score was lowest in the oldest old (≥85). The prevalence of a history of coronary heart disease, hypertension, and dementia increased, and the prevalence of diabetes mellitus decreased, with age. Polypharmacy, defined as six or more prescribed medications, decreased with age. To identify the factors influencing polypharmacy in frail older people in the community, logistic regression analysis was conducted (Table 2). Participants with congestive heart failure (odds ratio (OR) = 1.66, 95% confidence interval (CI) = 1.09–2.55), coronary heart disease (OR = 3.05, 95% CI = 2.16–4.31), and diabetes mellitus (OR = 1.51, 95% CI = 1.06–2.15) were more likely to be receiving multiple medications according to multivariate analysis. In contrast, participants with dementia were less likely to have been prescribed multiple medications (OR = 0.64, 95% CI = 0.48–0.84). The oldest old had less polypharmacy using univariate analysis (OR = 0.64, 95% CI = 0.49–0.82) and multivariate analysis (OR = 0.55, 95% CI = 0.39–0.77) controlled for sex, ADL dependency, and the presence of common chronic diseases.

Table 1. Characteristics of Community-Dwelling Frail Older People Stratified by Age

Characteristic	Total (N = 1,875)	Age 65–74 (n = 433)	Age 75–84 (n = 827)	Age ≥85 (n = 615)	*P*-value
Men/women (% of men/total)	632/1,243 (33.7)	191/242 (44.1)	275/552 (33.3)	166/449 (27.0)	<.001
Age, mean ± SD*	80.6 ± 7.7	70.5 ± 2.7	79.4 ± 2.8	89.3 ± 3.6	<.001
Activity of daily living score, mean ± SD (range 0–20)†	12.8 ± 6.6	12.6 ± 6.8	13.6 ± 6.3	11.8 ± 6.7	<.001‡
Charlson Comorbidity Index, mean ± SD†	2.0 ± 1.6	2.2 ± 1.7	1.9 ± 1.5	2.0 ± 1.5	.003§
GDS-15 score, mean ± SD (range 0–15)†	6.6 ± 3.6	6.8 ± 3.8	6.5 ± 3.6	6.5 ± 3.6	.38
Chronic diseases (% of total)					
Congestive heart failure	8.5	1.8	6.5	15.7	<.001
Coronary heart disease	12.2	7.0	12.5	15.2	<.001
Cerebrovascular disease	34.3	40.1	32.4	33.1	.03
Diabetes mellitus	12.0	16.1	12.3	8.9	.003
Dementia	34.4	24.8	31.0	45.7	<.001
Hypertension	24.3	19.4	24.5	27.3	.01
Depression (GDS-15 score ≥6)	57.2	58.4	56.7	57.1	.27
Cancer	9.1	9.1	8.8	9.6	.90
Use of medications (% of total)					
0	5.1	3.3	3.3	8.9	<.001
1–2	16.8	14.0	15.7	20.3	
3–5	41.9	41.9	43.2	40.2	
≥6	36.2	40.9	37.8	30.6	

* Analysis of variance or † Kruskal-Wallis was used for analysis; chi-square test was used for others.
‡ Aged 65–74 vs 75–84, $P = .007$; 65–74 vs ≥85, $P = .003$; 75–84 vs ≥85, $P < .001$.
§ Aged 65–74 vs 75–84, $P < .001$; 65–74 vs ≥85, $P = .02$; 75–84 vs ≥85, $P = .08$.
SD = standard deviation; GDS-15 = 15-item Geriatric Depression Scale.

Logistic regressions were conducted to evaluate the extent to which age group and the characteristics of older people were independent predictors of being prescribed essential medications. Univariate analysis showed the rates of prescription of antithrombotic agents (antiplatelet or warfarin), acetylcholinesterase inhibitors, and antidepressants in older people with a history of CVD (including coronary heart disease and stroke), dementia, and depressive symptoms, respectively, declined substantially with age (Table 3), but in participants with diabetes mellitus or hypertension, age did not influence hypoglycemic (oral hypoglycemic drugs or insulin) or antihypertension use. Being female was associated with the underuse of antithrombotic agents in older people with a history of CVD (male: OR = 1.80, 95%

