Table 3: Anthropometric, biochemical, and hematological characteristics of elderly women with high pulse pressure (≧65 mmHg).

	PP < 65 mmHg $n = 112$	PP ≧ 65 mmHg $n = 38$	P value
Age (years)	74.3 ± 8.4	78.9 ± 6.5	0.001
BMI (kg/m^2)	22.5 ± 2.8	22.0 ± 2.8	0.404
Body fat percentage (%)	32.5 ± 6.8	33.6 ± 7.1	0.417
Systolic blood pressure (mmHg)	136 ± 14	163 ± 15	0.000
Diastolic blood pressure (mmHg)	83 ± 10	89 ± 11	0.003
Pulse pressure (mmHg)	53 ± 8	75 ± 9	0.000
Plasma glucose (mg/dL)	86 ± 10	86 ± 8	0.736
Insulin (μU/mL)	5.5 ± 4.0	5.0 ± 3.9	0.511
HOMA-IR	1.2 ± 1.0	1.1 ± 0.9	0.446
Total cholesterol (mg/dL)	223 ± 31	216 ± 27	0.238
HDL-cholesterol (mg/dL)	69 ± 16	63 ± 13	0.026
LDL-cholesterol (mg/dL)	130 ± 30	131 ± 23	0.837
Triglyceride (mg/dL)	120 ± 70	113 ± 48	0.556
Serum uric acid (mg/dL)	4.7 ± 1.0	4.8 ± 1.1	0.462
Serum creatinine (mg/dL)	0.71 ± 0.14	0.75 ± 0.18	0.185
Cystatin C (mg/L)	0.81 ± 0.16	0.94 ± 0.25	0.005
eGFR (mL/min/1.73 m^2)	63 ± 12	60 ± 16	0.204
Leptin (ng/mL)	8.8 ± 5.9	8.9 ± 5.5	0.984
Adiponectin (μg/mL)	16.5 ± 7.9	15.2 ± 5.8	0.300
hsCRP (μg/dL)	171 ± 286	328 ± 491	0.069
Log hsCRP	1.81 ± 0.57	2.11 ± 0.61	0.007
TNF-α (pg/mL)	2.12 ± 0.97	2.63 ± 1.17	0.009
Log TNF-α	0.28 ± 0.21	0.38 ± 0.20	0.014
PAI-1 (ng/mL)	27.8 ± 10.3	29.7 ± 11.0	0.329
IL-6 (pg/mL)	4.20 ± 5.46	6.13 ± 7.09	0.084
Log IL-6 (pg/mL)	0.43 ± 0.37	0.58 ± 0.42	0.049
Neutrophils (×10^3/μL)	3.12 ± 0.97	3.72 ± 1.31	0.003
Lymphocytes	2.02 ± 0.57	2.06 ± 0.67	0.716
Monocytes (×10^3/μL)	0.29 ± 0.09	0.34 ± 0.11	0.011
Leukocytes (×10^3/μL)	5.61 ± 1.32	6.31 ± 1.48	0.007
Hemoglobin (g/dL)	13.0 ± 1.1	12.7 ± 1.1	0.094

Data are mean ± SD. Abbreviations are the same as in Table 1.

Biochemical parameters and blood pressure were measured only once and there was no follow-up data. Finally, we did not have detailed drug information although many participants were on antipressure drugs. It is known that some antipressure drugs reduce PP (e.g., angiotensin-converting enzyme inhibitors) and others increase it (e.g., vasodilators) [31].

5. Conclusions

The present studies have demonstrated an independent association of higher PP with local and systemic low-grade inflammation in community-living elderly women and suggest that low-grade inflammation may be one of the confounders for the association between high PP and incident type 2 diabetes in elderly patients with hypertension.

Abbreviations

CVD: Cardiovascular disease
DBP: Diastolic blood pressure
eGFR: Estimated glomerular filtration rate
hsCRP: High-sensitivity C-reactive protein
HOMA-IR: Homeostasis model assessment
IL-6: Interleukin-6
LPL: Lipoprotein lipase
PAI-1: Plasminogen activator inhibitor-1
PP: Pulse pressure

SBP: Systolic blood pressure
TNF-α: Tumor necrosis factor-α.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Eriko Yamada performed data collection, analysis, and interpretation; Mika Takeuchi performed data analysis; Miki Kurata participated in data collection, analysis, and interpretation; Tsutomu Kazumi performed data interpretation, preparation of draft manuscript, and overall scientific management; Keisuke Fukuo helped in conception and design and did revisions. All authors read and approved the final manuscript.

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《原　著》

若年女性におけるサーチュイン(SIRT1)遺伝子多型と生活習慣病関連指標と血清PAI-1濃度との関連

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要旨　【目的】生活習慣病関連指標とサーチュイン(Sirtuin 1, SIRT1)遺伝子多型との関連を検討した．【方法】若年女性414名において生活習慣病関連の臨床指標である体組成測定やアディポサイトカインを含む血液生化学検査を施行した．SIRT1遺伝子の代表的な一塩基多型(single nucleotide polymorphism; SNP)(rs7895833(A/G))を，TaqMan PCR法により解析し，臨床指標との関連を解析した．【結果】従来報告されているSIRT1遺伝子多型と拡張期血圧に加えて，今回，血清plasminogen activator inhibitor-1(PAI-1)濃度の間に有意な関連が認められることが明らかになった．また，SIRT1遺伝子多型とは無関係に，血清PAI-1濃度と，体重，BMI，収縮期血圧，拡張期血圧との間に，それぞれ正の相関関係が認められた．これに対して，体脂肪率と血清PAI-1濃度との関係においては，SIRT1遺伝子多型が影響し，マイナーアリル(AGもしくはAA)を持つAアリル群において，両者に有意な正の相関関係が認められたが，メジャーアリル(G)をホモで持つGG群では，このような関係を認めなかった．【結論】今回，我々は，若年女性において，SIRT1遺伝子多型rs7895833(A/G)が，血清PAI-1濃度や，体脂肪率と血清PAI-1濃度との関係に関連することを初めて報告した．今後，SIRT1遺伝子多型が，生活習慣病を引き起こす体脂肪を標的とした栄養食事指導に応用されることが期待される．

キーワード：生活習慣病，体脂肪，血清PAI-1濃度，SIRT1遺伝子

I. 緒　言

高血圧症，肥満症，糖尿病，脂質異常症といったいわゆる生活習慣病は，遺伝因子と食習慣などの環境因子が複雑に関与して発症する多因子遺伝疾患である．近年，多因子疾患の発症に影響を与える遺伝因子を検出する手法として一塩基多型(single nucleotide polymorphism; SNP)解析が注目されている．SNPとは，ゲノム上の塩基配列のうち1つの塩基変異が集団中に1%以上の頻度で存在しているものとして定義される．この塩基の変化により遺伝子がコードしている蛋白の機能や発現が変化することがあり，疾患の発症に影響を与えていると考えられている[1]．

サーチュインは，細胞核に発現するNicotinamide Adenine Dinucleotide$^+$(NAD^+)依存型ヒストン脱アセチル化酵素であり，摂取エネルギー量を著しく減らすカロリー制限(栄養素の摂取を20～40%減少させる)により活性化され[2]，Peroxisome proliferator-activated receptor (PPAR)-γを抑制し，インスリン分泌やアディポネクチンの発現などを介して，動脈硬化の予防や脂質代謝の改善など，生活習慣病を予防すると報告されている[2,3]．最近の研究で，サーチュイン遺伝子のサブタイプの一つであるSirtuin 1(SIRT1)遺伝子座の代表的なSNPであるrs7895833(A/G)が，オランダ人のBody Mass Index(BMI)と関連するという報告後[4]，日本人の血圧と関連することや[5]，Aアリル頻度が日本人透析患者で有意に低いことなどが報告されている[6]．しかし，これまでの報告は中高年の解析であり，データに環境因子の影響が出る可能性がある．

そこで，今回，我々は，サーチュイン遺伝子が，日本人生活習慣病リスクと関連するかを検討することを目的として，比較的環境因子が均一と考えられる若年女性を

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対象に，SIRT1 遺伝子座内に存在する SNP(rs7895833(A/G))遺伝子多型と生活習慣病に関連する臨床指標との関連を検討した．

