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(3) 今後の研究計画

成人健康調査では放射線影響を研究するため、被爆者と対照者からなるコホート集団の追跡調査を行っており、エンドポイントとして疾患発生、臨床検査データ、死亡の情報が得られている。がんや良性腫瘍で放射線被曝による死亡や発生の増加が確認され、また、白内障などがん以外の疾患の増加も示唆されている。放射線リスクの定量化に際し、喫煙や食事などの放射線以外の要因を考慮する事が真の放射線影響を評価する上で重要であり、放射線以外の要因について寄与の大きさについても評価している。また、現在最も若い対象者の年齢が 60 歳に達しており、高齢化集団における疾患の発生および進行に関する調査が行われている。

肥満は様々な生活習慣病を合併し、また生活習慣病の原因となることから、公衆衛生上の重要な課題として注目されている。しかし、日本人の肥満が糖尿病や心血管疾患の発症に与える影響の程度や、より妥当な肥満指標が何かを検討した前向き研究はまれである。また肥満指標と内臓脂肪や皮下脂肪の関係、加齢に伴う肥満指標の変化についても日本人でのエビデンスはほとんど得られていない。成人健康調査では横断的に体脂肪の測定を行っており肥満指標と体脂肪の関係を解析できる。また BMI と加齢の関係を横断的・縦断的に解析することが可能である。

慢性的炎症状態はがんや循環器疾患を始めとする種々の疾患を引き起こし得ることが注目されているが、日本人でのエビデンスはまれである。成人健康調査では横断的にウィルスや細菌の感染に関する調査が実施され、また、炎症指標として白血球数や CRP の縦断的データが得られている。慢性的炎症状態とがんや循環器疾患の関係についても検討したい。

7. 端野・壮瞥町研究

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1）最近の研究成果

研究報告 1：*Diabetes Care 2006; 29：1128-1129*

内臓脂肪蓄積型肥満（腹部肥満）はメタボリックシンドロームの基盤をなす病態であり、肥満細胞から分泌される生理活性物質がインスリン抵抗性、血圧上昇、耐糖能障害、脂質代謝異常などに関与することが明かにされつつある。本報告では地域住民の腹囲を測定、腹部肥満を同定し、その後の 10 年間の追跡により糖尿病発症を endpoint として、腹部肥満と糖尿病発症の関連を検討した。対象は一般住民男性 348 人、女性 523 人で早朝空腹時に標準法により腹囲周囲径を測定し、男性では WC≧85 cm，女性では WC≧90 cm を腹部肥満とした。また 10 年間の縦断調査により、新規糖尿病発症を登録した。その結果、173 名の腹部肥満の 12.7%、654 名の非腹部肥満の 5.9%に糖尿病発症があった。多重ロジスティック回帰分析で年齢、血圧、喫煙、BMI レベルを補正しても、腹部肥満は相対危険 2.07 で新規糖尿病発症を予測した。このとき BMI レベルは有意な因子とならなかった。日本人一般住民でも BMI よりも内臓脂肪蓄積型肥満が糖尿病発症に関連することが示された。

研究報告 2 ：*Hypertens. Research 2006; 29: 961-967*

これまでに、レニンーアンジオテンシン系（RA 系）遺伝子多型と高血圧の関連を検討した報告は多数なされているが、同系はインスリン抵抗性(IR)の病態にも密接に関与していることが知られている。IR は、心血管疾患の主要な危険因子の共通の遺伝的背景因子である。IR の疾患感受性遺伝子として、これまでにアドレナリン β3 受容体遺伝子、アディポネクチン遺伝子などが検討されているが、本邦の一般住民を対象に IR と感受性遺伝子の関連を検討した成績は少ない。そこで本報告では、RA 系の遺伝子多型と IR の関連を検討した結果を示す。住民検診を受診し、インフォームドコンセントを得た住民のうち心血管疾患罹患者、糖尿病治療者を除いた 550 名（男性 199 名、女性 351 名、平均年齢 63.6±0.4 歳）を対象とした。末梢血からゲノム DNA を抽出し、ACE 遺伝子 I/D 多型、AGT 遺伝子 Met235Thr 多型、AGTR1 遺伝子 A1166C 多型について遺伝子型の決定を行った。