Table 2. Logistic Regression Analysis for Polypharmacy

Characteristic	Univariate Odds Ratio (95% Confidence Interval)	Multivariate Odds Ratio (95% Confidence Interval)
Age (reference: 65–74)		
75–84	0.88 (0.69–1.11)	0.71 (0.53–0.95)
≥85	0.64 (0.49–0.82)	0.55 (0.39–0.77)
Male (reference: female)	1.26 (1.03–1.53)	1.05 (0.82–1.35)
Activity of daily living score (range 0–20) (reference: high function (≥18))		
Mid function (12–17)	1.26 (0.99–1.59)	1.35 (1.03–1.79)
Low function (≤11)	0.94 (0.74–1.19)	1.38 (0.99–1.89)
Presence of chronic diseases (reference: absence)		
Congestive heart failure	1.91 (1.36–2.68)	1.66 (1.09–2.55)
Coronary heart disease	2.65 (1.98–3.54)	3.05 (2.16–4.31)
Cerebrovascular disease	0.96 (0.78–1.18)	1.10 (0.84–1.43)
Dementia	0.59 (0.47–0.73)	0.64 (0.48–0.84)
Diabetes mellitus	1.59 (1.19–2.13)	1.51 (1.06–2.15)
Depression (Geriatric Depression Scale-15 score ≥6)	1.27 (1.02–1.58)	1.26 (0.99–1.59)
Hypertension	0.87 (0.69–1.08)	0.82 (0.62–1.08)

Table 3. Univariate Analysis of Characteristics Associated with Participants Receiving Medication

Characteristic	Antithrombotic Agent Use for History of CVD		Acetylcholinesterase Inhibitor Use for Dementia		Antidepressant Use for Depression		Hypoglycemic Use for Diabetes Mellitus		Antihypertensive Use for Hypertension	
	n, Odds Ratio (95% Confidence Interval)									
Age										
65–74	163,	1.00 —	83,	1.00 —	173,	1.00 —	60,	1.00 —	83,	1.00 —
75–84	290,	0.75 (0.51–1.10)	205,	1.00 (0.51–1.94)	348,	0.68 (0.34–1.35)	94,	1.56 (0.73–3.32)	196,	2.05 (0.99–4.27)
≥85	219,	0.48 (0.32–0.73)	202,	0.44 (0.21–0.93)	220,	0.40 (0.16–0.96)	48,	0.55 (0.25–1.26)	155,	1.92 (0.89–4.11)
Sex										
Female	391,	1.00 —	336,	1.00 —	465,	1.00 —	129,	1.00 —	302,	1.00 —
Male	281,	1.80 (1.32–2.46)	154,	0.79 (0.45–1.38)	276,	0.69 (0.36–1.35)	73,	0.75 (0.40–1.41)	132,	0.56 (0.31–1.03)
Activity of daily living score (range 0–20)										
High function (≥18)	167,	1.00 —	74,	1.00 —	237,	1.00 —	59,	1.00 —	160,	1.00 —
Mid function (12–17)	238,	1.14 (0.77–1.70)	180,	0.50 (0.27–0.93)	272,	0.93 (0.45–1.91)	71,	1.27 (0.56–2.88)	143,	0.88 (0.42–1.88)
Low function (≤11)	265,	0.81 (0.54–1.20)	233,	0.13 (0.06–0.28)	232,	0.88 (0.41–1.89)	72,	0.60 (0.28–1.29)	130,	0.57 (0.28–1.16)
Chronic diseases										
Congestive heart failure										
Absence	613,	1.00 —	438,	1.00 —	624,	1.00 —	189,	1.00 —	363,	1.00 —
Presence	59,	0.89 (0.52–1.54)	51,	0.22 (0.05–0.94)	63,	0.51 (0.12–2.15)	13,	0.30 (0.10–0.94)	42,	2.75 (0.64–11.78)
CVD										
Absence			221,	1.00 —	390,	1.00 —	110,	1.00 —	201,	1.00 —
Presence			268,	0.28 (0.16–0.48)	297,	0.48 (0.23–0.97)	92,	0.71 (0.38–1.31)	204,	1.12 (0.61–2.07)
Dementia										
Absence	380,	1.00 —			507,	1.00 —	130,	1.00 —	236,	1.00 —
Presence	268,	0.67 (0.49–0.93)			161,	1.49 (0.73–3.02)	65,	0.48 (0.25–0.91)	154,	0.62 (0.33–1.14)
Depression										
Absence	198,	1.00 —	120,	1.00 —			67,	1.00 —	154,	1.00 —
Presence	297,	1.26 (0.87–1.81)	161,	1.06 (0.54–2.08)			90,	0.65 (0.31–1.37)	184,	1.30 (0.64–2.62)
Diabetes mellitus										
Absence	580,	1.00 —	424,	1.00 —	597,	1.00 —			328,	1.00 —
Presence	92,	0.89 (0.57–1.40)	65,	0.96 (0.45–2.03)	90,	1.18 (0.48–2.90)			77,	0.72 (0.35–1.48)
Hypertension										
Absence	468,	1.00 —	336,	1.00 —	557,	1.00 —	125,	1.00 —		
Presence	204,	0.54 (0.38–0.76)	154,	0.50 (0.27–0.93)	184,	1.14 (0.58–2.27)	77,	1.15 (0.61–2.19)		