II. 対象と方法

1. 調査対象

本研究の対象者は，平成16年度から平成20年度の生活習慣病オープンリサーチ研究に参加した武庫川女子大学の学生のうち遺伝子多型解析に同意した学生414名である．本研究は，本学倫理委員会の承認を得たのち，研究目的，内容についての説明後，研究参加者全員から文書による同意を得た．

2. 調査内容

1)身体計測と体組成分析

身長，体重，血圧の測定を行い，体組成分析は，Dual-energy X-ray Absorption(DXA)法を用いた．

2)血液生化学検査

総コレステロール，HDL コレステロール，LDL コレステロール，中性脂肪，HbA1c，グルコース，インスリン，plasminogen activator inhibitor-1(PAI-1)を測定した．なお，採血は朝食前の空腹時に実施し，測定は三菱化学メディエンス株式会社に委託した．

3)SIRT1 遺伝子多型解析

対象者の血液から QIAamp DNA Mini Kit(株式会社キアゲン)を用いて DNA を抽出し，Real-time PCR 装置(Applied Biosystems 7500 リアルタイム PCR システム，Applied Biosystems 社)を用いて TaqMan PCR 法により，SIRT1 遺伝子 rs7895833 多型の解析を行った．

3. 統計解析

統計解析には，SPSS Statistics version 20 を用いた．SIRT1 遺伝子多型と臨床指標の比較は，Kruskal-Wallis test を用いた．アリルと身体計測値，血液生化学検査値との相関関係については，Spearman's rank correlation coefficient を用いた．各検定における統計学的有意水準は5%未満とした．

III. 結　果

1. 対象者の属性

対象者の属性を表1に示す．対象者の平均年齢は，20.4±1.2歳で，BMI と体脂肪率の平均値は，それぞれ，20.9±2.3 kg/m^2，25.9±6.4%であった．平均収縮期血圧と平均拡張期血圧は，それぞれ107.0±9.1 mmHg，61.4±7.3 mmHg であった．糖関連検査(HbA1c，グルコース，インスリン)，脂質関連検査(総コレステロール，HDL コレステロール，LDL コレステロール，中性脂肪)，および血清 PAI-1 濃度の平均値はそれぞれすべて正常範囲内であった．

表1　対象者の属性

	n	全体
年齢(歳)	414	20.4 ± 1.2
身長(cm)	414	161.1 ± 6.1
体重(kg)	414	54.4 ± 7.9
BMI(kg/m^2)	414	20.9 ± 2.3
体脂肪率(%)	414	25.9 ± 6.4
収縮期血圧(mmHg)	412	107.0 ± 9.1
拡張期血圧(mmHg)	412	61.4 ± 7.3
総コレステロール(mg/dl)	414	181.8 ± 28.0
HDL コレステロール(mg/dl)	414	75.2 ± 13.7
LDL コレステロール(mg/dl)	414	95.2 ± 23.0
中性脂肪(mg/dl)	414	57.3 ± 30.7
HbA1c(JDS)(%)	414	4.8 ± 0.2
グルコース(mg/dl)	414	83.7 ± 7.0
インスリン(μU/ml)	409	6.0 ± 3.5
PAI-1(ng/ml)	412	21.1 ± 12.3

平均値±標準偏差

2. SIRT1 遺伝子 rs7895833 多型のジェノタイプ頻度

SIRT1 SNP rs7895833 の遺伝子型(AA/AG/GG)の頻度は，それぞれ AA 型が0.10，AG 型が0.42，GG 型が0.48であった．マイナーアリル A の頻度は0.312であった．遺伝子型解析結果は，集団遺伝学のハーディー・ワインベルクの法則(Hardy-Weinberg principle)に従っていた．

3. 若年女性における SIRT1 遺伝子多型と臨床指標との関連

SIRT1 遺伝子 rs7895833 多型と生活習慣病リスクとの関連を明らかにする目的で，多型別の臨床指標の相違を検討した．表2に示すように，各遺伝子型(AA/AG/GG)に応じて，対象を3群間に分類して各種形質を比較したところ，若年女性においてもこれまでの報告と同様に，rs7895833 の遺伝子型(AA/AG/GG)と拡張期血圧の間に有意な関連が認められた(64.4±7.1(AA)，61.4±7.3(AG)，60.8±7.3(GG)，P=0.011)．一方，興味深いことに，rs7895833 の遺伝子型(AA/AG/GG)と血清 PAI-1 濃度の間に有意な関連が認められた(23.4±11.3(AA)，21.8±11.9(AG)，20.0±12.8(GG)，P=0.035)．しかし，他の形質，臨床指標や生化学検査値との間には有意な関連は認められなかった．インスリン抵抗性の指標である HOMA-IR と SIRT1 遺伝子多型との間にも有意な関連は認められなかった．

4. 血清 PAI-1 濃度と体脂肪率の相関における，SIRT1 遺伝子多型の影響

次に，血清 PAI-1 濃度と臨床指標との相関関係を検討した．表3に示すように，年齢，身長と血清 PAI-1 濃度との間には，相関は認められなかったが，体重，BMI，体脂肪率，収縮期血圧，拡張期血圧は血清 PAI-1 濃度と

表 2　SIRT1 遺伝子多型別の臨床指標の相違

	n	GG	n	AG	n	AA	P-value
年齢(歳)	198	20.4 ± 1.3	174	20.5 ± 1.2	42	20.3 ± 1.1	0.653
身長(cm)	198	161.3 ± 5.9	174	161.0 ± 6.2	42	160.5 ± 6.9	0.894
体重(kg)	198	54.4 ± 7.5	174	54.7 ± 8.2	42	52.6 ± 8.0	0.766
BMI(kg/m^2)	198	20.9 ± 2.1	174	21.1 ± 2.5	42	20.3 ± 2.4	0.593
体脂肪率(%)	198	25.5 ± 5.9	174	26.4 ± 7.0	42	25.5 ± 6.1	0.181
収縮期血圧(mmHg)	196	106.5 ± 9.0	174	106.8 ± 8.7	42	110.3 ± 10.4	0.072
拡張期血圧(mmHg)	196	60.8 ± 7.3	174	61.4 ± 7.3	42	64.4 ± 7.1	0.011*
総コレステロール(mg/dl)	198	181.4 ± 28.9	174	180.8 ± 26.3	42	188.0 ± 30.4	0.258
HDL コレステロール(mg/dl)	198	75.5 ± 14.0	174	74.9 ± 13.5	42	74.8 ± 13.7	0.848
LDL コレステロール(mg/dl)	198	94.8 ± 23.1	174	94.0 ± 22.0	42	101.8 ± 25.7	0.212
中性脂肪(mg/dl)	198	55.5 ± 35.2	174	59.4 ± 27.0	42	57.5 ± 20.2	0.146
HbA1c(JDS)(%)	198	4.8 ± 0.2	174	4.8 ± 0.2	42	4.8 ± 0.3	0.415
グルコース(mg/dl)	198	84.1 ± 7.3	174	83.4 ± 6.3	42	82.8 ± 8.3	0.384
インスリン(μU/ml)	193	5.9 ± 3.1	174	6.1 ± 3.8	42	6.0 ± 4.0	0.976
PAI-1(ng/ml)	196	20.0 ± 12.8	174	21.8 ± 11.9	42	23.4 ± 11.3	0.035*

Kruskal-wallis test(P-value *<0.05)

表 3　SIRT1 遺伝子多型別の PAI-1 と臨床指標との相関関係

	全体	GG	A アリル
年齢(歳)	0.044	0.057	0.023
身長(cm)	0.064	0.048	0.079
体重(kg)	0.195**	0.155*	0.247**
BMI(kg/m^2)	0.208**	0.190**	0.241**
体脂肪率(%)	0.150**	0.071	0.206**
収縮期血圧(mmHg)	0.309**	0.301**	0.306**
拡張期血圧(mmHg)	0.428**	0.403**	0.445**

Spearman's rank correlation coefficient(P-value **<0.01, *<0.05)

