III. 研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kurata M, Fukuo K, et al.	Association of Metabolic Syndrome with Serum Adipokines in Community-Living Elderly Japanese Women: Independent Association with Plasminogen Activator-Inhibitor-1.	Metab Syndr Relat Disord.	14(1):	40-5	2015
Takeuchi M, Fukuo K, et al.	Association of Metabolic Syndrome with Serum Adipokines in Community-Living Elderly Japanese Women Independent Association with Plasminogen Activator-Inhibitor-1 Metab Syndr Relat Disord.	Metab Syndr Relat Disord.	13(9)	415-21	2015
Yamada E, Fukuo K, et al.	Low haemoglobin levels contribute to low grip strength independent of low-grade inflammation in Japanese elderly women.	Asia Pac J Clin Nutr.	24(3)	444-51	2015
Takenouchi A, Fukuo K, et al.	Direct association of visit-to-visit HbA1c variation with annual decline in estimated glomerular filtration rate in patients with type 2 diabetes.	J Diabetes Metab Disord.	14	69	2015

Tsuboi A, Fukuo K, et al.	Associations of decreased serum transthyretin with elevated high-sensitivity CRP, serum copper and decreased hemoglobin in ambulatory elderly women.	Asia Pac J Clin Nutr.	24(1)	83-9	2015
Takata K, Tomita T, Sakoda S, et al.	Dietary yeasts reduce inflammation in central nervous system via microflora.	Ann Clin Transl Neurol.	16	1040	2015
Endo T, Sakoda S, et al.	Parkinsonian Rigidity Depends on the Velocity of Passive Joint Movement.	Parkinsons Dis.	2015	961790 - 961794	2015
Hirano H, Maeda H, Sakoda S, et al.	Survivin expression in lung cancer: Association with smoking, histological types and pathological stages.	Oncol Lett.	10	1456-1462	2015
Arima Y, Sakoda S, et al.	A pain-mediated neural signal induces relapse in murine autoimmune encephalomyelitis, a multiple sclerosis model.	eLife	10	7554/eLife.08733	2015
Mori C, Sakoda S, et al.	Two cases of hereditary motor and sensory neuropathy with proximal dominant involvement (HMSN-P).	Rinsho Shinkeigaku.	55	401-5	2015
Inoue K, Sakoda S, et al.	An autopsy case of neuronal intermediate filament inclusion disease with regard to immunophenotypic and topographical analysis of	Neuropathology	35	545-52	2015

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Yamadera M, Sakoda S, et al.	Microvascular disturbance with decreased pericyte coverage is prominent in the ventral horn of patients with amyotrophic lateral sclerosis.	Amyotroph Lateral Scler Frontotemporal Degener.	16	393-401	2015
Takata K, Sakoda S, et al.	Dietary Yeasts Reduce Inflammation in Central Nerve System via Microflora.	Ann Clin Transl Neurol.	2	56-66	2015
Harada M, Sakoda S, et al.	Temporal expression of growth factors triggered by epiregulin regulates inflammation development.	J Immunol.	194	1039-1046	2015
Enomoto H, et al.	Development of risky varices in alcoholic cirrhosis with a well-maintained nutritional status.	World J Hepatol.	2	2358-2362,	2015

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Association of Metabolic Syndrome with Chronic Kidney Disease in Elderly Japanese Women: Comparison by Estimation of Glomerular Filtration Rate from Creatinine, Cystatin C, and Both

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Abstract

Background: Associations between metabolic syndrome (MetS) and chronic kidney disease (CKD) has not been extensively studied in elderly Asians, who in general have lower body mass index (BMI) than European populations.

Methods: A cross-sectional analysis was conducted including 159 community-living elderly Japanese women. MetS was defined by the modified National Cholesterol Education Program Adult Treatment Panel III criteria, but using a BMI ≥25 kg/m^2 instead of waist circumference and renal function was assessed according to the Kidney Disease Outcomes Quality Initiative CKD classification. Creatinine-based and cystatin C-based estimated glomerular filtration rate (eGFR) and the average of the two eGFRS were used.

Results: Prevalence of CKD was much higher when creatinine-based eGFR was used than the prevalence obtained when cystatin-C based equations were used (46.5% vs. 12.6%, $P<0.001$). Eighteen (11.3%) women met MetS criteria. Both the presence of MetS and the number of MetS components were associated with higher prevalence of CKD using the average eGFR (all $P<0.05$) but not using creatinine-based ($P=0.86$) and cystatin C-based ($P=0.12$) eGFR alone. Lower average eGFR and higher prevalence of CKD using average eGFR were evident in even women with only one MetS component, 89% of whom had elevated blood pressure.

Conclusions: Prevalence of CKD varied substantially depending on the used equation. In nonobese, elderly Japanese women, both the presence of MetS and the number of MetS components were associated with higher prevalence of CKD and elevated blood pressure may play an important role in these associations. These findings should be confirmed in studies employing more participants with MetS diagnosed using standard criteria (waist circumference instead of BMI).

Introduction

CHRONIC KIDNEY DISEASE (CKD)[1–4] and metabolic syndrome (MetS)[5–7] are each independently associated with cardiovascular disease and are also positively associated with each other. Several prospective studies indicated that MetS is a risk factor for CKD in the general population.[8–10] CKD is a frequent disease in the elderly.[11,12] CKD is currently defined as a creatinine-based estimated glomerular filtration rate (eGFRcreat) of less than 60 mL/min/1.73 m^2 or a urine albumin to creatinine ratio of 30 mg/gram or higher.2 Serum creatinine levels are affected by muscle mass, age, and race,[13] and eGFR calculated using creatinine is less reliable for assessing renal function when GFR is more than 60 mL/min/1.73 m^2.[3,14]

Studies suggest that cystatin C is less dependent upon diet and muscle mass than creatinine and it is assumed that it should provide more accurate GFR estimates, particularly in populations with reduced muscle mass, such as the elderly.[15,16] Recently, the average (eGFRaver) of creatinine-based and cystatin-C based eGFR (eGFRcys) has been reported to have greater precision and accuracy than the individual creatinine and cystatin C equations.[17] The eGFRaver provides the most precise and accurate GFR among people who have mildly decreased GFR (eGFRcreat of 60 to 74 mL/min/1.73 m^2).[17] Further, it has been reported

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that the mortality risk was higher across all levels of decreased eGFR with cystatin C-based equations compared with creatinine-based equations.[18] However, as far as we know there is no report that studied kidney function employing average eGFR in the Asian elderly and hence that examined the relationship between CKD diagnosed using eGFRaver and MetS. Therefore, we evaluated these issues in community-living elderly Japanese women.

Methods

We examined 159 free-living elderly women whose details have been reported elsewhere.[19,20] They were residents in Nishinomiya City and were recruited as volunteers by local welfare commissioners from the city of Nishinomiya, Hyogo, Japan. Subjects with clinically diagnosed acute or chronic inflammatory diseases and cancer were excluded from the study. Although 43, 9, and 58 women (27.0%, 5.7%, and 36.5%, respectively) reported to be receiving statins, antidiabetic drugs, and antihypertensive drugs, respectively, detailed drug information was not available. Hypertension (58 women on antihypertensive medication and 46 women with systolic/diastolic BP ≥140/90 mmHg without medication) was found in 104 women (65.4%), whereas diabetes mellitus was found in 12 women (7.5%) [9 on antidiabetic medication and 3 with hemoglobin A1c (HbA1c) ≥6.5% without medication]. This research followed the tenets of the Declaration of Helsinki. The design of this study was approved by the Ethical Committees of Mukogawa Women's University and written informed consent was obtained from all participants.