CVD = cardiovascular disease.

Table 4. Multivariate Analysis of Characteristics Associated with Participants Receiving Medication

Characteristic	Antithrombotic Agent Use for History of CVD (n = 480)	Acetylcholinesterase Inhibitor Use for Dementia (n = 280)	Antidepressant Use for Depression (n = 668)	Hypoglycemic Use for Diabetes Mellitus (n = 154)	Antihypertensive Use for Hypertension (n = 302)
	Odds Ratio (95% Confidence Interval)				
Age (reference 65–74)					
75–84	0.90 (0.56–1.42)	0.67 (0.25–1.82)	0.59 (0.28–1.26)	1.39 (0.54–3.54)	1.98 (0.75–5.19)
≥85	0.53 (0.32–0.90)	0.21 (0.06–0.71)	0.33 (0.12–0.91)	0.53 (0.19–1.49)	1.48 (0.55–3.98)
Sex (reference female)					
Male	1.57 (1.08–2.30)	1.20 (0.54–2.68)	0.74 (0.35–1.57)	0.93 (041–2.13)	0.79 (0.34–1.80)
Activity of daily living score (reference high function (≥18))					
Mid function (12–17)	1.17 (0.74–1.84)	0.56 (0.24–1.29)	0.91 (0.40–2.03)	1.73 (0.66–4.53)	0.94 (0.39–2.27)
Low function (≤11)	0.94 (0.58–1.54)	0.07 (0.02–0.26)	0.96 (0.40–2.31)	1.28 (0.49–3.58)	0.80 (0.29–2.20)
Presence of chronic disease (reference absence)					
Congestive heart failure	1.18 (0.61–2.27)	0.29 (0.03–2.46)	0.63 (0.14–2.82)	0.26 (0.06–1.01)	1.93 (0.41–9.14)
CVD		0.26 (0.12–0.57)	0.45 (0.21–0.97)	0.95 (0.43–2.10)	1.70 (0.75–3.86)
Dementia	0.92 (0.61–1.39)		1.88 (0.87–4.05)	0.68 (0.28–1.66)	0.73 (0.32–1.65)
Depression	1.25 (0.85–1.84)	1.75 (0.80–3.82)		0.62 (0.27–1.39)	1.54 (0.72–3.29)
Diabetes mellitus	0.56 (0.32–0.99)	0.73 (0.23–2.34)	0.85 (0.31–2.29)		0.77 (0.30–1.97)
Hypertension	0.69 (0.45–1.05)	0.37 (0.15–0.92)	1.40 (065–2.99)	1.28 (0.54–3.03)	

CVD = cardiovascular disease.

CI = 1.32–2.46), and having a higher ADL dependency was associated with the underuse of acetylcholinesterase inhibitors in those with dementia (low ADL function: OR = 0.13, 95% CI = 0.06–0.28). In older people with dementia or diabetes mellitus, those with heart failure were less likely to be prescribed acetylcholinesterase inhibitors (OR = 0.22, 95% CI = 0.05–0.94) and hypoglycemics (OR = 0.30, 95% CI = 0.10–0.94). In older people with dementia or depression, those with a history of CVD were less likely to be prescribed acetylcholinesterase inhibitors (OR = 0.67, 95% CI = 0.49–0.93) and antidepressants (OR = 0.48, 95% CI = 0.23–0.97). The presence of dementia was associated with the underuse of antithrombotic agents and hypoglycemic drugs in older people with a history of CVD (OR = 0.67, 95% CI, 0.49–0.93) and those with diabetes mellitus (OR = 0.48, 95% CI = 0.25–0.91).