の間に，それぞれ，正の相関関係が認められた．さらに，血清 PAI-1 濃度と各種臨床指標との関係に SIRT1 SNP rs7895833 の遺伝子多型が影響を与える可能性を検討する目的で，遺伝子多型別の血清 PAI-1 濃度と各種臨床指標との相関関係を検討した．体重，BMI，収縮期血圧，拡張期血圧に関しては，SIRT1 遺伝子多型とは無関係に，それぞれ血清 PAI-1 濃度との間に有意な正の相関関係が認められた．これに対して，体脂肪率と血清 PAI-1 濃度との間においては，rs7895833 のメジャーアリル(G)をホモで持つ GG 群においては有意な相関が認められないのに対して，rs7895833 のマイナーアリル(AG もしくは AA)を持つ A アリル群においては，有意な正の相関関係が認められた．

IV. 考　察

本研究は，若年女性において，SIRT1 遺伝子多型と血清 PAI-1 濃度が関連すること，さらに，SIRT1 遺伝子多型が，体脂肪と血清 PAI-1 濃度との関係に影響を与えることを明らかにした．我々の知る限り，これらの知見は初めての報告である．現在のところこのメカニズムは不明であるが，最近，サーチュインを活性化させるレスベラトロールが，脂肪細胞において PAI-1 産生を低下させる可能性が報告されている[7]．また，SIRT1 遺伝子を内皮細胞で過剰発現させると，高血糖下での PAI-1 の発現，翻訳が低下することが報告されている[8]．これらの報告は SIRT1 が PAI-1 の産生に影響を与える可能性を示しており，SIRT1 遺伝子多型と血清 PAI-1 濃度との関連性を支持するもので，興味深い．

PAI-1 は，脂肪細胞によって産生され[9]，体脂肪の蓄積により血清 PAI-1 濃度が上昇するが，本研究の対象である若年女性においても，体脂肪率と血清 PAI-1 濃度との間に弱い正の相関が認められた．しかし，注目すべきことに，体脂肪率と血清 PAI-1 濃度の間に，rs7895833 のメジャーアリル(G)をホモで持つ GG 群においては有意な

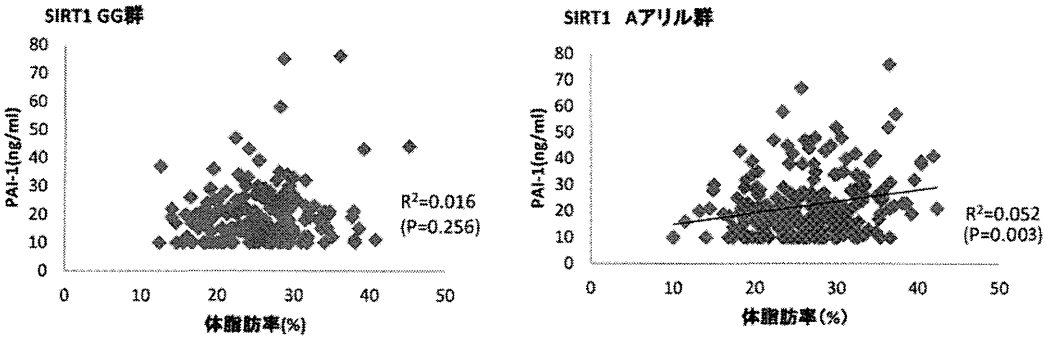

図1　SIRT1ジェノタイプにおけるPAI-1と体脂肪率との関係

相関が認められず，マイナーアリル(AGもしくはAA)を持つAアリル群においてのみ，有意な相関が認められた．このことは，血清PAI-1濃度と体脂肪率との相関に，SIRT1遺伝子多型が影響を与える可能性を示すものである．すなわち，Aアリル群では，GG群に比し体脂肪率の増加が，血清PAI-1濃度の上昇を誘導しやすい可能性があり，体脂肪率と血清PAI-1濃度とSIRT1遺伝子多型との間に何らかの機能的関連が存在することを示唆するものである．

本研究におけるSIRT1 SNP rs7895833(A/G)のマイナーアリルAの頻度は0.312であり，下山らの報告による日本人のマイナーアリルAの頻度0.290[5]と同様の値を示した．しかしながら，オランダにおけるロッテルダム研究(Aアリル頻度：0.798)[4]や米国ピマインディアン(Aアリル頻度：0.48)[10]よりマイナーアリルA頻度が低く，人種間でアリル頻度に差がある可能性が考えられる．

本研究では，SIRT1遺伝子多型と拡張期血圧との間に有意な関連を認めた．この結果は，日本人中高年女性を対象とした下山らの報告[5]と一致する．最近，拡張期血圧が，血管内皮機能と関連すること[11]，また，サーチュイン遺伝子が，高血糖下における血管内皮機能異常を予防する作用があることが報告されている[12]．今後，サーチュイン遺伝子多型が，血管内皮機能と拡張期血圧との関係に影響を与えるかなどの検討が必要と思われる．

ところで，体脂肪の蓄積は，血清PAI-1濃度の上昇とともに，インスリン抵抗性を増加させ体液量の増加を引き起こす．また，脂肪細胞からは，昇圧物質であるアンギオテンシノーゲンが分泌されるため，血圧が上昇する．本研究では，SIRT1遺伝子多型とは無関係に，血清PAI-1濃度と血圧との間に有意な相関関係が認められたが，PAI-1と血圧との関係については，一定の見解が得られていない．すなわち，高血圧患者におけるアディポサイトカイン濃度との相関の検討では，アディポネクチン濃度と血圧との相関は認められたが[13]，血清PAI-1濃度との相関は認められなかったという報告がある[14]．

一方，最近，PAI-1の酵素活性の阻害は，高血圧に対して予防的に働くという報告があり[15]，PAI-1が新たな高血圧治療標的分子として注目されている[16]．また，PAI-1遺伝子座に存在するSNPが，男性の拡張期血圧と関連するという報告もある[17]．しかし，血圧への作用が，PAI-1の直接作用なのか，肥満や体脂肪増加を介した間接的な作用なのかは不明であり，今後，詳細な検討が必要である．

以上，我々は，若年女性において，SIRT1遺伝子多型rs7895833(A/G)が，血清PAI-1濃度や，体脂肪率と血清PAI-1濃度との関係に関連することを初めて報告した．PAI-1は，高血圧などの生活習慣病や血栓性疾患の発症に関与する可能性があるため，今後，本研究の結果が，体脂肪を標的とした栄養食事指導に応用されることが期待される．

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Genetic variation in sirtuin 1 (SIRT1) gene may influence serum PAI-1 concentrations in Japanese young women

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Polymorphism in sirtuin 1 (SIRT1) gene (*SIRT1*) has been associated with body fat and blood pressure in Japanese. Our objective was to examine whether polymorphism of *SIRT1* is associated with body fat-related clinical parameters in Japanese young women. We carried out a cross-sectional genetic association study with genotyping of a single nucleotide polymorphism (SNP) of the *SIRT1* region (rs 7895833). A total of 414 young women with a mean age 20.4 ± 1.2 years participated in this study. We used a DXA device to assess the body composition parameters. Genotyping was performed by using the TaqMan PCR method. There was a significant association of genotypes with serum PAI-1 concentrations as well as diastolic blood pressure levels. Interestingly, a significant correlation of serum PAI-1 concentrations with % body fat was observed in carriers with A alleles but not in carriers with homozygote of the major G allele. In conclusion, we have shown for the first time that the *SIRT1* SNP (rs7895833) is associated with serum PAI-1 concentrations and may influence correlation between body fat composition and serum PAI-1 concentrations. These results suggest that the *SIRT1* SNP (rs7895833) is a useful genetic parameter for dietary interventions in obese young women.

Key words: life-style disease, body fat composition, plasma PAI-1 concentration, SIRT1 gene

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Association of Adiponectin with Serum Preheparin Lipoprotein Lipase Mass in Women Independent of Fat Mass and Distribution, Insulin Resistance, and Inflammation

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Abstract

Background: Substantially increased lipoprotein lipase (LPL) activity was reported in mice overexpressing adiponectin.
Methods: Associations of serum adiponectin with serum preheparin LPL mass (serum LPL), fat mass, and fat distribution and markers of insulin resistance and inflammation were examined in 311 young and 148 middle-aged women.
Results: In young women, serum adiponectin was positively associated with high-density lipoprotein cholesterol (HDL-C) and serum LPL and inversely with body mass index (BMI), abdominal girth, trunk fat mass, trunk/lower-body fat ratio, serum leptin, and log high-sensitivity C-reactive protein. These associations were confirmed in middle-aged women. Adiponectin showed positive association with the Matsuda insulin sensitivity index and inverse associations with homeostasis model assessment of insulin resistance, serum triglycerides, leukocyte count, interleukin-6, and plasminogen activator inhibitor-1 in middle-aged women but not in young women. Multivariate analysis revealed that serum LPL and trunk/lower-body fat ratio were significant determinants of adiponectin, not only in young women but also in middle-aged women. These associations were independent of markers of inflammation and insulin sensitivity/resistance.
Conclusions: LPL mass in preheparin serum was associated with adiponectin levels independently of fat mass and distribution, systemic inflammation, and insulin resistance in healthy women. Therefore, LPL may represent a link between low adiponectin and dyslipidemia found in metabolic syndrome and type 2 diabetes mellitus.