Anthropometric indices and BP were measured after an overnight fasting. Thereafter, blood samples were obtained from the cubital vein. We evaluated routine chemical parameters, including glucose, insulin, liver enzymes, and complete blood count as previously reported.[20,21] HbA1c was measured by an immunoassay using an autoanalyzer (JCA-BM9030, JEOL Ltd.). Insulin resistance (IR) was evaluated using homeostasis model assessment (HOMA-IR).[22]

Metabolic syndrome was defined using the modified criteria of the National Cholesterol Education Program Adult Treatment Panel III guidelines.[23] Because body mass index (BMI) is not inferior to waist circumference as a practical marker of the metabolic risk clustering in Japanese[24] and because BMI may be better than waist circumference for defining metabolic syndrome in Japanese women,[25] adiposity was defined using Asian criteria as BMI ≥25.0 kg/m^2.[26] Elevated BP was defined as systolic/diastolic BPs of 130/85 mmHg or greater and/or current use of antihypertensive medicine. Hypertriglyceridemia was defined as a serum triglyceride level of 150 mg/dL or greater. Low high-density lipoprotein (HDL) cholesterol level was defined as less than 50 mg/dL for all participants are women. Impaired fasting glucose (IFG) was defined as fasting plasma glucose levels of 100 mg/dL or greater and/or current use of antidiabetic medicine. Metabolic syndrome was defined as the presence of three or more components.[23]

TABLE 1. CLINICAL FEATURES OF JAPANESE ELDERLY WOMEN WITH METABOLIC SYNDROME

	Metabolic syndrome		
	No (n=*141*)	*Yes* (n=*18*)	P *values*
Age (years)	76±8	76±7	0.782
BMI (kg/m^2)	22.2±2.7	25.7±3.1	0.000
Antidiabetic drug users (*n*, %)	4, 2.8	5, 27.8	0.000
Antihypertensive drug users (*n*, %)	47, 33.3	11, 61.1	0.021
Elevated BP (*n*, %)	105, 74.5	17, 94.4	0.059
Systolic BP (mmHg)	143±19	146±19	0.466
Diastolic BP (mmHg)	84±11	84±7	0.978
Fasting glucose (mg/dL)	86±10	101±22	0.009
Fasting insulin (μU/mL)	5.2±3.3	8.1±7.2	0.114
HbA1c (%)	5.6±0.4	6.1±0.6	0.003
HOMA-IR	1.12±0.79	2.13±2.2	0.069
Log insulin	0.65±0.22	0.8±0.29	0.013
Log HOMA-IR	−0.02±0.24	0.19±0.33	0.001
AST (IU/l)	24±8	27±15	0.417
ALT (IU/l)	18±9	24±17	0.189
GGT (IU/l)	24±15	32±19	0.047
Log ALT	1.22±0.17	1.31±0.22	0.043
Log GGT	1.33±0.19	1.44±0.23	0.024
Creatinine (mg/dL)	0.72±0.15	0.75±0.24	0.594
Cystatin C (mg/l)	0.8±0.2	1.0±0.3	0.183
eGFRcreat (mL/min/1.73m^2)	62±13	62±21	0.916
eGFRcys (mL/min/1.73m^2)	81±19	75±24	0.198
eGFRaver (mL/min/1.73m^2)	72±15	69±22	0.576
eGFRcreat <60 mL/min/1.73m^2 (n, %)	65, 46.1	9, 50.0	0.755
eGFRcys <60 mL/min/1.73m^2 (n, %)	16, 11.3	4, 22.2	0.190
eGFRaver <60 mL/min/1.73m^2 (n, %)	25, 17.7	7, 38.9	0.035

Data presented as mean±standard devition or *n*, %.

ALT, alanine-aminotransferase; AST, asparate-aminotransferase; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; eGFRcreat, creatinine-based eGFR; eGFRcys, cystatin-C based eGFR; eGFRaver, average of eGFRcreat and eGFRcys; GGT, gamma-glutamyltransferase; HOMA-IR, homeostasis-model insulin resistance.

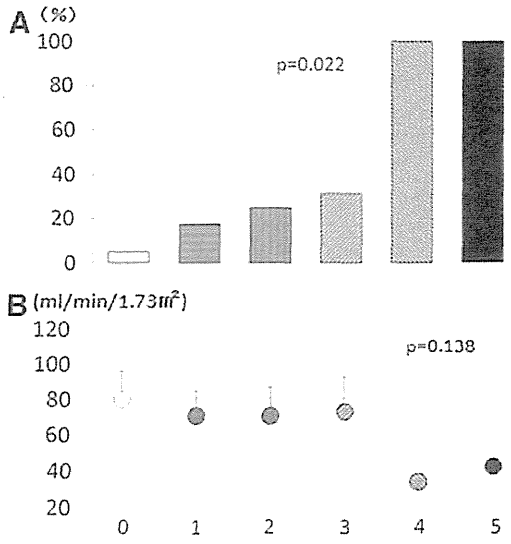

FIG. 1. The prevalence of chronic kidney disease (**A**) and means ± standard deviaiton of the average of creatinine-based and cystatin C-based estimated glomerular filtration rate (**B**) as a function of the number of components of metabolic syndrome. The number of participants with 0, 1, 2, 3, 4, and 5 components was 21, 80, 40, 16, 1, and 1, respectively. Although differences in eGFRaver among the six groups were not significant ($P=0.138$) when using the Jonckheeree-Terpstra test, differences among the six groups were highly significant when analysis of variance was applied ($P=0.009$).

Serum creatinine was measured enzymatically using an autoanalyzer (AU 5200, Olympus) and cystatin C by latex immunoassay using a commercially available kit (IatroCys-C, Mitsubishi Chemical Medience). Coefficients of variation of creatinine and cystatin were 1.0% at 0.71 mg/dL and 1.2% at 0.70 mg/L, respectively. The eGFR was calculated using the equation recommended by the Japanese Society for Nephrology.[27,28] The average (eGFRaver) of eGFRcreat and eGFRcys[17] were calculated in each participant and was used in analysis. CKD was defined as an eGFR of less than 60 mL/min/1.73 m^2.[2]

Data are presented as mean ± standard deviation unless otherwise stated. Due to deviation from normal distribution, serum levels of liver enzymes, insulin, and HOMA-IR were logarithmic transformed for analysis. Comparisons between the control and MetS groups were made with two-sample *t*-tests. The association of CKD and eGFR with the number of components of MetS and P_{trend} were derived using Jonckheeree-Terpstra test or analysis of variance when appropriate. A two-tailed $P<0.05$ was considered statistically significant. All calculations were performed with SPSS system 15.0 (SPSS Inc.).

Results

As previously reported,[19,20] participants were relatively healthy, community-living, ambulatory elderly women. Estimated GFRcreat (62 ± 14 mL/min/1.73m^2) was substantially lower than eGFRcys (80 ± 20 mL/min/1.73m^2, $P<0.001$), and hence, prevalence of CKD was much higher when using eGFRcreat than using eGFRcys (46.5% vs. 12.6%, $P<0.001$). Of 159 women, 31 (20.1%) had CKD using eGFRaver and 18 (11.3%) met MetS criteria. Among components of MetS, elevated BP was the most prevalent (122 women; 76.7%; hypertension in 104 women), followed by hypertriglyceridemia and obesity in 28 (17.6%) and 26 women (16.4%), respectively. Low HDL cholesterol and IFG were found in 23 (14.5%) and 19 women (11.9%), respectively, and BMI ≥30.0 kg/m^2 in only 3 women (1.9%).

Women with as compared with without MetS had a higher prevalence of CKD using eGFRaver whereas no significant difference was found in prevalence of CKD with either eGFRcreat or eGFRcys between the two groups of women (Table 1). There was no significant difference in serum concentrations of creatinine and cystatin C, and three estimates of GFR. By definition, BMI, fasting glucose, and triglycerides were higher and HDL cholesterol was lower in women with

TABLE 2. CHARACTERISTICS OF ELDERLY WOMEN ACCORDING TO THE NUMBER OF COMPONENTS OF METABOLIC SYNDROME PRESENT

	Number of components						
	0 (n=*21*)	*1* (n=*80*)	*2* (n=*40*)	*3* (n=*16*)	*4* (n=*1*)	*5* (n=*1*)	P *value*
BMI (kg/m^2)	21.3±2.2	21.5±2.1	23.9±3.1	25.4±3.1	29.1	26.1	0.000
FMI (kg/m^2)	6.2±1.9	6.7±1.7	8.5±2.6	9.7±2.6	12.0	12.3	0.000
Systolic BP (mmHg)	120±8	148±18	144±17	145±18	177	134	0.007
Diastolic BP (mmHg)	75±7	86±11	85±10	83±6	102	83	0.036
Fasting glucose (mg/dL)	82.7±8.2	85.0±8.5	89.4±12.1	97.1±15.0	169.0	102.0	0.000
Fasting insulin (μU/mL)	4.8±3.1	4.9±3.5	5.9±3.0	5.9±2.8	18.4	32.4	0.003
HbA1c (%)	5.5±0.2	5.5±0.3	5.7±0.5	6.1±0.5	7.4	5.4	0.000
HOMA-IR	1.0±0.7	1.1±0.8	1.3±0.8	1.4±0.7	7.7	8.2	0.000
Log insulin	0.61±0.25	0.63±0.21	0.72±0.21	0.72±0.21	1.26	1.51	0.003
Log HOMLA-IR	−0.08±0.27	−0.05±0.22	0.06±0.23	0.10±0.22	0.89	0.91	0.000
Cystatin C (mg/L)	0.8±0.1	0.8±0.2	0.9±0.2	0.9±0.3	1.5	1.4	0.053
eGFRcreat (mL/min/1.73m^2)	65±12	61±13	62±14	66±19	29	42	0.405
eGFRcreat <60 mL/min/1.73m^2 (%)	38.1	47.5	47.5	43.8	100	100	0.701

Data presented as mean ± standard deviation or *n*, %.