Multivariable analysis showed that the oldest age group received fewer antithrombotic agents (OR = 0.53, 95% CI = 0.32–0.90), acetylcholinesterase inhibitors (OR = 0.21, 95% CI = 0.06–0.71), and antidepressants (OR = 0.33, 95% CI = 0.12–0.91) among older people with a history of CVD and those diagnosed with dementia and with depressive symptoms, respectively (Table 4). When a separate analysis was conducted of the participants with a history of stroke and those with a history of coronary heart disease, the oldest age group was less likely to be prescribed antithrombotic agents in subjects with a history of coronary heart disease (OR = 0.29, 95% CI = 0.09–0.91) but not with stroke (OR = 0.66, 95% CI = 0.37–1.19). Analysis also showed that women with a history of CVD were less likely than men with CVD to be prescribed antithrombotic agents (male: OR = 1.57, 95% CI = 1.08–2.30) and that having a low ADL function was associated with the underprescription of acetylcholinesterase inhibitors (low ADL function: OR = 0.07, 95% CI = 0.02–0.26) in older people with dementia. In older people with hypertension or diabetes mellitus, none of the factors studied were associated with the underprescription of antihypertensive or hypoglycemic drugs, respectively. In older people with depressive symptoms, those with a history of CVD were less likely to be prescribed antidepressants (OR = 0.45, 95% CI = 0.21–0.97).

DISCUSSION

In the present study, the presence of various chronic diseases, including congestive heart failure, coronary heart disease, and diabetes mellitus, was demonstrated to influence multiple medication use in community-dwelling frail older people. In contrast, participants with dementia were less likely to be prescribed multiple medications. Whether doctors prescribe differently for patients with cognitive impairment is a controversial issue. Some studies have shown that fewer drugs are prescribed for patients with dementia than for those without,[12,13] but other studies have demonstrated no significant difference between patients with and without dementia in the average number of medications prescribed.[14,15] Nevertheless, it is more important to know the influence of the presence of cognitive impairment on the use of beneficial medication for specific chronic diseases than that on the total number of prescribed medications. This study also showed, using a multivariate logistic regression model controlling for other confounding factors, that the oldest age group (≥85) is less likely to be prescribed multiple medications. It is possible that these oldest patients do not see their primary care physician, even if they have chronic diseases or conditions, but this was found not to be true, because the number of visits they made to their primary physician per month was not a predictor of underuse of medication for chronic diseases and conditions (data not shown). These results prove the hypothesis that the rate of multiple medication use is lower in the oldest community-dwelling frail older people (≥85) than in the younger old.

Previous studies have showed that nursing home residents aged 85 and older are less likely to be treated than those aged 65 to 74 for stroke secondary prevention[16] and that there is a marked underuse of aspirin in the treatment of older patients with documented prior myocardial infarction at the time of admission to a nursing home.[17] In agreement with these studies based at the nursing home, the present study targeting community-dwelling older people demonstrated that the oldest subjects with a history of CVD were less likely to be prescribed antithrombotic agents for secondary prevention. Nevertheless, when a separate analysis was conducted of the participants with a history of stroke and those with a history of coronary heart disease, older age was still a predictor of nonuse of antithrombotic agents in subjects with a history of coronary heart disease but not with stroke. In the present survey, hemorrhagic and ischemic stroke were not differentiated between in the stroke diagnosis. Although ischemic strokes account for 85% of all strokes of persons aged 65 and older according to the Japanese national survey, it is possible the inclusion of hemorrhagic stroke affected the analysis.