Introduction

LIPOPROTEIN LIPASE (LPL) IS a key enzyme in lipid metabolism. Low LPL activity may be associated with dyslipidemia characterized by low high-density lipoprotein cholesterol (HDL-C) and high serum triglycerides (TGs) seen in insulin-resistance and type 2 diabetes mellitus (T2DM).[1] Measurement of LPL activity or mass in postheparin plasma is a standard procedure for evaluation of the enzyme *in vivo*. However, there was a relatively large amount of LPL protein compared with LPL activity in preheparin plasma, indicating that the majority of circulating LPL is catalytically inactive.[2,3] Studies have shown that preheparin plasma LPL (from now on referred to as serum LPL) has significant relationships with serum lipids and lipoproteins, abdominal obesity, and insulin resistance (see ref. 4). Recently, a prospective study has demonstrated that reduced levels of serum LPL are associated with an increased risk for future coronary artery disease.[5]

Previously, we reported that decreased serum adiponectin is associated more closely with adiposity and dyslipidemia than with insulin resistance in male college students.[6] Recently, we showed in female college students that high lower-body fat mass was associated with higher adiponectin and HDL-C and lower TGs, whereas high trunk fat mass was associated with lower adiponectin and higher TGs.[7]

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Therefore, we investigated whether adiponectin influenced serum LPL and whether this relationship was affected by systemic inflammation, insulin resistance, or fat distribution. These analyses were done in young and middle-aged women, populations in which confounding factors are so scarce.[8]

Subjects and Methods

We examined 311 young and 148 middle-aged women. Young women were female Japanese students of Department of Food Sciences and Nutrition, School of Human Environmental Sciences, Mukogawa Women's University, and middle-aged women were the biological mothers of the 148 students who participated in the study. The characteristics of students and mothers are described in detail elsewhere.[7–9] Subjects with clinically diagnosed acute or chronic inflammatory diseases, endocrine, cardiovascular, hepatic, renal diseases, hormonal contraception, and unusual dietary habits 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 consents were obtained from all participants.

Anthropometric indices were measured after an overnight fast. Whole-body dual-energy X-ray absorptiometry (DXA) (Hologic QDR-2000, software version 7.20D, Waltham, MA) was used to measure lean tissue mass, fat mass, and bone mineral mass for arms, lower body, trunk, and the total body.[9]

Blood samples were obtained in the morning after 12-hr overnight fast. The oral glucose tolerance test (OGTT) was performed with 75-grams of glucose administration in 118 female students and 66 mothers. Blood samples were taken at 0, 30, 60, and 120 min for glucose and insulin analysis. Plasma glucose was determined by the hexokinase/glucose-6-phosphate dehydrogenase method [interassay coefficient of variation (CV) < 2%]. Serum insulin was measured by an enzyme-linked immunosorbent assay (ELISA) method with a narrow specificity excluding des-31, des-32, and intact proinsulin (interassay CV < 6%).Insulin resistance was determined by both homeostasis model assessment (HOMA-IR) using fasting plasma glucose and insulin levels[10] and the Matsuda index [insulin-sensitive index (ISI)] using glucose and insulin levels during the OGTT.[11] The area under the curve (AUC) during OGTT was calculated using the trapezoidal method. Diagnosis of diabetes and impaired glucose tolerance was according to the American Diabetes Association criteria.[12]

Serum TGs, total cholesterol, HDL-C, and liver enzymes were measured using an autoanalyzer (AU5232, Olympus, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula.[13] Adiponectin, leptin, and inflammatory markers were measured as previously reported in detail.[9] The complete blood cell count was analyzed using an automated blood cell counter (Sysmex XE-2100, Sysmex, Kobe, Japan).

Serum LPL was measured by the sandwich ELISA using a specific monoclonal antibody against bovine milk LPL, as described by Kobayashi et al.[14] A commercial kit from Daiichi Pure Chemicals (Tokyo, Japan; interassay CV = 2.8%) was used.

Data were presented as mean ± standard deviation (SD) unless otherwise stated. Due to deviation from normal distribution, high-sensitivity C-reactive protein (hsCRP) was logarithmically transformed for analysis. Bivariate correlations of adiponectin with cardiometabolic parameters 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 adiponectin. A two-tailed $P < 0.05$ was considered statistically significant. All calculations were performed with the SPSS system 15.0 (SPSS Inc., Chicago, IL).

Results

On average, middle-aged mothers and their daughters were nonobese and rather slim and had normal blood pressure and high HDL-C. OGTT revealed that impaired glucose

TABLE 1. ANTHROPOMETRIC AND BIOCHEMICAL CHARACTERISTICS OF YOUNG AND MIDDLE-AGED WOMEN STUDIED

	Young women	*Middle-aged women*	
	n = *311*	n = *148*	P *value*
Age (years)	20.5 ± 1.2	49.8 ± 3.6	0.000
BMI (kg/m^2)	20.4 ± 2.3	22.0 ± 2.8	0.000
Waist circumference (cm)	71.2 ± 5.7	78.7 ± 8.1	0.000
Total fat mass (kg)	14.4 ± 4.4	16.1 ± 5.9	0.001
Trunk fat mass (kg)	7.0 ± 2.5	8.7 ± 3.6	0.000
Lower-body fat mass (kg)	5.6 ± 1.5	5.3 ± 1.8	0.080
Percentage body fat (%)	27.8 ± 5.5	30.1 ± 7.2	0.000
Percentage trunk fat (%)	28.6 ± 6.6	32.9 ± 8.7	0.000
Percentage lower-body fat (%)	30.6 ± 5.2	30.3 ± 6.7	0.617
Trunk/lower-body ratio	1.25 ± 0.25	1.64 ± 0.39	0.000
Fasting glucose (mg/dL)	83 ± 7	89 ± 14	0.000
2-hr glucose (mg/dL)	93 ± 23	113 ± 28	0.000
Fasting insulin (μU/mL)	6.2 ± 3.4	5.4 ± 2.8	0.022
Matsuda Index	9.92 ± 4.40	10.5 ± 5.05	0.449
HbA1c (%)	4.8 ± 0.2	5.1 ± 0.4	0.000
HOMA-IR	1.27 ± 0.87	1.21 ± 0.71	0.448
Triglycerides (mg/dL)	58 ± 34	81 ± 36	0.000
Total cholesterol (mg/dL)	182 ± 28	224 ± 35	0.000
HDL-C (mg/dL)	75 ± 13	77 ± 16	0.045
Leptin (ng/mL)	8.6 ± 3.9	7.6 ± 4.9	0.019
Adiponectin (μg/mL)	11.5 ± 4.3	11.8 ± 4.9	0.479
Preheparin LPL (ng/mL)	72 ± 18	80 ± 22	0.000
White blood cells (× 10^3/μL)	6.0 ± 1.6	5.3 ± 1.6	0.000
PAI-1 (ng/mL)	21.0 ± 12.9	23.9 ± 15.0	0.034
hsCRP (μg/dL)	28.9 ± 68.5	17.1 ± 53.9	0.067
log hsCRP	1.05 ± 0.50	1.41 ± 0.53	0.000
TNF-α (pg/mL)	0.68 ± 0.48	0.77 ± 0.38	0.051
IL-6 (pg/mL)	1.47 ± 1.81	1.02 ± 1.07	0.037
8-epi-PGF2α (pg/mg · creatinine)	328 ± 107	367 ± 186	0.013
Systolic blood pressure (mmHg)	106 ± 10	121 ± 16	0.000
Diastolic blood pressure (mmHg)	61 ± 8	74 ± 11	0.000

Data are means ± standard deviation (SD).