MetS than in those without MetS. However, there was no difference in systolic and diastolic BP levels, and the percentage of women with hypertension (63% and 83%, respectively, $P=0.09$) between women with and without MetS. Log alanine-aminotransferase, log gamma-glutamyltransferase, HbA1c, log fasting insulin and log HOMA-IR were higher in women with than those without MetS.

The number of participants with 0, 1, 2, 3, 4, and 5 components were 21 (13.2%), 80 (50.3%), 40 (25.2%), 16 (10.1%), 1 (0.6%), and 1 (0.6%), respectively. As the number of components of MetS increased (Fig. 1), the prevalence of CKD using eGFRaver increased from 4.8% in women without MetS component to 31.3% in those with 3 components of MetS. Both of the women with 4 or 5 components had CKD. As compared with women without component, the mean of eGFRaver decreased to the same extent among three groups of women with 1–3 components of MetS (Fig. 1). Therefore, differences in eGFRaver among the six groups were not significant ($P=0.138$) when using Jonckheeree-Terpstra test. However, differences among 6 groups were highly significant when analysis of variance was applied ($P=0.009$). Analysis of variance excluding 2 participants with 4 and 5 components showed no difference in eGFRaver among 4 groups with 0–3 components ($P=0.115$), demonstrating no association between the number of MetS components and eGFRaver values. There was no difference in the prevalence of CKD using eGFRcreat and mean eGFRcreat among the six groups (Table 2). As the number of components of MetS increased, fat mass index, fasting glucose and insulin, and HOMA-IR increased. HbA1c and serum cystatin C were higher in women with 2 or more components than women with 1 or fewer MetS component (Table 2). Women with 1 or more MetS component had higher systolic and diastolic BP as compared with women who had none of MetS components. Elevated BP was found in 89%, 85%, and 94% of women with 1 component, 2 components, and those with MetS, respectively, and 75%, 73%, and 83%, respectively, had hypertension.

Discussion

Prevalence of CKD varied substantially depending on the used equation in community-living, ambulatory Japanese elderly women; 46.5% of CKD prevalence using eGFRcreat was much higher than 12.6% of prevalence using eGFRcys. Both the presence of MetS and the number of MetS components were associated with the higher prevalence of CKD even in elderly Japanese women, who have the highest life expectancy in the world.[29] However, this was evident only when CKD was assessed by the average of eGFRcreat and eGFRcys. We confirmed graded relations between the number of clinical traits of MetS and the prevalence of CKD. It is noted that these findings were observed in community-living ambulatory elderly women in whom 65.4% had hypertension and only 7.5% had diabetes.

CKD prevalence was substantially higher when using eGFRcreat as compared with eGFRcys in Japanese elderly women. It is consistent with results from studies conducted in elderly Western individuals[31–33] and has been a consistent feature in a systematic review.[34] In the present study, associations of the presence of MetS and the number of MetS components with the higher prevalence of CKD were found only when CKD was diagnosed using eGFRaver. These findings may be in line with the observation that hypertension was significantly associated with CKD progression using the combined cystatin C and creatinine equation but not using the creatinine equation in the Multi-Ethnic Study of Atherosclerosis.[35]

The present study confirmed results of meta-analysis using 11 prospective observational studies in middle-aged people[36] and extended that both the presence of MetS and the number of MetS components were associated with the higher prevalence of CKD even in elderly Japanese women. Lower eGFR and higher CKD prevalence were evident in 80 women with only one MetS component, of whom 71 (88.8%) women had elevated BP; 60 had hypertension and 11 had high-normal BP, defined as systolic/diastolic BP of 130–139/85–89 mmHg. High-normal blood pressure has recently been reported to be an independent predictor of impaired renal function, defined as eGFR <60 mL/min/1.73 m^2, in a population-based cohort of 1307 subjects free of diabetes, cardiovascular and renal disease.[37] Failure to detect significant association between the presence of MetS and the mean of average eGFR in the present study may be due to the small number of women with MetS. Failure to detect significant association between the number of MetS components and the mean of average eGFR may be due in part to the predominance of elevated BP over metabolic components (IFG, obesity, and dyslipidemia). Approximately 90% of women with one or more components had elevated BP.

Our study has several limitations. We are unable to determine either the direction of association or the causal pathway given the cross-sectional design of our study. The recruitment procedure may also have had an effect on the results. As participation was voluntary, women who pay more attention to their health may have been more likely to participate. Biochemical parameters, including cystatin levels, were measured only once. Although 43, 9, and 58 women (27.0%, 5.7%, and 36.5%, respectively) reported to be receiving statins, antidiabetic drugs, and antihypertensive drugs, respectively, detailed drug information was not available. These drugs may have substantial effects on serum cystatin C levels.[38–40] In addition, a more direct measurement of GFR, such as inulin clearance, was not used in this study as a gold standard for comparison. We also lacked measures of urine albumin excretion. Finally, BMI in lieu of waist circumference was used for defining MetS, and participants were relatively small in number and were all females.

In conclusion, the present studies have demonstrated associations of the presence of MetS and the number of MetS components with higher prevalence of CKD in nonobese elderly Japanese women and suggest that elevated blood pressure may play an important role in these associations. These findings should be confirmed in studies employing more participants with MetS diagnosed using standard criteria (waist circumference instead of BMI).

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Author Disclosure Statement

No competing financial interests exist.

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Association of Metabolic Syndrome with Serum Adipokines in Community-Living Elderly Japanese Women: Independent Association with Plasminogen Activator-Inhibitor-1

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Abstract

Background: Associations between metabolic syndrome (MetS) with serum adipokines and basal lipoprotein lipase mass (serum LPL) have not been extensively studied in elderly Asians, who in general have lower body mass index than European populations.
Methods: A cross-sectional analysis was conducted including 159 community-living elderly Japanese women whose age averaged 77 years. MetS was defined by the modified National Cholesterol Education Program Adult Treatment Panel III criteria, but using a body mass index ≥25 kg/m^2 instead of waist circumference. Serum LPL, leptin, adiponectin, plasminogen activator inhibitor 1 (PAI-1), interleukin-6, tumor necrosis factor-alpha, and high-sensitivity C-reactive protein were measured.
Results: Both the presence of MetS and the number of MetS components were associated with higher homeostasis assessment of insulin resistance, serum levels of leptin, PAI-1, and tumor necrosis factor-alpha and with lower serum levels of LPL and adiponectin (all $P<0.05$), but not with high-sensitivity C-reactive protein and interleukin-6. Among six biomarkers of MetS, PAI-1 remained associated with MetS independent of fat mass index and insulin resistance.
Conclusions: Although proinflammatory, prothrombotic, and anti-inflammatory states were associated with MetS, higher PAI-1 was associated with MetS independent of fat mass index and insulin resistance in elderly Japanese women, in whom obesity is rare.