In the present study, the oldest group univariate and multivariate analyses indicated underuse of acetylcholinesterase inhibitors by older people with dementia. It is possible that a higher proportion of the oldest elderly might have a severe form of Alzheimer's disease and therefore not be eligible for treatment with acetylcholinesterase inhibitors. Few published studies on the use of antidepressants have focused on the older population, even though the prevalence of depression is high in community-dwelling elderly persons. In the present study, 57.2% of the participants had a GDS-15 score of 6 or higher, although only 2% of the subjects were diagnosed with depression in primary care settings, consistent with reports from other countries that the majority of older people with depression are not diagnosed in primary care.[18,19] Alternatively, potentially effective antidepressant medications are also used inadequately in older populations. According to the data from a national survey in Canada, the rate of antidepressant use was 3.1% in older people in the community. Of those who were depressed, 4.2% were taking an antidepressant.[20] In the current survey, only 5.9% of subjects who had depressive symptoms received antidepressants, and univariate analysis showed that the oldest old with depression were less likely to use antidepressants than those who were younger. The multivariate analysis confirmed this association.

Only several reports on the rate of drug treatment for diabetes mellitus in older people have been found. One cross-sectional study demonstrated that the likelihood of drug treatment for people with diabetes with insulin or oral

hypoglycemics declined substantially with increasing age.[21] In the present study, the use of hypoglycemic agents by older people diagnosed with diabetes mellitus was the lowest in the oldest persons, and in comparison with persons aged 65 to 74, the OR of hypoglycemic use in the oldest old was 0.53 using multivariable logistic regression analysis, although the *P*-value did not reach statistical significance ($P = .23$).

In this population of frail older people living at home, the nonuse of antihypertensive medication was relatively low in older people with hypertension, and no difference in the ratio of the prescription of antihypertensive drugs between age categories was found. Furthermore, no association was detected between the nonuse of antihypertensive medication and any factors tested, not only in the univariate analysis but also in the multivariate analysis. This is in contrast to previous studies showing that older people were likely to be undertreated for hypertension.[22,23]

It has been suggested that ADL impairment, cognitive impairment, and comorbid conditions are factors influencing the underprescription of beneficial agents in older people associated with chronic diseases: the underuse of antithrombotic agents by stroke patients with severe cognitive or physical impairment,[16,24] hypoglycemic agents underuse by older people with diabetes mellitus with higher levels of comorbidity,[21] and the underuse of antihypertensive medication by older people with cognitive impairment or comorbidity.[22,23] Nevertheless, in the current study, even after controlling for ADL dependency and the presence of dementia, age was still a significant predictor of the nonuse of antithrombotic agents by older people with a history of CVD, acetylcholinesterase inhibitors by older people with dementia, and antidepressants by older people with depression. In addition, the present study suggests that the influence of ADL dependency, cognitive impairment, and comorbid conditions on the underuse of beneficial medications was also dependent on each chronic disease/condition. The lowest category of ADL function was only associated with the nonuse of acetylcholinesterase inhibitors by the demented elderly using multivariable logistic regression analysis. The presence of dementia was associated with the nonuse of antithrombotic agents by the participants with a history of CVD in univariate analysis, but multivariate analysis did not confirm this association. Furthermore, no association was detected between the nonuse of antihypertensive medication and the presence of dementia in univariate and multivariate analysis. It is possible that, to avoid the risk of adverse drug reactions, physicians decide not to use beneficial medications for the oldest old, although multiple medication use may not always be a disadvantage for older people with comorbid conditions when drugs with proven efficacy in elderly patients are available. These results suggest again that it is not easy to predict the underuse of prescribed beneficial medication in older persons but is instead complex and dependent on each chronic disease/condition. The history of CVD was associated with the nonuse of acetylcholinesterase inhibitors by older people with dementia using univariate and multivariate logistic regression analysis. It is possible that the origin of dementia for most of them might be vascular.