BMI, body mass index; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment insulin resistance; HDL-C, high-density lipoprotein cholesterol; LPL, lipoprotein lipase; PAI-1, plasminogen activator inhibitor-1; hsCRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; 8-epi-PGF2α, 8-epi-prostaglandin F2α.

tolerance was found in 4 of 118 young women and 11 of 66 middle-aged women, whereas none had diabetes. Middle-aged mothers had a higher percentage of body fat, waist circumference, trunk fat, and trunk/lower-body fat ratio, a marker of central or abdominal obesity,[15] than the daughters (Table 1). Mothers, however, had lower serum leptin and there was no difference in percentage of lower-body fat, serum adiponectin, Matsuda index, and HOMA-IR between the two groups. Serum LPL and HDL-C were slightly, but significantly, higher in mothers than in daughters even in the presence of higher TGs in mothers.

By univariate linear analysis (Table 2), serum adiponectin was positively associated with HDL-C and serum LPL, and inversely with body mass index (BMI), waist circumference, trunk fat mass, trunk/lower-body fat ratio, serum leptin, and log hsCRP in both young and middle-aged women. In middle-aged women, but not in young women, adiponectin showed a positive association with the Matsuda insulin sensitivity index and inverse associations with serum insulin, HOMA-IR, TGs, leukocyte count, interleukin-6 (IL-6), and plasminogen activator inhibitor-1 (PAI-1). Young women showed no association between adiponectin and TGs.

Because young women as well as middle-aged women showed the strongest associations between adiponectin and trunk/lower-body fat ratio, we adjusted for the ratio (Table 2, partial correlation). After adjustment for trunk/lower-body fat ratio, associations with HDL-C and serum LPL remained significant in both young and middle-aged women. In middle-aged women, associations with serum insulin, HOMA-IR, leukocyte count, and IL-6 remained significant.

We have done multivariate analysis for adiponectin as a dependent variable (Table 3). The model included all variables that showed significant associations with adiponectin as independent variables. We found that serum LPL and trunk/lower-body fat ratio were significant determinants of adiponectin, accounting for 18% and 19% of the variation in adiponectin in young and middle-aged women, respectively.

TABLE 2. CORRELATION COEFFICIENTS OF SERUM ADIPONECTIN WITH ANTHROPOMETRIC AND BIOCHEMICAL CHARACTERISTICS OF YOUNG AND MIDDLE-AGED WOMEN

	Adiponectin			
	Young women		*Middle-aged women*	
	Simple	*Partial*	*Simple*	*Partial*
Trunk/lower-body ratio	−0.371**	Adjusted	−0.357**	Adjusted
Age (years)	−0.044	−0.025	0.084	0.119
BMI (kg/m^2)	−0.204**	−0.066	−0.252**	−0.125
Waist circumference (cm)	−0.174*	−0.069	−0.266**	−0.111
Total fat mass (kg)	−0.215**	−0.078	−0.113	0.033
Trunk fat mass (kg)	−0.275**	−0.080	−0.179*	0.029
Lower-body fat mass (kg)	−0.079	−0.062	0.011	0.016
Total fat mass (%)	−0.199**	−0.063	−0.115	0.038
Trunk fat mass (%)	−0.268**	−0.068	−0.177*	0.033
Lower-body fat mass (%)	−0.046	−0.045	0.016	0.030
Fasting glucose (mg/dL)	−0.042	−0.057	−0.206*	−0.148
2-hr glucose (mg/dL)	−0.193*	−0.155	−0.112	−0.031
Fasting insulin (μU/mL)	−0.069	−0.017	−0.270**	−0.170*
Matsuda Index	0.132	0.113	0.375**	0.218
HbA1c (%)	−0.004	−0.016	−0.108	−0.054
HOMA-IR	−0.068	−0.013	−0.296**	−0.195*
Triglyceride (mg/dL)	−0.095	−0.025	−0.178*	−0.086
Total cholesterol (mg/dL)	0.090	0.130*	0.100	0.139
HDL-C (mg/dL)	0.286**	0.222**	0.322**	0.250**
Leptin (ng/mL)	−0.182**	−0.088	−0.185*	−0.088
Adiponectin (μg/mL)	1.000	1.000	1.000	1.000
Preheparin LPL (ng/mL)	0.311**	0.259**	0.255**	0.252**
White blood cells (×10^3/μL)	−0.145*	−0.087	−0.269**	−0.232**
PAI-1 (ng/mL)	−0.103	−0.055	−0.318**	−0.246**
hsCRP (μg/dL)	−0.078	−0.025	0.144	0.151
log hsCRP	−0.138*	−0.063	−0.181*	−0.101
TNF-α (pg/mL)	0.046	0.065	−0.071	0.018
IL-6 (pg/mL)	0.081	0.040	−0.307**	−0.356**
8-epi-PGF2α (pg/mg·creatinine)	0.154*	0.152*	0.033	0.020
Systolic blood pressure (mmHg)	−0.074	−0.039	−0.148	−0.068
Diastolic blood pressure (mmHg)	0.045	0.071	−0.123	−0.042

*$P < 0.05$.
**$P < 0.01$.
BMI, body mass index; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment insulin resistance; HDL-C, high-density lipoprotein cholesterol; LPL, lipoprotein lipase; PAI-1, plasminogen activator inhibitor-1; hsCRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; 8-epi-PGF2α, 8-epi-prostaglandin F2α.

TABLE 3. STEPWISE MULTIPLE REGRESSION ANALYSIS FOR ADIPONECTIN IN YOUNG AND MIDDLE-AGED WOMEN

	Standardized β	P *value*	*Cumulative* R^2
A. Young women			
Trunk/lower-body ratio	−0.322	0.000	0.132
phLPL	0.240	0.004	0.181
B. Middle-aged women			
Trunk/lower-body ratio	−0.329	0.001	0.113
phLPL	−0.300	0.002	0.194

Model A included trunk/lower-body ratio, phLPL, percentage body fat, fasting glucose, 2-hr glucose, fasting insulin, Matsuda index, G-AUC, triglycerides, HDL-C, ApoB, white blood cells, PAI-1, and Log(hsCRP) as independent variables. Model B included trunk/lower-body ratio, phLPL, percentage body fat, fasting glucose, fasting insulin, triglycerides, HDL-C, white blood cells, PAI-1, and Log(hsCRP) as independent variables.

phLPL, post heparin lipoprotein lipase; G-AUC, glucose area under the curve; HDL-C, high-density lipoprotein cholesterol; ApoB, apolipoprotein B; PAI-1, plasminogen activator inhibitor-1.

These associations were independent of markers of inflammation and insulin sensitivity/resistance.

Associations between middle-aged mothers and their daughters were significant for serum adiponectin ($r=0.305$, $P<0.01$) and borderline significant for serum LPL ($r=0.198$, $P=0.06$).

Discussion

The current study demonstrates an association of decreased serum adiponectin levels with low serum LPL mass in healthy, slim women in early adult life. Low adiponectin was also associated with higher trunk/lower-body fat mass, a marker of abdominal fat accumulation. These associations were independent of known determinants of serum adiponectin, including fat mass, HDL-C, systemic inflammation, and insulin resistance and were confirmed in nonobese middle-aged women.

An association of low adiponectin with decreased postheparin LPL activity or serum LPL mass has been reported in male patients who had diagnosed or suspected coronary artery disease[16,17] and in patients with T2DM[16,18] and hyperlipidemia.[19] In the present study, an association between decreased serum LPL mass and adiponectin was demonstrated in young, slim women and confirmed in the second group of apparently healthy, middle-aged nonobese women. A previous study has reported that adiponectin increased TGs clearance by increasing LPL expression in white adipose tissue in female adiponectin transgenic mice.[20] Increased LPL expression and activity have been reported in skeletal mouse from mice with short-term elevations of adiponectin.[21] A prospective population-based study reported that reduced levels of serum LPL are associated with an increased risk for future coronary artery disease.[22]

Recently, we demonstrated that trunk fat mass was negatively associated with serum adiponectin, whereas lower-body fat mass was positively associated after mutual adjustment in 481 young female university students,[7] who consisted of 170 athletes and 311 nonathletes.[23] In the present study, adiponectin was inversely associated with trunk/lower-body fat ratio, a marker of abdominal fat accumulation, in the 311 young nonathletes and was confirmed in middle-aged women. Young athletes were included in the former study[7] because we wanted a wide range of body fat distribution, whereas young athletes were excluded from the present study because of possible effects of endurance training on serum adiponectin.[24] Some studies in women reported an independent association between adiponectin and waist-to-hip ratio, another marker of abdominal fat accumulation.[25–27] In 837 French Caucasian subjects,[28] genetic variations in the adiponectin gene have been reported to affect the future gain of abdominal fat, assessed using waist-to-hip ratio, over the life span.