Introduction

METABOLIC SYNDROME (MetS) describes the clustering of obesity, dyslipidemia, elevated blood pressure (BP) and hyperglycemia,[1] and it is a strong, independent contributor to the onset of type 2 diabetes mellitus and cardiovascular disease.[1,2] Although current evidence supports that insulin resistance plays a central role in MetS,[3] a proinflammatory state and a prothrombotic state are usually present with MetS[4]; the former is indicated by increased circulating C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) and the latter by elevated plasminogen activator-inhibitor-1 (PAI-1). In contrast, serum levels of adiponectin, an anti-inflammatory and antithrombotic adipokine, were low in insulin resistance and MetS.[4,5]

It has been hypothesized that adipokines are a possible link between obesity and other components of MetS.[5,6] For example, previously, we showed that low serum adiponectin was more closely related to adiposity and dyslipidemia than insulin resistance in young healthy men.[7] Recently, we found that decreased serum adiponectin was associated more closely with abdominal obesity and low serum lipoprotein lipase mass than with insulin resistance and inflammation.[8] Serum levels of proinflammatory, procoagulant, and anti-inflammatory biomarkers differ significantly in the context of sex and ethnicity.[9] Because obesity and ageing are known to affect glucose and lipid metabolism, specifically in women,[10] and because very little information is available on the relationship between circulating adipokines and MetS in Asian elderly populations, we evaluated these issues in community-living elderly Japanese women. Kidney function was evaluated in the present study as well, because it is also well known that declining kidney function is common in the elderly and is associated with dysregulation of serum adipokines.[11–13]

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Methods

We examined 159 community-living elderly women whose details have been reported elsewhere.[13] They were residents in Nishinomiya, an urban area of Hyogo, Japan and were recruited as volunteers by local welfare commissioners from the city of Nishinomiya. Although 43, 9, and 58 women (27.0%, 5.7%, and 36.5%, respectively) reported to be receiving statins, antidiabetic drugs, and antihypertensive drugs, respectively, detailed drug information was not available. Subjects with clinically diagnosed acute or chronic inflammatory diseases and cancer were excluded from the study. This research followed the tenets of the Declaration of Helsinki. The design of this study was approved by the Ethical Committees of Mukogawa Women's University and written informed consent was obtained from all participants.

Anthropometric indices were measured after an overnight fasting. Thereafter, blood samples were obtained from the cubital vein. Fat mass was measured using an impedance method (InBody 430, Biospace). Fat mass index (FMI) was calculated as fat mass dividing by the square of height (kg/m^2). We evaluated routine chemical parameters, including glucose, insulin, serum lipids, and lipoproteins as previously reported.[13] Insulin resistance was determined by homeostasis model assessment of insulin resistance (HOMA-IR) using fasting plasma glucose and insulin levels.[14] Lipoprotein lipase mass in preheparin serum (from now on referred to as basal LPL) was measured by the sandwich enzyme-linked immunosorbent assay (ELISA) with a specific monoclonal antibody against bovine milk LPL[15]using commercially available kits from Daiichi Pure Chemicals [interassay coefficient of variation (CV) = 2.8%].

Adiponectin was assayed by a sandwich ELISA (Otsuka Pharmaceutical Co., Ltd.). Intra- and interassay CV were 3.3% and 7.5%, respectively. Leptin was assessed using a Radioimmunoassay (RIA) Kit from LINCO research (interassay CV = 4.9%). High-sensitivity CRP was measured by an immunoturbidometric assay with the use of reagents and calibrators from Dade Behring Marburg GmbH (interassay CV <5%). TNF-α was measured by immunoassays (R&D Systems, Inc., Minneapolis, MN; interassay CV <6%). IL-6 was measured by a commercially available kit (IL-6, Human, ELISA Kit, QuantiGlo, 2nd Generation, Funakoshi Co., Ltd.). PAI-1 was measured by an ELISA method (Mitsubishi Chemicals; interassay CV <8%).

Metabolic syndrome was defined using the modified criteria of the National Cholesterol Education Program Adult Treatment Panel III guidelines.[16] Because body mass index (BMI) is not inferior to waist circumference as a practical marker of the metabolic risk clustering in Japanese[17] and because BMI may be better than waist circumference for defining metabolic syndrome in Japanese women,[18], adiposity was defined using Asian criteria as BMI ≥25.0 kg/m^2.[19] Elevated BP was defined as systolic/diastolic BPs of 130/85 mmHg or greater and/or current use of antihypertensive medicine. Hypertriglyceridemia was defined as a serum triglyceride level of 150 mg/dL or greater. Low high-density lipoprotein (HDL) cholesterol level was defined as less than 50 mg/dL since all participants are women. Impaired fasting glucose was defined as fasting blood plasma level of 100 mg/dL or greater and/or current use of antidiabetic medicine. Metabolic syndrome was defined as the presence of three or more components.[16]

Serum creatinine was measured enzymatically using an autoanalyzer (AU 5200, Olympus) and cystatin C by latex immunoassay using a commercially available kit (IatroCys-C, Mitsubishi Chemical Medience). The estimated glomerular filtration rate (eGFR) was calculated using the equation recommended by the Japanese Society for Nephrology.[20,21] The average of creatinine-based and cystatin C-based eGFR was calculated in each participant[22] and was used in analysis.

Data were presented as mean ± standard deviation unless otherwise stated. Due to deviation from normal distribution, high-sensitivity CRP (hsCRP) and IL-6 were logarithmic transformed for analysis. Z-scores were calculated for inflammatory markers in each participants. Comparisons between women with and without MetS were made with two-sample *t*-tests and analysis of covariance to adjust for FMI and BMI. The association of adipokine levels with the number of components of MetS and *P* values for trend were derived using Jonckheeree-Terpstra test. Pearson correlation coefficients were computed to assess the association between HOMA-IR and anthropometric and metabolic variables. Bivariate and multivariate logistic regression with forward stepwise selection were performed to further identify the most significant variables contributing to MetS. Potential confounders of interest were forced into the model and odds ratios and 95% confidential intervals were calculated. A two-tailed $P < 0.05$ was considered statistically significant. All calculations were performed with SPSS system 15.0 (SPSS Inc.).

Results

As previously reported,[13] participants were relatively healthy, community-living, ambulatory elderly women (Table 1). HOMA-IR was positively associated with BMI and FMI and inversely with HDL cholesterol while HOMA-IR was not significantly associated with serum triglycerides (TG) and LPL (Table 1). In addition, HOMA-IR showed negative association with average eGFR ($eGFR_{aver}$). Further, it was associated positively with serum leptin, TNF-α, and PAI-1, whereas associations with adiponectin, hsCRP, and IL-6 were not significant.

Eighteen women (11.3%) met MetS criteria. Women with MetS had higher BMI and FMI compared with those without MetS (Table 2). As expected, those with MetS had higher TG and lower HDL. In addition, women with MetS had lower basal LPL. Further, they had higher serum leptin, TNF-α, PAI-1, and lower adiponectin. However, HOMA-IR, log hsCRP, and log IL-6 did not differ significantly between the two groups. Adjustment for eGFR had no effect on the results. However, associations with serum LPL, adiponectin, and leptin disappeared after adjustment for FMI, a better indicator in the screening of MetS than BMI and percentage body fat.[23] In contrast, associations remained significant with HDL cholesterol (59 ± 3 [SE] vs. 69 ± 1 mg/dL, $P = 0.001$), TG (145 ± 12 vs. 111 ± 5 mg/dL, $P = 0.009$), TNF-α (3.2 ± 0.2 vs. 2.2 ± 0.1 pg/mL, $P < 0.001$) and PAI-1 (33.1 ± 1.7 vs. 27.8 ± 0.8 ng/mL, $P = 0.005$) even after adjustment for FMI.

The number of participants with 0, 1, 2, 3, 4, and 5 components were 21 (13.2%), 80 (50.3%), 40 (25.2%), 16 (10.1%), 1 (0.6%), and 1 (0.6%), respectively. Among components of MetS, elevated BP was the most prevalent (122 women; 76.7%). Hypertriglyceridemia and obesity were found in 28 (17.6%) and 26 women (16.4%),

TABLE 1. ANTHROPOMETRIC AND BIOCHEMICAL FEATURES OF ELDERLY WOMEN STUDIED AND CORRELATION COEFFICIENTS OF HOMEOSTASIS MODEL ASSESSMENT OF INSULIN RESISTANCE

Feature	*Mean ± SD*	*HOMA-IR rho*	P *value*
Age (years)	76 ± 8	−0.012	0.882
Body mass index (kg/m^2)	22.6 ± 2.9	0.322	0.000
Fat mass index (kg/m^2)	7.5 ± 2.4	0.333	0.000
Fasting glucose (mg/dL)	88 ± 13	0.519	0.000
Fasting insulin (μU/mL)	5.5 ± 4.0	0.961	0.000
HOMA-IR	1.23 ± 1.08	1.000	—
Total cholesterol (mg/dL)	221 ± 33	−0.106	0.182
HDL cholesterol (mg/dL)	66 ± 16	−0.246	0.002
Non-HDL cholesterol (mg/dL)	154 ± 32	0.011	0.886
Triglyceride (mg/dL)	119 ± 65	0.147	0.065
Basal LPL (ng/mL)	93 ± 26	−0.057	0.473
$eGFR_{aver}$ (mL/min/1.73m^2)	71 ± 16	−0.215	0.007
Leptin (ng/mL)	9.2 ± 6.2	0.524	0.000
Adiponectin (μg/mL)	15.8 ± 7.4	−0.142	0.073
hsCRP (μg/dL)	214 ± 369	−0.025	0.757
TNF-α (pg/mL)	2.3 ± 1.2	0.224	0.004
PAI-1 (ng/mL)	29.0 ± 10.9	0.345	0.000
IL-6 (pg/mL)	5.5 ± 12.0	−0.039	0.626
Leukocytes (×10^3/μL)	5.8 ± 1.4	−0.060	0.449

$eGFR_{aver}$, the average of creatinine-based cystatin C-based estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance; hsCRP, high-sensitivity C-reactive protein; LPL, lipoprotein lipase; TNF, tumor necrosis factor; PAI-1, plasminogen activator inhibitor-1; IL-6, interleukin-6.

respectively. However, only 3 women (1.9%) had BMI ≥30.0 kg/m^2. Low HDL cholesterol and impaired fasting glucose were in 18 (11.3%) and 19 women (11.9%), respectively.