There are many factors that contribute to the underuse of beneficial medications in the oldest old. The use of age as an indicator of benefit of care is imprecise, in that elderly persons differ appreciably in physical, mental, and cognitive status and in life expectancy. It is of concern that the very population that receives the most medications may not always have a favorable risk/benefit ratio. Physicians may decide not to use a medication, because patients may not benefit from treatment (e.g., the low use of acetylcholinesterase inhibitors by demented older people with the lowest ADL function). In fact, geriatric therapeutics must also take into account specific geriatric diseases (e.g., dementia, CVD) and syndromes (e.g., falls, gait and balance disturbances, incontinence, ADL impairment). As proposed by others,[25] the lack of high-quality evidence derived from clinical studies with relevance to treating older patients with multiple chronic medical conditions may be one of the factors that contribute to the underuse of beneficial medications in the oldest old. In fact, clinical evidence often does not provide a definitive answer on the benefits or risks of many drug therapies in older people, especially in those aged 75 and older.[26] Of a number of chronic diseases common in older people, the evidence for drug therapy has been accumulating in the field of hypertension faster than with other diseases. This may be one of the reasons that the highest prescription rate is for antihypertensive medication and the reason there is no restriction of treatment in the oldest patients.

A recent study indicated that the cost of prescription drugs is another problem contributing to the undertreatment of diseases in older people.[27] These cost-related problems seem to be dependent on health insurance systems, which vary between countries. In Japan, universal mandatory health insurance, which covers nearly all regular health care, including prescription drugs, covers the entire population. Elderly health insurance for people aged 75 and older or aged 65 and older with some impairments covers health care, including prescription drugs, with a 10% copayment. Therefore, it is unlikely that cost problems influenced these results or that their influence, if any, was great.

The major limitation of this study was that diagnoses of chronic diseases were based solely on information available in the care-managing centers' records, which were based on the data provided by primary care physicians every 6 months. The accuracy of the diagnosis of chronic diseases by these physicians was not evaluated. It was also not discovered how severe these chronic conditions, which included dementia, diabetes mellitus, and hypertension, were. The results may not be representative of frail older Japanese in the community as a whole, because the subjects in this study represented an urban population. In addition, these findings may not be generalizable to other populations given that health practices, ethnic attitudes about treating very old people, and cost/access to medications may influence these results. Because of the small numbers of participants with each chronic condition, these observations cannot be commented on conclusively. The findings of this study need to be reproduced in a larger sample of practices.

In summary, it was demonstrated that, among community-dwelling frail older people, the rate of multiple medication use is lower in the oldest persons than in the younger ones. In addition, the underuse of beneficial medication for the oldest persons in this group was observed: antithrombotic agents by subjects with a history of CVD,

acetylcholinesterase inhibitors by subjects with dementia, and antidepressants by subjects with depression. Nevertheless, the oldest persons with diabetes mellitus and hypertension were not associated with the underuse of hypoglycemic and antihypertensive agents, respectively. Thus, the underuse of prescribing medication for chronic diseases/conditions of frail older people living in the community is common but not for all conditions.

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Author Contributions: Masafumi Kuzuya: study concept, design, conduct of study, interpretation of data, and preparation of manuscript. Yuichiro Masuda and Yoshihisa Hirakawa: conduct of study, interpretation of data. Mitsunaga Iwata: analysis and interpretation of data. Hiromi Enoki: statistical analysis and interpretation of data. Jun Hasegawa and Xian Wu Cheng: acquisition of data. Akihisa Iguchi: study concept and study supervision.

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ORIGINAL ARTICLE

Lack of correlation between total lymphocyte count and nutritional status in the elderly

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KEYWORDS
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Summary *Background & aims*: Malnutrition is a widespread but largely unrecognized problem in aged people. Although absolute total lymphocyte count (TLC) has been proposed as a useful indicator of nutritional status, there is little evidence that low TLC levels reflect malnutrition in the elderly. To examine whether TLC is a suitable marker of malnutrition in the elderly.

Methods: A total of 161 elderly subjects (44 males and 117 females, mean age±SD: 77.9±7.4; range: 65–95 years) were enrolled from geriatric clinical settings. The participants were categorized according to severely low, low, or normal TLC. Anthropometry measurements, serum albumin, total cholesterol levels, and total score on the mini-nutritional assessment (MNA) were determined.

Results: There were no significant differences among the three TLC groups with regard to anthropometry measurements, serum albumin, total cholesterol levels, or MNA score. There was a significant negative correlation of TLC with age, but not with other nutritional markers. The clinical nutritional screening tool, MNA score, was well correlated with all of the nutritional parameters used in the present study except for TLC.

Conclusion: TLC is not a suitable marker of malnutrition in the elderly.