It has been shown that associations among biomarkers of metabolic syndrome are stronger in obese women than in lean women.[29] This may be in line with our observation that adiponectin was inversely associated with TGs in middle-aged women but not in young women, because middle-aged women had a greater percentage of body fat, waist circumference, and trunk fat than did younger women, although the middle-aged women were not obese.

Mother–daughter regressions, which provide an estimate of heritability, were significant for adiponectin in the present study, as previously reported.[30,31] Associations of serum LPL between mothers and daughters were borderline significant in the present study. Although, to the best of our knowledge, there was no study on parent–offspring regressions of serum LPL, our finding may be in line with familial resemblance of postheparin LPL activities reported in the HERITAGE Family Study.[32]

The results in this article are subject to several limitations. The cross-sectional design of the present study complicates the drawing of causal inferences, and a single measurement of biochemical variables may be susceptible to short-term variation, which would bias the results toward the null. We used several surrogates in the present study, which may be less accurate. The main limitation of our study is that DXA is unable to distinguish between subcutaneous and intramuscular fat in the legs or between visceral and subcutaneous fat in the trunk. The contribution of subcutaneous fat to the total amount of fat in the legs, however, is relatively large.[33] Therefore, the associations found in our study with fat mass in the lower body are probably mainly due to the subcutaneous fat depot. Another possible limitation of DXA is that gluteal fat and abdominal fat cannot be perfectly distinguished. Possibly, part of the gluteal fat was included in the trunk region and part of the abdominal fat was included in the leg region. Therefore, we may have underestimated the true associations. Finally, we did not measure LPL activity or heparin-stimulated LPL mass and activity. In the above-mentioned prospective population-based study,[22] however, although corrections for systolic blood pressure, diabetes, smoking, BMI, and LDL-C levels did not strongly affect the relationship between serum LPL concentration and coronary artery disease, correction of HDL-C and TG levels rendered loss of statistical significance, indicating that the relationship of serum LPL concentration with coronary artery disease is largely explained by these factors. These findings, coupled with an independent association of serum LPL with HDL in the present study, lead to the proposal of Tornval et al. that serum LPL may represent a catabolic product of biologically active LPL.[2]

In conclusion, LPL mass in preheparin serum was associated with adiponectin levels independently of fat mass and distribution, systemic inflammation, and insulin resistance in healthy women. LPL may represent a link between low adiponectin and dyslipidemia found in metabolic syndrome and T2DM.

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Author Disclosure Statement

No competing financial interests exist.

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Original Article

Serum copper, zinc and risk factors for cardiovascular disease in community-living Japanese elderly women

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Background: Associations of copper (Cu) and zinc (Zn) serum levels with risk factors for cardiovascular disease (CVD) have not been extensively studied in elderly Asian people. Methods: Relationships to CVD risk factors were examined in 202 freely-living elderly Japanese women. Results: By univariate analysis, log high-sensitivity C-reactive protein (hsCRP) and non-HDL cholesterol were associated with serum Cu concentrations. An independent predictor of Cu was log hsCRP. Serum Zn concentrations decreased with age. After adjustment for age, serum albumin, HDL cholesterol and red blood cell (RBC) were positively and serum insulin and log hsCRP were inversely associated with serum Zn. In stepwise multiple regression analysis (model 1), serum albumin and HDL cholesterol were associated with serum Zn. In analysis excluding albumin from model 1 (model 2), independent determinants were log hsCRP (inverse) and the total number of RBC. In analysis including serum creatinine in model 2, creatinine has emerged as a determinant in addition to log hsCRP and RBC number. In analysis including estimated glomerular filtration rate (eGFR) instead of creatinine and excluding age in model 2, eGFR has emerged as a determinant of serum Zn in addition to log hsCRP and RBC number. Conclusions: Systemic low-grade inflammation may contribute to elevated serum Cu and decreased serum Zn concentrations in the elderly, and may represent an important confounder of the relationship between the serum trace elements and mortality in this population.

Key Words: Cu, Zn, inflammation, women, elderly

INTRODUCTION

Although diabetes, hypertension, smoking and dyslipidemia are major risk factors for atherosclerotic cardiovascular disease (CVD), they cannot fully explain variation in the incidences of the diseases. In addition, these traditional risk factors are useful for predicting incident CVD in younger populations whereas their predictive values decrease with age.[1] Recent attention has focused on the discriminative ability of novel risk markers in elderly cohort.[2] Animal and human studies have shown the role of essential trace elements, copper (Cu) and zinc (Zn), in atherogenesis and carcinogenesis.[3-5]

Several studies reported that high serum Cu and low serum Zn have been associated with risk factors for and mortality from CVD.[6-11] However, majority of these studies were done in younger population of western countries. In contrast, these associations have not been extensively studied in community-living elderly population of Asian origin. Because women as compared to men as well as older as compared to younger persons had higher serum Cu and lower Zn,[6,12] we examined relationships between these 2 variables and traditional and non-traditional risk factors for CVD in 202 community-living Japanese elderly women.

PARTICIPANTS AND METHODS

We examined 202 free-living elderly Japanese women whose details have previously been reported elsewhere.[13] They were all Japanese, participated on foot and were residents in Nishinomiya, Hyogo, Japan. Nobody reported to have cancer, or clinically diagnosed acute or chronic inflammatory diseases. Of 202 elderly women, 54 (26.7%), 12 (5.9%), and 74 women (36.6%) reported to be receiving statins, antidiabetic and antihypertensive drugs, respectively. 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 blood pressure were meas-

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ured between breakfast and lunch and thereafter, blood-samples were obtained from the cubital vein. Fat mass was measured using an impedance method (InBody 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. Muscle strength was assessed by handgrip strength using a handheld dynamometer (T.K.K.5401, Takei Scientific Instruments, Tokyo, Japan). Two trials for the dominant hand were performed and the stronger result was used in analyses.

Plasma glucose, serum insulin, lipids and lipoproteins were assayed as previously reported.[14,15] Because of non-fasted blood sampling; non-HDL cholesterol was calculated as the difference between total and HDL cholesterol. Serum albumin[16] and prealbumin[17] were measured as previously reported. Adiponectin, leptin and hsCRP were assayed by commercially available kits as previously reported.[14,15] Complete blood cell count was analyzed using an automated blood cell counter (Sysmex XE-2100, Sysmex, Kobe, Japan). Serum copper was measured using a commercially available kit (Quick-auto Neo Cu 7070, Shino-test Co, Tokyo, Japan). Serum zinc was measured using atomic absorption spectrophotometry (AA240FS/Agilent, Agilent Technologies Japan, Hachioji, Japan).

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] and participants with eGFR<60 mL/min/1.73 m^2 was considered as having chronic kidney disease (CKD). Women with hemoglobin level <12 g/dL were considered as anemic.[19]

Data were presented as mean±SD unless otherwise stated. Due to deviation from normal distribution, hsCRP was logarithmic transformed for analysis. Differences between 2 groups were analyzed by t test and frequencies of conditions by Chi-square tests. Differences among 3 groups 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 regression analyses were performed to further identify the most significant variables contributing to the variation of Cu and Zn. 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

Table 1 shows anthropometric, biochemical and hematological characteristics of Japanese elderly women studied. Underweight (BMI<18.5 kg/m^2) and hypoalbuminemia (albumin<3.5 g/dL), both of which are considered a hallmark of malnutrition, were found in 18 (7.9%) and only 1 (0.4%) out of 202 women, respectively. No subjects had total cholesterol<130 mg/dL, another marker of malnutrition. Anemia and CKD were found in 11 (20.3%) and 33 (33.7%) women, respectively, in the current study and were similar in prevalence to Japanese women aged 70 and older in the general population (21.1 and 31.3%, respectively).[20,21]

Serum Cu was positively associated with log hsCRP, total and non-HDL cholesterol and platelet count (Table 1). Stepwise multiple regression analysis revealed an independent association of serum Cu with log hsCRP (standardized β= 0.395, R^2=0.152, $p<0.0001$).