As the number of components of MetS increased (Table 3), serum leptin, TNF-α, and PAI-1 increased and serum adiponectin decreased, whereas there was no significant difference in hsCRP, IL-6, and leukocyte count. Associations with TNF-α, PAI-1, leptin, and adiponectin remained significant after adjustment for kidney function (Fig. 1). In contrast, associations with adiponectin and leptin disappeared after adjustment for FMI (data not shown).

As the number of components of MetS increased (Table 3), HDL cholesterol and basal LPL decreased, whereas FMI, HOMA-IR, and serum TG increased. There was no significant difference in total and non-HDL cholesterol and $eGFR_{aver}$. The association with HDL cholesterol remained but associations with TG and basal LPL disappeared after adjustment for FMI (data not shown).

Bivariate logistic regression analysis demonstrated significant associations with MetS of HOMA-IR, all lipid variables, and adipokines examined except for hsCRP and IL-6 (Table 4, model A). Among six adipokines, PAI-1 only showed association with MetS independent of FMI and HOMA-IR (Table 4, model B). Among lipid variables, HDL cholesterol showed association with MetS independent of FMI and HOMA-IR (model C). In a model that included all lipid variables and adipokines (model D), only HDL cholesterol remained associated with MetS independent of FMI and HOMA-IR. Addition of percentage body fat to model D did not change the results (data not shown).

TABLE 2. ANTHROPOMETRIC AND BIOCHEMICAL FEATURES OF ELDERLY WOMEN WITH METABOLIC SYNDROME

Feature	*MetS(−)* n = *141*	*MetS(+)* n = *18*	P *value*
Age (years)	76 ± 8	76 ± 7	0.782
Body mass index (kg/m^2)	22.2 ± 2.7	25.7 ± 3.1	0.000
Fat mass index (kg/m^2)	7.1 ± 2.2	10.0 ± 2.5	0.000
Fasting glucose (mg/dL)	86 ± 10	101 ± 22	0.009
Fasting insulin (μU/mL)	5.2 ± 3.3	8.1 ± 7.2	0.114
HOMA-IR	1.12 ± 0.79	2.13 ± 2.20	0.069
Total cholesterol (mg/dL)	222 ± 31	209 ± 42	0.118
HDL cholesterol (mg/dL)	69 ± 15	49 ± 12	0.000
non-HDL cholesterol (mg/dL)	153 ± 30	161 ± 41	0.364
Triglyceride (mg/dL)	113 ± 57	171 ± 95	0.020
Basal LPL (ng/mL)	95 ± 26	75 ± 20	0.002
eGFRaver (mL/min/1.73m^2)	71 ± 15	68 ± 21	0.576
Leptin (ng/mL)	8.6 ± 5.3	13.7 ± 9.9	0.045
Adiponectin (μg/mL)	16.3 ± 7.5	11.8 ± 5.0	0.015
hsCRP (μg/dl)	226 ± 387	119 ± 150	0.032
TNF-α (pg/mL)	2.2 ± 1.0	3.4 ± 2.4	0.048
PAI-1 (ng/mL)	27.7 ± 10.0	39.2 ± 12.5	0.000
IL-6 (pg/mL)	5.7 ± 12.7	3.7 ± 2.8	0.495
hsCRP *z*-score	0.03 ± 1.05	−0.27 ± 0.40	0.024
TNF-α *z*-score	−0.12 ± 0.77	0.85 ± 1.89	0.046
PAI-1 *z*-score	−0.12 ± 0.92	0.94 ± 1.14	0.000
IL-6 *z*-score	0.01 ± 1.06	−0.16 ± 0.24	0.511
log(hsCRP)	1.88 ± 0.62	1.90 ± 0.37	0.864
log(TNF-α)	0.30 ± 0.20	0.44 ± 0.28	0.005
log(IL-6)	0.49 ± 0.42	0.46 ± 0.31	0.799
Leukocytes (×10^3/μL)	5.8 ± 1.4	5.8 ± 1.5	0.923

MetS, metabolic syndrome.

Discussion

The present study shows that the presence and severity (number of components) of the metabolic syndrome are highly associated with circulating levels of adipokines (TNF-α, PAI-1, leptin, and adiponectin) and basal LPL in well-functioning older Japanese women after adjustment for kidney function. These data extend previous reports done predominantly in young or middle-aged subjects[24–28] and show that these relationships still hold in these older Japanese women, in whom the prevalence of obesity, defined by the World Health Organization as body mass index (BMI) ≥30 kg/m^2, is no more than 2%–3%.[29] Among six biomarkers of MetS, PAI-1 remained associated with MetS independent of fat mass index and insulin resistance.

In addition to sex and ethnicity,[9] reduced kidney function, which is common in the elderly, is known to be associated with dysregulation of serum proinflammatory, procoagulant, and anti-inflammatory biomarkers.[11–13] You et al.[30] and Stenholm et al.[31] examined associations of metabolic syndrome with a total of more than six proinflammatory, prothrombotic, and anti-inflammatory cytokines in well-functioning older adults. Although they found associations between the presence of metabolic syndrome and dysregulation of a broad range of the above-mentioned cytokines,[30,31] no information was available on kidney

TABLE 3. ANTHROPOMETRIC AND BIOCHEMICAL FEATURES OF ELDERLY WOMEN AS A FUNCTION OF THE NUMBER OF COMPONENTS OF METABOLIC SYNDROME

	Number of components				
Feature	*0 (n=21)*	*1 (n=80)*	*2 (n=40)*	*3–5 (n=18)*	P *value*
Age (years)	69±8[a]	78±7[b]	75±8[b]	76±7[b]	0.000
BMI (kg/m^2)	21.3±2.2[a]	21.5±2.1[a]	23.9±3.1[b]	25.7±3.1[b]	0.000
Fat mass index (kg/m^2)	6.2±1.9[a]	6.7±1.7[a]	8.5±2.6[b]	10.0±2.5[b]	0.000
Total cholesterol (mg/dL)	226±38	222±29	220±33	209±42	0.398
HDL cholesterol (mg/dL)	74±17[a]	71±13[a]	61±14[b]	49±12[c]	0.000
non-HDL cholesterol (mg/dL)	152±39	151±27	159±32	161±41	0.434
Triglyceride (mg/dL)	85±27[a]	102±39[a]	148±80[b]	171±95[b]	0.000
Basal LPL (ng/mL)	110±37[a]	95±22[a,b]	88±26[b,c]	75±20[c]	0.000
eGFR$_{aver}$ (mL/min/1.73m^2)	79±16	70±14	70±16	68±21	0.145
Leptin (ng/mL)	7.1±4.3[a]	7.8±4.7[a]	11.1±6.2[b]	13.7±9.9[a,b]	0.004
Adiponectin (μg/mL)	17.1±7.5[a,b]	17.2±7.6[a]	14.1±6.9[a,b]	11.8±5.0[b]	0.011
hsCRP (μg/dL)	174±343	202±334	300±492	119±150	0.156
TNF-α (pg/ mL)	1.9±0.8	2.2±1.0	2.3±0.9	3.4±2.4	0.060
PAI-1 (ng/ mL)	24.4±6.5[a]	25.9±8.8[a]	32.9±11.9[b]	39.2±12.5[b]	0.000
IL-6 (pg/ mL)	4.1±4.8	5.2±6.5	7.6±21.8	3.7±2.8	0.583
Log hsCRP	1.71±0.64	1.86±0.60	2.02±0.64	1.90±0.37	0.266
Log TNF-α	0.24±0.18[a]	0.30±0.21[a]	0.33±0.18[a,b]	0.44±0.28[b]	0.017
Log IL-6	0.44±0.39	0.50±0.41	0.48±0.46	0.46±0.31	0.938
Leukocytes (×10^3/ μL)	5.5±1.3	5.8±1.5	6.0±1.4	5.8±1.5	0.669