Introduction

Malnutrition is a common finding in the elderly, not only in institutionalized populations but also in community-dwelling elderly, with prevalence rates ranging from 12% to 85%.[1,2] Malnutrition is associated with increased hospitalizations, increased susceptibility to infection, decreased wound healing, reduced quality-of-life, and increased mortality in the elderly.[3,4] However, it remains difficult to define malnutrition for the elderly precisely. Therefore, malnutrition is

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often unrecognized and subsequently goes untreated.

Anthropometry measurements such as body mass index (BMI), mid-arm circumference (MAC), calf circumference (CC), and skin fold thickness are generally considered as the single most easily obtainable, inexpensive, and noninvasive method by which to assess nutritional state. Biochemical measurements such as serum albumin and total cholesterol are also well known as markers for the protein energy malnutrition (PEM), and are the most commonly used laboratory tests.[5,6]

Multidimensional screening tools for nutritional assessments in the clinical situation have been developed. Among those, the mini-nutritional assessment (MNA) is a simple clinical scale for the evaluation of the nutritional status of frail elderly subjects.[7,8] It has been validated in various countries by comparing its results with a clinical assessment performed by expert geriatric nutritionists.

Total lymphocyte count (TLC) has been also proposed as a useful indicator of nutritional status and outcome. It has been proposed that TLC decreases with progressive malnutrition and correlates with morbidity and mortality in hospitalized patients.[5,6] It has also been proposed that regardless of age, a decrease in TLC to less than 1500/mm^3 or less than 900/mm^3 reflects malnutrition or severe malnutrition, respectively.[5,6] Although TLC is one of the most commonly obtained nutritional markers, there is little evidence that low TLC levels reflect malnutrition in the elderly, and it remains uncertain whether TLC can be used as a marker of malnutrition in elderly subjects.

In the present study, we evaluated the relationship of TLC with other nutritional markers including MNA score, anthropometry measurements, serum albumin, and total cholesterol levels as an indicator of nutritional status in the Japanese elderly.

Methods

Subjects

We enrolled 235 elderly subjects (67 males and 168 females, mean age $\pm$ SD: 78.6 $\pm$ 7.6; range: 65–95 years) from our geriatric outpatient clinic ($n = 69$), a nursing home ($n = 56$), geriatric hospitals ($n = 72$), and home care patients ($n = 38$). All participants provided written informed consent. Subjects diagnosed with infection, inflammation, liver disorders, kidney disorders, cancer, or bone marrow proliferative disorders were not included in the 235 participants. The analysis on TLC described herein was limited to the 161 (44 male and 117 female) participants (mean $\pm$ SD: 77.9 $\pm$ 7.4 years; range: 65–95 years) whose TLC measurements were obtained, since some participants did not approve blood sampling for TLC measurement.

Anthropometric measurements and biochemical markers

BMI is defined as weight in kilograms divided by height in meters squared. Triceps skinfold (TSF) was measured with Harpenden callipers over the triceps muscle at the midway point between the acromion and the olecranon process. MAC and CC were measured on the left arm and calf with a tape measure. Three repeat measurements were taken to the nearest 0.5 mm, with the mean taken as the true value. All anthropometric measurements were taken at least twice by two different investigators, and the reported values are the means of the repeated measurements. Blood samples were collected after an overnight fast. Serum albumin and total cholesterol levels were determined using automated analysers. Blood was collected into tubes containing EDTA, and TLC was measured with use of a Coulter counter.

Definition of malnutrition

A BMI of less than 20 is widely accepted to indicate that the subject is underweight, particularly in well-developed countries, and 18.5 is recommended as a practical lower limit for most populations.[9] Therefore, a diagnosis of malnutrition was made when BMI was less than 18.5 kg/m^2. Serum albumin and total cholesterol levels were used as the biochemical markers of undernutrition: levels less than 3.5 g/dl of albumin or 150 mg/dl of total cholesterol were taken to indicate malnutrition. Participants were categorized into three groups according to lymphocyte count, as follows: severely low lymphocyte (<900 count/mm^3), low lymphocyte (900–1499 count/mm^3), and normal lymphocyte count ($\geqslant 1500$ count/mm^3). The relationship of each group to various respective nutritional markers has been examined. In addition, participants were classified according to the cutoff of each nutritional parameter and comparisons were made among groups in terms of anthropometric markers, nutritional proteins, and MNA score.