Serum Zn was associated negatively with age and positively with handgrip strength and serum albumin (Table 1). In addition, serum Zn was associated positively with HDL cholesterol and eGFR and negatively with serum insulin and creatinine. Further, it showed positive associations with red blood cell count (RBC), hemoglobin (Hb) and hematocrit (HCT). After adjustment for age (Table 1), association of serum Zn with log hsCRP became significant. Associations remained significant with albumin, HDL cholesterol, insulin and RBC count.

Stepwise multiple regression analysis for serum Zn as a dependent variable were done which included age and all variables that showed significant associations with serum Zn after controlling for age as independent variables i.e. albumin, HDL cholesterol, insulin, log hsCRP and RBC (Table 2, model 1). Independent determinants of serum Zn were serum albumin and HDL cholesterol. Because strong association between serum Zn and albumin may result from the fact that most Zn is bound primarily to albumin in the circulation, multiple regression analysis was done in which independent variables were limited to variables of interests (model 2). Log hsCRP, RBC and serum creatinine emerged as independent determinants of serum Zn. These 3 variables explained 11.6% of serum Zn variability. In model 3, creatinine was replaced by eGFR, and age was excluded from independent variables because eGFR was calculated using age. EGFR has emerged as an independent determinant of serum Zn in addition to HDL cholesterol and RBC number.

Elderly women were divided into 3 groups according to tertiles of Zn (Table 3). Women in the lowest as compared to the highest third of serum Zn were older and had weaker handgrip strength. In addition, they had lower serum albumin and HDL cholesterol. Further, they had higher insulin, creatinine and hsCRP and lower eGFR. Finally, they had lower RBC, Hb, HCT and hence higher prevalence of anemia (30.0, 21.6 and 10.3% in the lowest, median and highest serum Zn tertiles, respectively, p=0.02) and of eGFR<45 mL/min/1.73 m^2 (11.7, 5.4 and 1.5%, respectively, p=0.05). After taking into accounting age, differences remained significant in handgrip strength, albumin, RBC and Hb (data not shown).

Neither serum Zn nor serum Cu was associated with BMI, percentage fat mass, abdominal circumference, blood pressure, serum leptin and adiponectin.

In stepwise multiple regression analysis for hsCRP as a dependent variable and serum Zn and serum Cu as independent variables, serum Cu was a determinant of log hsCRP (standardized β=0.402) and explained 15.8% of the variability.

DISCUSSION

To the best of our knowledge, this is the first report to date to assess the relationship of serum Cu and Zn with a broad range of risk factors for CVD in elderly women of Asian origin. The present study has demonstrated that

serum Cu was independently associated with hsCRP, a marker of systemic low-grade inflammation, in the elderly. Determinants of serum Zn were HDL cholesterol and serum albumin. It is noted that these findings were observed in community-living elderly women in whom prevalence of anemia and CKD were similar to Japanese women aged 70 and older in the general population.

Increases in serum copper concentrations have been cited in response to inflammation and infections and in various chronic diseases such as arthritis and cancer.[22] The present study has shown in community-living elderly women that modest increase in serum Cu has been associated with systemic low-grade inflammation, i.e. higher hsCRP, as previously reported in elderly community-living Italian people[12,23,24] and in the hospitalized Japanese elderly.[25] It has been proposed that increased mortality in subjects with increased serum Cu is unlikely to result from dietary imbalance but rather from secondary compartmentalization in the body caused by inflammatory processes.[7]

It is well-known that Zn deficiency causes impairments in both adaptive and innate immune responses, and pro

Table 1. Anthropometric and biochemical characteristics of 202 free-living elderly women studied and correlation coefficients of serum copper and zinc

Variables	Mean±SD	Zinc		Copper
		Simple	Partial	Simple
Age (years)	76.3 ± 8.2	-0.256***	adjusted	-0.032
BMI (kg/m^2)	22.5 ± 3.1	-0.035	0.035	-0.012
Body fat percentage (%)	31.8 ± 7.1	-0.021	0.051	0.049
Abdominal circumference (cm)	86.5 ± 9.3	-0.036	0.088	-0.016
Hand grip strength (kg)	20.4 ± 5.3	0.280***	0.121	-0.062
Systolic blood pressure (mmHg)	143 ± 22	-0.054	-0.029	0.011
Diastolic blood pressure (mmHg)	84 ± 13	0.009	0.043	-0.027
Albumin (g/dL)	4.4 ± 0.3	0.411***	0.430***	-0.010
Plasma glucose (mg/dL)	100 ± 29	0.019	-0.111	0.010
Insulin (μU/mL)	8.3 ± 7.5	-0.178*	-0.217*	-0.046
Total cholesterol (mg/dL)	219 ± 31	0.166*	0.146	0.162*
HDL-cholesterol (mg/dL)	64 ± 14	0.186**	0.190*	-0.010
nonHDL-cholesterol (mg/dL)	155 ± 33	0.078	0.052	0.158*
TG (mg/dL)	142 ± 79	-0.056	-0.156	-0.074
Serum creatinine (mg/dL)	0.69 ± 0.15	-0.195**	-0.180	0.021
eGFR (mL/min/1.73m^2)	65 ± 13	0.215**	0.174	-0.051
Iron (μg/dL)	94 ± 28	0.133	0.085	-0.119
Copper (μg/dL)	109 ± 15	-0.020	-0.013	1.000***
Zinc (μg/dL)	78 ± 12	1.000***	1.000	-0.020
hsCRP (μg/dL)	85 ± 109	-0.173*	-0.239**	0.356***
log hsCRP	1.7 ± 0.4	-0.109	-0.191*	0.402***
TNF-α (pg/mL)	1.6 ± 1.0	-0.131	-0.155	0.081
Leptin (ng/mL)	7.7 ± 4.7	-0.055	0.020	-0.026
Adiponectin (μg/mL)	14.1 ± 7.8	0.010	0.150	0.085
PAI-1 (ng/mL)	26.5 ± 16.5	0.179*	-0.003	0.108
White blood cells (×10^3/μL)	6.1 ± 1.6	-0.078	-0.115	0.089
Red blood cells (×10^4/μL)	424 ± 38	0.272***	0.198*	0.105
Hemoglobin (g/dL)	12.9 ± 1.2	0.325***	0.169	0.041
Hematocrit (%)	40.9 ± 3.4	0.279***	0.121	0.083
Platelets (×10^4/μL)	22.9 ± 5.6	-0.056	0.012	0.164*

BMI: body mass index, eGFR: estimated glomerular filtration rate, hsCRP: high-sensitivity C-reactive protein, TNF-α: tumour necrosis factor-α, PAI-1: plasminogen activator inhibitor-1. Associations of zinc were adjusted for age. Blood was drawn between breakfast and lunch. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Table 2. Stepwise multiple regression analysis for serum zinc as a dependent variable in freely-living elderly women

Independent variables	Model 1	p values	Model 2	p values	Model 3	p values
Age	ns		ns		not included	
Serum albumin	0.398	<0.001	not included		not included	
HDL cholesterol	0.151	0.02	ns		0.196	0.021
Red blood cell count	ns		0.205	0.018	0.221	0.011
log hsCRP	ns		-0.200	0.019	ns	
Serum insulin	ns		ns		ns	
eGFR	not included		not included		0.190	0.028
Serum creatinine	ns		-0.176	0.044	not included	
Cumulative R^2	0.183		0.116		0.121	

Data is standardized beta. ns: not significant. Abbreviations are the same as in Table 1.
In model 1, eGFR was not included because it was calculated using age and creatinine.
In model 2 and 3, variables of interest were included as independent variables.