Means not sharing common letters are significantly different from each other at $P<0.05$ or less.
BMI, body mass index.

function. In the present study, associations between the presence of metabolic syndrome and dysregulation of proinflammatory, prothrombotic, and anti-inflammatory cytokines still hold even after taking into account the average of creatinine-based and cystatin C-based eGFR, which has been reported to have greater precision and accuracy than the individual creatinine and cystatin C equations in the elderly.[22]

Whether metabolic syndrome is linked with adipokines in well-functioning elderly persons has been reported in two studies only. Studies from the United States[30] and Italy[31] have shown that circulating CRP and IL-6 were significantly higher among older persons with MetS compared with those without MetS. Big differences between the previous and the present Japanese study are in BMI and fat mass. BMI in persons without MetS averaged 22.2 and 25.7 kg/m^2, and fat mass averaged 15.9 and 23.3 kg in Japanese and American studies[30] respectively. The prevalence of obesity (BMI ≥30 kg/m^2) is 15.0% in older Italians[31] in the absence of MetS, whereas it is 1.9% in Japanese elderly women even in the presence of MetS. Although the differences in CRP and IL-6 levels between persons with and without MetS were pronounced in the highest body fat percentage tertile in older Americans,[30] the difference in IL-6 in the lowest body fat percentage tertile is modest and the difference in CRP is no longer significant between the two groups.[30] These findings are consistent with results in the present study. It has been shown that associations among biomarkers of metabolic syndrome are stronger in obese women than in lean women.[32]

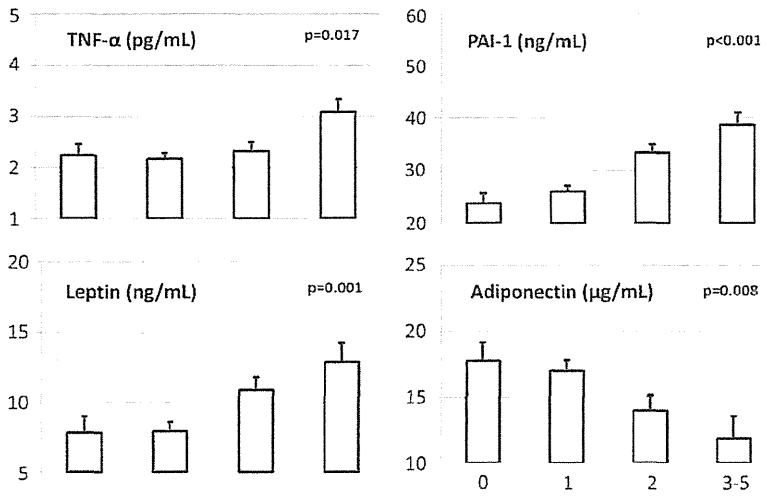

FIG. 1. Serum levels of tumor necrosis factor-α (TNF-α), plasminogen activator-inhibitor-1 (PAI-1), leptin, and adiponectin after adjustment for the average of creatinine-based and cystatin C–based estimated glomerular filtration rate as a function of the number of metabolic syndrome components. The number of participants with 0, 1, 2, 3, 4, and 5 components was 21, 80, 40, 16, 1, and 1, respectively. Data are presented as means ± standard error.

TABLE 4. BIVARIATE (MODEL A) AND MULTIVARIATE (MODELS B–D) STEPWISE LOGISTIC REGRESSION ANALYSIS FOR METABOLIC SYNDROME ACCORDING TO ADIPOKINES AND SERUM LIPID VARIABLES

	OR (95% CI)	P
Model A		
FMI (kg/m^2)	1.660 (1.306–2.110)	0.000
HOMA-IR	1.696 (1.179–2.441)	0.004
Adiponectin (μg/mL)	0.887 (0.804–0.979)	0.017
Leptin (ng/mL)	1.107 (1.035–1.183)	0.003
Log hsCRP	1.050 (0.462–2.391)	0.907
Log IL-6	0.851 (0.248–2.919)	0.798
Log TNF-α	35.0 (2.6–471)	0.007
PAI-1 (ng/mL)	1.085 (1.038–1.134)	0.000
Basal LPL (ng/mL)	0.957 (0.930–0.984)	0.002
HDL cholesterol (mg/dL)	0.863 (0.809–0.920)	0.000
TG (mg/dL)	1.010 (1.004–1.016)	0.002
Model B		
FMI (kg/m^2)	1.429 (1.108–1.843)	0.006
PAI-1 (ng/mL)	1.087 (1.024–1.155)	0.007
Model C		
FMI (kg/m^2)	1.703 (1.249–2.321)	0.001
HDL cholesterol (mg/dL)	0.845 (0.778–0.918)	0.000
Model D		
FMI (kg/m^2)	1.703 (1.249–2.321)	0.001
HDL cholesterol (mg/dL)	0.845 (0.778–0.918)	0.000

Covariates included in each model are as follows: *Model B*, fat mass index (FMI), HOMA-IR, leptin, adiponectin, PAI-1, log(hsCRP), log(TNF-α), log(IL-6); *Model C*, FMI, HDL cholesterol, triglycerides (TG), serum LPL; *Model D*, all variables in *Model A*.

CI, confidence interval; OR, odds ratio.

There was a strong relationship between the number of metabolic syndrome components and these inflammatory and fibrinolytic variables even after controlling for FMI in the present study. However, the relationship with serum leptin, adiponectin, and basal LPL appeared to be largely the result of fat mass, as it was markedly attenuated, becoming nonsignificant after adjusting for FMI, although LPL is most abundant in adipose tissue and skeletal muscle.[33] It is well known that leptin[34] and adiponectin[35] are secreted from adipocytes.

Among six biomarkers of MetS, PAI-1 remained associated with MetS on multivariable logistic regression in community-dwelling Japanese women in the present study. Increased PAI-1 levels have even been proposed to be a true component of MetS.[36,37] In addition, a higher PAI-1 level also has been demonstrated to predict the incidence of diabetes[38] and hypertension[39] in community-based samples. Furthermore, higher plasma PAI-1 was associated with future development of MetS and with longitudinal changes in metabolic risk factors.[40]

Insulin resistance as assessed using HOMA-IR was not independently associated with MetS in the present study (Table 4, model B), although insulin resistance increased as the number of components of MetS increased. This may be due in part to lower BMI in our elderly women as described above because adiposity is one of major determinants of insulin resistance.

Our study has several limitations. BMI instead of waist circumference was used for defining metabolic syndrome. Therefore, metabolic syndrome in our participants is not standard but modified. We are unable to determine either the direction of association or the causal pathway given the cross-sectional design of our study. The recruitment procedure may also have had an impact on the results. As participation was voluntary, women who pay more attention to their health may have been more likely to participate. Biochemical parameters, including cystatin levels, were measured only once. Although 43, 9, and 58 women (27.0%, 5.7%, and 36.5%, respectively) reported to be receiving statin, antidiabetic, and antihypertensive drugs, respectively, detailed drug information was not available. These drugs may have effects on serum adipokine levels. For example, clinical data support a role for the thiazolidinediones in correcting dyslipidemia, decreasing inflammation, and improving the adipokine profile.[41] In addition, post-heparin LPL activity was not measured. Finally, participants were relatively small in number and were all females.

Conclusion

Although proinflammatory, prothrombotic, and anti-inflammatory states were associated with MetS, higher PAI-1 was associated with the presence of MetS independent of fat mass index and insulin resistance in elderly women in Japan, where obesity is rare.