MNA, a comprehensive, noninvasive, well-validated screening tool for malnutrition in elderly persons, has been also used as an indicator of

malnutrition. The MNA includes 18 items, including the anthropometrical measurements BMI, MAC, and CC, weight loss, a global assessment (six questions related to lifestyle, medication, and mobility), a dietary questionnaire (eight questions related to the number of meals, types of food, and fluid intake), and a subjective assessment (self-perception of health and nutrition). The MNA assigns points on nutritional adequacy with a maximum score of 30 points.[7] The MNA score distinguishes between elderly patients with adequate nutrition (scores of 24 and up), protein-calorie undernutrition (lower than 17), and risk of malnutrition (between 17 and 23.5).[7]

Statistical analysis

Differences between groups (TLC: <900, 900–1499, $\geqslant 1500$) were determined by one-way analysis of variance, Chi-square test or the Kruskal–Wallis test, as appropriate. The Kolmogorov–Smirnov test was used to check the normal distribution of variables. Chi-square test, Mann–Whitney U test, or Student's unpaired t-test was used to test differences between normal and malnutrished groups, as appropriate. Partial rank correlation coefficients adjusted for age were used to measure the relationships between TLC and variables, or between MNA score and variables. The significance level was set at 0.05. Data evaluation was carried out using the SPSS software package (SPSS Inc., Chicago, USA).

Results

Table 1 shows the mean results of variables, which are expressed according to the classification of lymphocyte count ($<$ 900, 900–1499, $\geqslant 1500$). There were significant differences between classes with regard to MAC, but there was no trend toward greater MAC values in the group with 900–1499 TLC compared to those in the <900 TLC group. No significant differences were observed between classes in terms of age, BMI, TSF, CC, serum albumin, total cholesterol, or MNA score. There was a weak but statistically significant negative correlation between lymphocyte count and age ($r = -0.21$, $P = 0.0006$). There were no correlations between TLC and any other nutritional indices.

When levels of less than 18.5 kg/m^2 of BMI, 3.5 g/dl of albumin or 150 mg/dl total cholesterol, and 17 points on MNA score were taken to indicate malnutrition, the relationship among these parameters and anthropometric measurements were examined (Table 2). The groups with <18.5 kg/m^2 of BMI, <3.5 g/dl of serum albumin, <150 mg/dl of total cholesterol, and <17 of MNA score had significantly lower values than those of the well-nourished groups with respect to most of the nutrition-related variables except for lymphocyte count.

The score on MNA, a commonly used comprehensive malnutrition screening for the elderly, was correlated with BMI, MAC, TSF, CC, serum albumin, and total cholesterol levels ($r = 0.52$, 0.36, 0.26, 0.28, 0.61, and 0.34, respectively; $P \leqslant 0.0001$

Table 1 Lymphocyte count and nutritional characteristics.

	Lymphocyte (count/mm^3)			P-value*
	<900	900–1499	$\geqslant 1500$	
n	9	51	101	
Men/women	1/8	12/39	31/70	0.343
	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)	79.1 (9.8)	79.1 (6.4)	77.2 (7.7)	0.287
BMI (kg/m^2)	21.8 (3.1)	21.2 (3.9)	22.6 (3.7)	0.074
MAC (cm)	25.0 (2.8)	23.6 (3.1)	25.2 (3.2)	0.013
TSF (mm)	10.6 (4.9)	11.4 (8.2)	14.6 (8.6)	0.052
CC (cm)	30.3 (2.2)	31.2 (3.8)	31.5 (4.0)	0.666
Albumin (g/dl)	4.0 (0.4)	4.1 (0.3)	4.1 (0.5)	0.526
Total cholesterol (mg/dl)	186.4 (38.3)	203.8 (33.8)	205.3 (41.3)	0.380
MNA score	20.9 (2.3)	20.6 (4.2)	21.0 (4.1)	0.901

BMI: body mass index; MAC: midarm circumference; TSF: triceps skinfold; CC: calf circumference; MNA: mini-nutritional assessment.

*One-way analysis of variance was conducted except for the gender difference (χ^2-test) and MNA score (Kruskal–Wallis test).