Table 3. Anthropometric, biochemical and hematological characteristics of elderly women grouped according to tertiles of serum zinc concentrations

Variables	Serum zinc (μg/dL)		
	Low 44-71 n=60	Medium 72-81 n=74	High 82-127 n=68
Age (years)	79.1 ± 6.6[a]	75.9 ± 8.5[b]	74.3 ± 8.6[b]
BMI (kg/m^2)	22.5 ± 3.2	22.8 ± 3.0	22.4 ± 3.1
Body fat percentage (%)	31.2 ± 8.0	32.1 ± 7.1	31.8 ± 6.2
Abdominal circumference (cm)	86.5 ± 9.8	86.4 ± 9.7	86.8 ± 8.6
Handgrip strength (kg)	18.4 ± 4.5[a]	20.7 ± 5.8[b]	21.8 ± 4.9[b]
Systolic blood pressure (mmHg)	144 ± 26	143 ± 17	143 ± 23
Diastolic blood pressure (mmHg)	83 ± 14	85 ± 12	84 ± 13
Albumin (g/dL)	4.2 ± 0.3[a]	4.4 ± 0.2[b]	4.5 ± 0.2[c]
Plasma glucose (mg/dL)	102 ± 38	97 ± 21	101 ± 26
Insulin (μU/mL)	10.9 ± 9.5[a]	7.4 ± 6.2[b]	7.1 ± 6.7[b]
Total cholesterol (mg/dL)	209 ± 31[a]	220 ± 32[b]	225 ± 30[b]
HDL-cholesterol (mg/dL)	60 ± 13[a]	64 ± 15[ab]	66 ± 14[b]
non-HDL-cholesterol (mg/dL)	149 ± 31	156 ± 36	159 ± 31
TG (mg/dL)	136 ± 74	155 ± 89	132 ± 71
Serum creatinine (mg/dL)	0.72 ± 0.17[a]	0.71 ± 0.17[a]	0.65 ± 0.10[b]
eGFR (mL/min/1.73 m^2)	62 ± 13[a]	64 ± 13[a]	69 ± 11[b]
Iron (μg/dL)	92 ± 28	94 ± 28	96 ± 29
Copper (μg/dL)	108 ± 18	111 ± 14	108 ± 14
Zinc (μg/dL)	65 ± 5[a]	76 ± 3[b]	91 ± 9[c]
Copper/zinc ratio	1.67 ± 0.32[a]	1.47 ± 0.19[b]	1.20 ± 0.19[c]
hsCRP (μg/dL)	118 ± 148[a]	71 ± 86[b]	71 ± 86[b]
log hsCRP	1.8 ± 0.5	1.7 ± 0.4	1.7 ± 0.4
TNF-α (pg/mL)	1.8 ± 1.4	1.5 ± 0.9	1.5 ± 0.8
Leptin (ng/mL)	8.1 ± 4.3	7.6 ± 5.1	7.4 ± 4.6
Adiponectin (μg/mL)	14.0 ± 8.7	14.3 ± 7.3	14.0 ± 7.4
PAI-1 (ng/mL)	25.4 ± 12.0	27.0 ± 16.8	26.9 ± 19.4
White blood cells (×10^3/μL)	6.2 ± 1.7	6.1 ± 1.5	5.9 ± 1.5
Red blood cells (×10^4/μL)	409 ± 34[a]	426 ± 40[b]	435 ± 34[b]
Hemoglobin (g/dL)	12.5 ± 1.1[a]	13.0 ± 1.2[b]	13.2 ± 1.1[b]
Hematocrit (%)	39.8 ± 3.4[a]	41.0 ± 3.5[b]	41.8 ± 3.2[b]
Platelets (×10^4/μL)	22.9 ± 6.2	23.2 ± 5.8	22.6 ± 4.7

Data are means±SD. Abbreviations are the same as in Table 1.
Means not sharing common alphabetical letters are significantly different each other at $p<0.05$ or less.

motes systemic inflammation.[26] We confirmed in Japanese elderly women that a subtle decrease in serum Zn was associated with higher hsCRP in the present study. Indeed, Zn supplementation has been shown to increase antioxidant power and decreased hsCRP in elderly subjects.[27] However, association of serum Zn with hsCRP abolished after controlling for albumin, suggesting that the association may be weak if any.

Although a close association between serum Zn and albumin found in the present study appeared to be due to the fact that Zn is primarily bound to albumin (70%) in the circulation,[28] a low concentration of serum albumin, commonly used as a nutritional marker, has been shown to be associated with a higher risk of myocardial infarction in men and women and all-cause mortality in women in the Framingham Offspring Study.[29] In addition, a decrease over time in serum albumin, even within the normal range, has been shown to be associated with a higher risk for incident CVD.[30] These findings suggest that serum albumin seems to be not only a nutritional marker but a CVD risk factor.

Low serum Zn levels have been reported in patients with nephrotic syndrome.[31] A linear correlation between proteinuria and urinary zinc excretion suggests that low zinc in nephrotic syndrome may be related in part to increased urinary zinc losses.[31] However, there was only one participant with serum albumin <3.5 g/dL, a diagnostic criteria for nephrotic syndrome in the present study although urine tests were not undergone.

Low serum Zn has been reported in patients with end-stage renal failure.[32-34] Low Zn might occur because of anorexia, alterations in gastrointestinal absorption, inflammation, hypoalbuminemia and the dialysis procedure per se.[34] In the present study, there was no participant with end-stage renal failure (eGFR <30 mL/min/1.73 m^2). In addition, the association of serum Zn with eGFR was independent of serum albumin and hsCRP. We have no explanation for the association. However, we have confirmed independent association of Zn with cystatin C-based eGFR, a superior marker of renal function than creatinine-based eGFR, in a separate group of elderly Japanese women (paper in preparation).

Zn is clearly involved in several aspects of normal haematopoiesis by virtue of its role in many enzyme systems involved with DNA synthesis,[35,36] and are key structural components of a large number of proteins. As previously reported in Japanese middle-aged women and endurance female runners,[37,38] association between marginal

Zn deficiency and anemia has been confirmed in elderly Japanese women in the present study.

Serum Zn concentrations readily decrease after a meal,[39] This decrease represents a redistribution of Zn from the small, vulnerable pool to the tissue.[40] In the present study, blood was taken in the fed state in the morning in elderly women and the serum Zn averaged 77.3 μg/dL. This is consistent with the observation by Kubori et al,[41] who measured serum Zn in blood samples taken in the morning and found that mean concentrations ranged from 75.6 to 78.3 μg/dL in a total of 1017 the Japanese elderly recruited from 6 areas in Nagano, Japan.

Although animal and human studies suggest that zinc has the potential to affect lipoprotein metabolism, significant effects of Zn supplementation were not observed for serum lipids and lipoproteins in a meta-analysis of 33 randomized controlled trials.[42] We found a positive association between serum Zn and HDL cholesterol in community-dwelling elderly women, as previously reported in 189 employees in UK.[6] However, serum Zn correlated with LDL cholesterol in 778 US adults.[43] As far as we know, there was no data on the relationship between serum trace elements and lipids and lipoproteins in healthy Japanese population.

Several limitations must be acknowledged. We did not have information on food intake, which is one of major determinants of Cu and Zn serum levels.[6,11] The cross-sectional design did not allow causal relationship. The recruitment procedure may also have some potential impact on the results. As the participation was voluntary, women who pay more attention to health may be more likely to participate. Participants were recruited from one area in Japan. Those on medication for cardiovascular diseases were excluded. Therefore, the generalization of the results is limited. Biochemical parameters were measured only once. We did not measure erythrocyte zinc concentrations which do not change significantly in response to meal or inflammation.[44] We did not have detailed information on drugs and supplements, which may contain Zn and/or Cu. Finally, Cu has both prooxidant and antioxidant effects, as reviewed by Ferns et al.[45] Copper ions can catalyse the oxidative modification of LDL whereas they also form an intrinsic constituent of superoxide dismutase and caeruloplasmin, enzymes that may be involved in preventing oxidative injury. Also, Cu is an essential component of lysyl oxidase, an enzyme involved in the biosynthesis of collagen, which is a major constituent of the extracellular matrix of arterial wall.

In conclusion, the present studies have demonstrated that both serum Cu and Zn were associated with traditional and novel CVD risk factors, specifically hsCRP, in freely- and community-living elderly Japanese women. Higher hsCRP has been shown to be associated with higher CVD mortality in Japanese whose median CRP levels are low by western standards.[46] Therefore, systemic low-grade inflammation may contribute to elevated serum Cu and decreased serum Zn levels in the elderly, and may represent an important confounder of the relationship between the serum trace elements and mortality in this population.

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AUTHOR DISCLOSURES

There were no conflicts of interest.

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