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Author Disclosure Statement

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Original Article

Low haemoglobin levels contribute to low grip strength independent of low-grade inflammation in Japanese elderly women

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Muscle strength declines with age. However, factors that contribute to such declines are not well documented and have not been extensively studied in elderly populations of Asian origin. Correlations of grip strength with a broad range of factors associated with declines in muscle strength were examined in 202 community-living elderly Japanese women. After adjustment for age, grip strength was positively correlated with body weight, height, serum albumin, haemoglobin, high-density lipoprotein cholesterol (HDL-C) and serum iron and inversely with serum copper, and log high-sensitivity C-reactive protein (hsCRP). Multiple linear regression analysis with grip strength as a dependent variable showed that 47.0% of variability of grip strength could be accounted for by height, age and haemoglobin in order of increasing R^2. In conclusion, low haemoglobin may contribute to low muscle strength independently of age, anthropometric, nutritional, and inflammatory markers in the elderly, and may represent an important confounder of the association between grip strength and functional decline in community-living Japanese elderly women.

Key Words: grip strength, haemoglobin, height, age, elderly women

INTRODUCTION

The rapid increase in the prevalence of older persons in the general population has been accompanied by substantial interest in identifying those biomarkers which are able to predict functional decline and mortality in the elderly.[1] Among the biomarkers which predict functional decline in the elderly population, C-reactive protein (CRP), an inflammatory marker, and serum albumin, a laboratory variable commonly used to assess nutritional status, seem to play a major role.[2,3] We have recently shown that a modest increase in serum copper and subtle decreases in serum iron and zinc are associated with inflammatory markers in community-living Japanese elderly women.[4,5]

Grip strength, an approximation of total body muscle strength, has been found to be a robust predictor of functional decline, frailty and mortality.[6-13] However, underlying mechanisms of these associations are poorly understood and have not been extensively studied in elderly populations of Asian origin. Women as compared to men and older people as compared to younger people are known to have lower absolute and relative muscle strength.[14] Therefore, we examined relationships between grip strength and a broad range of factors associated with declines in muscle strength in community-living Japanese elderly women.

PARTICIPANTS AND METHODS

We examined 202 free-living elderly women whose details have previously been reported elsewhere.[4,5,15] Participants were residents in central urban area of Nishinomiya, Hyogo, Japan and were recruited as volunteers by local welfare commissioners. None of the subjects reported to have cancer, or clinically diagnosed acute or chronic inflammatory diseases. However, information was not available on drugs and nutritional supplements which the subjects consumed. Of 202 women, 105 had hypertension (systolic/diastolic blood pressure ≥140/90 mmHg) and 14 had glucose dysregulation (casual plasma glucose ≥140 mg/dL). This research followed the tenets of the Declaration of Helsinki. The study was approved by the Ethics

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Committees of Mukogawa Women's University (No.11-07) and written informed consent was obtained from each participant.

Anthropometric indices and blood pressure were measured between breakfast and lunch and thereafter, and the blood samples were obtained from the cubital vein. Fat mass was measured using an impedance method (In Body 430, Biospace, Tokyo, Japan). Blood pressure was measured using an automated sphygmomanometer (BP-203RV II, Colin, Tokyo, Japan) after participants had rested at least 5 mins.

Grip strength was measured with a handheld dynamometer (T.K.K.5401, Takei Scientific Instruments, Tokyo, Japan). The participant was asked to stand up and hold the dynamometer in the dominant hand with the arm parallel to the body without squeezing the arm against the body. The width of the handle was adjusted to the size of the hand to make sure that the middle phalanx rested on the inner handle. Two trials for the dominant hand were performed and the stronger results were used in analyses. Grip strength was expressed in kilograms (Kg).

Plasma glucose, serum insulin, lipids and lipoproteins were assayed as previously reported.[16,17] Because of non-fasted blood sampling, non-HDL cholesterol was calculated as the difference between total and HDL cholesterol. Adiponectin, leptin and high-sensitivity CRP (hsCRP), a marker of very low levels of inflammation in healthy individuals,[16] were assayed by respective commercially available kits as previously reported.[16,17] Complete blood cell count was analyzed using an automated blood cell counter (Sysmex XE-2100, Sysmex, Kobe, Japan). Serum iron, zinc and copper were measured as previously reported.[4,5]

Serum creatinine was measured enzymatically using an autoanalyzer (AU 5200, Olympus, Tokyo, Japan). The estimated glomerular filtration rate (eGFR) was determined using the equation recommended by the Japanese Society for Nephrology.[18]

Data were presented as mean±SD unless otherwise stated. Due to deviation from normal distribution, hsCRP was logarithmically transformed for analysis. Differences between 2 groups were analyzed by *t* test and frequencies of conditions by Chi-square tests. Differences among 3 groups or more were analyzed using analysis of variance. When *p* values in analysis of variance were $p<0.05$, Bonferroni's multiple comparison procedure was performed. Bivariate correlations were evaluated by Pearson correlation analysis. Stepwise multiple linear regression analyses were performed to further indentify the most significant variables contributing to the variation of grip strength. Confounder variables included were age, comorbidities (hypertension and glucose dysregulation) and variables which showed significant associations with grip strength after adjustment for age. The independent association of low grip strength with non-parametric data was examined by multiple logistic analysis. A two-tailed $p<0.05$ was considered statistically significant. All calculations were performed with SPSS system 15.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

As previously reported,[4,5] participants were apparently healthy, ambulatory elderly women with a similar prevalence of anaemia (haemoglobin <12 g/dL) and reduced renal function (eGFR <60 mL/min/1.73 m^2) to Japanese women aged 70 years and older in the general population. Severe anaemia (haemoglobin <11.0 g/dL) was found only in 11 women (5.4%) and moderate-severe renal insufficiency (eGFR <45 mL/min/1.73 m^2) only in 12 women (5.9%).

Mean grip strength in our participants (20.4±5.3 kg) was slightly lower than an average of 22.2±3.9 kg of Japanese women aged 75-79 years.[19] In Pearson's correlation analysis (Table 1), height and body weight were strongly and positively, and age was strongly and inversely correlated with grip strength. Although there was a modest correlation between grip strength and BMI, there was no correlation with percentage body fat, abdominal circumference and serum leptin. Grip strength showed a positive correlations with serum albumin, iron and zinc. Further, grip strength was inversely correlated with serum TNF-α and adiponectin, and positively correlated with PAI-1. Finally, grip strength showed positive correlations with red blood cell count, haemoglobin and haematocrit.

After adjustment for age (Table 1), correlations with body weight, height, serum albumin, iron and haemoglobin remained significant whereas correlations with BMI, serum zinc, TNF-α, adiponectin, PAI-1, red blood cell count and haematocrit were abolished. Correlations became significant with HDL cholesterol (positive), serum copper (negative), log hsCRP (negative) after controlling for age. Although there was no significant correlation between grip strength and eGFR ($r=0.13$, not significant) in simple regression analysis, the correlation became significant after adjustment for age but it was inverse ($r=-0.20$, $p<0.05$). From the pathophysiological point of view, the direction of the correlation is the opposite of what is expected. It may result from over-adjustment for age because eGFR is calculated using the formula including age.

Multiple linear regression analysis was conducted with grip strength as a dependent variable (Table 2). Confounder variables included were age, comorbidities (hypertension and glucose dysregulation) and variables which showed significant associations with grip strength after adjustment for age. We found that 47.0% of variability of grip strength can be accounted for by height, age and blood haemoglobin in order of increasing R^2. We excluded eGFR from dependent variables due to reasons described above.

In our samples, 40 women (19.8%) had anaemia by the WHO criteria (haemoglobin <12 g/dL). Women with anaemia were older and had lower grip strength compared with women without anaemia (Table 3). After adjustment for the same confounder variables as in Table 2, women with anaemia had weaker grip strength than women without anaemia (17.6±0.9 [SE] vs 20.7±0.4 kg, $p=0.003$). As previously reported[15] and confirmed in the present study, anaemic women had elevated serum adiponectin and creatinine, and lower eGFR and higher prevalence of eGFR <45 mL/min/1.73 m^2 (22.5 vs 1.9%, $p<0.0001$). However, there was no difference in serum levels of inflammatory markers and the prevalence of eGFR ≤45-<60 mL/min/1.73 m^2 between the 2 groups of women.

In order to further confirm the relationship between haemoglobin levels and grip strength, women were divided into quintiles of haemoglobin. This was done because anaemic women belonged to the lowest quintile of haemoglobin. Anaemic women (the lowest quintile; haemoglobin <12.0 g/dL) had lower grip strength than women in the highest (≥13.8 g/dL) and second highest quintiles (13.2-13.9 g/dL) after adjustment for the same confounder variables as in Table 2 (Figure 1). Women in the median quintile (12.6-13.2 g/dL) also had lower grip strength than women in the highest quintile. However, there was no difference in grip strength between anaemic women and non-anaemic women in the second lowest (12.0-12.5 g/dL) and median quintiles of haemoglobin.

Elderly women were divided into 2 groups according to a median value of grip strength; low and high groups. Multiple logistic regression analysis was conducted with low grip strength as a dependent variable. Independent variables included were the same as in the multiple regression analysis in Table 2, except for haemoglobin. Instead anaemia was included as an independent variable. In this model, height, weight and serum albumin were independently associated with low grip strength, but not anaemia (Table 4).

Table 1. Anthropometric and biochemical characteristics of Japanese elderly women studied and correlation coefficients of grip strength before (simple) and after (partial) adjustment for age

Variables	Mean±SD	Grip strength	
		Simple	Partial
Age (years)	76.3±8.2	-0.55***	adjusted
Height (cm)	149±6.2	0.58***	0.47***
Body weight (kg)	49.9±7.7	0.43***	0.33***
BMI (kg/m^2)	22.5±3.1	0.15*	0.09
Body fat percentage (%)	31.8±7.1	-0.01	-0.06
Abdominal circumference (cm)	86.5±9.3	0.03	0.12
Grip strength (kg)	20.4±5.3	1.00	1.00
Systolic blood pressure (mmHg)	143±22	-0.11	-0.10
Diastolic blood pressure (mmHg)	84±13	-0.03	-0.10
Albumin (g/dL)	4.39±0.26	0.39***	0.19
Plasma glucose (mg/dL)	100±29	-0.03	-0.12
Insulin (μU/mL)	8.3±7.5	-0.17	-0.15
Total cholesterol (mg/dL)	219±31	0.12	-0.03
HDL-cholesterol (mg/dL)	64±14	0.09	0.20
Non-HDL-cholesterol (mg/dL)	155±33	0.07	-0.12
TG (mg/dL)	142±79	0.10	-0.09
Serum creatinine (mg/dL)	0.69±0.15	-0.11	0.11
Iron (μg/dL)	94±28	0.18*	0.20*
Copper (μg/dL)	109±15	-0.06	-0.20*
Zinc (μg/dL)	78±12	0.28***	0.12
hsCRP (μg/dL)	85±109	-0.11	-0.18*
Log hsCRP	1.7±0.4	-0.09	-0.20*
TNF-α (pg/mL)	1.6±1.0	-0.22**	-0.12
Leptin (ng/mL)	7.7±4.7	0.03	-0.10
Adiponectin (μg/mL)	14.1±7.8	-0.24***	-0.09
PAI-1 (ng/mL)	26.5±16.5	0.16*	0.07
White blood cells (×10^3/μL)	6.1±1.6	-0.04	-0.12
Red blood cells (×10^4/μL)	424±38	0.30***	0.12
Haemoglobin (g/dL)	12.9±1.2	0.33***	0.21*
Haematocrit (%)	40.9±3.4	0.29***	0.12
Platelets (×10^4/μL)	22.9±5.6	-0.06	-0.17

hsCRP: high-sensitivity C-reactive protein; TNF-α: tumor necrosis factor-α; PAI-1:plasminogen activator inhibitor-1.
Blood was drawn between breakfast and lunch.
*p<0.05, **p<0.01, ***p<0.001.

Table 2. Stepwise multiple linear regression analysis for grip strength as a dependent variable in Japanese elderly women

	Standardized β	p	Cumulative R^2
Height	0.41	<0.001	0.34
Age	-0.32	<0.001	0.44
Haemoglobin	0.17	0.003	0.47

Model included as independent variables age, comorbidities (hypertension and glucose dysregulation) and all variables that showed significant associations with grip strength after age adjustment; height, weight, albumin, iron, haemoglobin, HDL cholesterol, serum creatinine, copper and log high-sensitivity CRP.

Table 3. Characteristics of Japanese elderly women with anaemia using the World Health Organization criteria (haemoglobin <12.0 g/dL)

	Anaemic (n=40)	Non-anaemic (n=162)	*p*
Age (years)	79.9±8.6	75.4±7.9	0.002
Height (cm)	147±6.4	150±6.0	0.045
Body weight (kg)	48.1±8.2	50.5±7.4	0.068
BMI (kg/m^2)	22.1±3.0	22.7±3.1	0.325
Body fat percentage (%)	30.8±7.5	32.0±7.0	0.356
Abdominal circumference (cm)	86.9±8.7	86.4±9.5	0.779
Grip strength (kg)	18.1±5.9	20.9±5.1	0.003
Systolic blood pressure (mmHg)	141±23	144±22	0.415
Diastolic blood pressure (mmHg)	80±12	85±13	0.022
Albumin (g/dL)	4.24±0.27	4.43±0.24	<0.0001
Plasma glucose (mg/dL)	102±44	99±23	0.615
Insulin (μU/mL)	8.2±6.4	8.3±7.9	0.933
Total cholesterol (mg/dL)	206±25	222±32	0.003
HDL-cholesterol (mg/dL)	64±13	64±15	0.984
Non-HDL-cholesterol (mg/dL)	142±25	158±34	0.005
TG (mg/dL)	129±73	145±81	0.276
Serum creatinine (mg/dL)	0.77±0.25	0.67±0.11	0.000
Iron (μg/dL)	80±27	98±27	0.000
Copper (μg/dL)	109±15	109±15	0.715
Zinc (μg/dL)	73±10	79±12	0.002
hsCRP (μg/dL)	79±95	87±113	0.688
Log hsCRP	1.7±0.4	1.7±0.4	0.862
TNF-α (pg/mL)	1.7±0.8	1.6±1.1	0.540
Leptin (ng/mL)	7.1±4.6	7.8±4.7	0.404
Adiponectin (μg/mL)	18.2±10.4	13.1±6.6	<0.0001
PAI-1 (ng/mL)	20.5±7.4	28.0±17.7	0.010
White blood cells (×10^3/μL)	5.9±1.5	6.1±1.6	0.360
Red blood cells (×10^4/μL)	382±31	434±31	<0.0001
Haemoglobin(g/dL)	11.3±0.6	13.3±0.9	<0.0001
Haematocrit (%)	36.5±2.1	42.0±2.7	<0.0001
Platelets (×10^4/μL)	23.1±5.2	22.9±5.7	0.833

Mean±SD or n, %.
hsCRP: high-sensitivity C-reactive protein; TNF-α:tumor necrosis factor-α; PAI-1:plasminogen activator inhibitor-1.

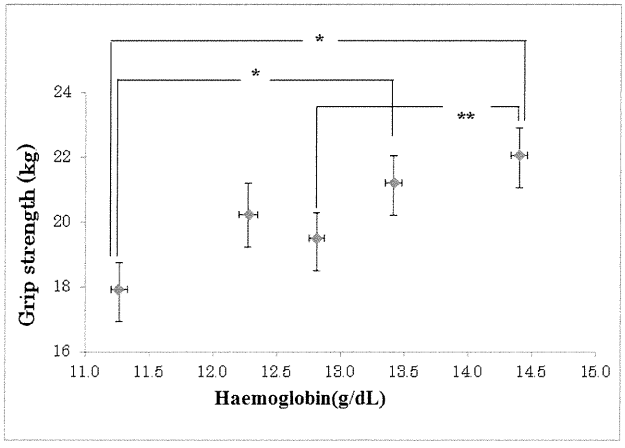

Figure 1. Relationship between grip strength and quintiles of blood haemoglobin in community-living elderly Japanese women. Data are mean±SE after adjustment for the same confounder variables as in Table 2. $^*p<0.01$, $^{**}p<0.05$

DISCUSSION

In the present study, we showed that grip strength was correlated with higher hsCRP and serum copper, and lower HDL cholesterol, serum albumin and haemoglobin in community-living elderly women. Among those, low blood haemoglobin was correlated with low grip strength independently of age, comorbidities, anthropometric, nutritional, and inflammatory markers although the relationship between grip strength and haemoglobin was not linear. It is noted that these findings were observed in free- and community-living elderly women who had fewer indicators of disease or malnutrition, such as low BMI, hypoalbuminemia, hypocholesterolemia or renal failure, which are usually considered hallmarks of malnutrition and frailty.

Anaemia has been shown to be associated with weaker muscle strength and poorer physical function in older adults.[20-23] In addition, in older women who are not