全対象の HOMA 指数の平均値は 1.30±0.05 であり、HOMA 指数は BMI、高血圧の有無、TG、HDL コレステロール、hs-CRP と有意な相関を示した。HOMA 指数 1.73 を基準に IR の有無で分類（IR 群、非 IR 群）、IR 群は 116 名、非 IR 群は 434 名であった。ACE 遺伝子多型、AGT 遺伝子多型、AGTR1 遺伝子の遺伝子型の頻度は、これまでの日本人の報告と同様である。このうち AGTR1 遺伝子の遺伝子型の頻度は、AA /

AC / CC がそれぞれ 84.4%/ 14.9%/ 0.7%である。IR 群では AGTR1 遺伝子の A アレル保有者が有意に高率で、AGTR1 遺伝子が AA 型の場合、AC, CC 型と比較して IR のオッズ比は 2.25 倍となった。この関連は、多変量で補正した検討でも同様であり、IR は AGTR1 遺伝子の A1166C 多型は有意な関連を示し、AA 型が IR のリスクであるという結果が得られた。AGTR1 遺伝子 A1166C 多型では、AA 型が AC, CC 型と比較して AII に対する受容体の反応性が高いことがこれまでに報告されており、このことからAA型保有者ではAIIの機能が発揮されやすく、IRが発現する可能性が考えられた。

研究報告３：*Medical Hypotheses and Research 2006; 3: 751-759*

メタボリックシンドローム（MetS）は動脈硬化性疾患の基本病態として検討されている。一方、アディポネクチン（ADP）は脂肪細胞由来で血中に高濃度で存在し動脈硬化病変の修飾物質として注目されている。本総説では日本人の MetS と ADP の関連を報告した。対象は地域住民検診受診者のうち高血圧、糖尿病、高脂血症治療者を除いた男女 1,067 名（平均年齢 59.8 ± 12.4 歳）。測定項目は body mass index (BMI)、腹囲径 (WC)、血圧値 (SBP/DBP)、空腹時血糖値 (FPG)、総コレステロール値 (TC)、中性脂肪値 (TG)、HDL コレステロール値 (HDL)、ADP。MetS は 2005 年日本内科学会基準によった。すなわち腹囲：男性では WC≧85 cm，女性では WC≧90 cm を必須とし、TG≧150 mg/dl かつ／または HDL < 40 mg/dl，SBP≧130 mmHg かつ／または DBP≧85 mmHg，FPG≧110 mg/dl，以上の 2 項目以上満たすものを MetS 群、それ以外を Non-MetS 群に分類した。ADP は F 分布を示したため自然対数変換し、年齢調整した ADP を MetS 群、Non-MetS 群で比較した。また ADP を従属変数として重回帰分析を行った。男女とも ADP は年齢，HDL と有意な正の相関を認め、BMI、WC、FPG、TG とは有意な負の相関を認めた。ADP を従属変数とした重回帰分析では年齢、BMI、WC、FPG、TG、HDL が有意な独立変数として採択された。ADP は Non-MS 群に比し MS 群で有意に低下しており、年齢調整後もその関係は保たれた（それぞれ男性:$p < 0.001$；女性：$p = 0.001$）。ADP は MS で有意に低下しており、MS 発症、進展に影響を及ぼしている可能性が示された。

２）今後の研究計画

端野・壮瞥町研究は 1976 年に開始され日本人の循環器疾患の病態生理を理解するために企画された前向き疫学研究である。メタボリックシンドロームは循環器疾患の基本病態として想定され、現在、種々の病態が検討され、疫学的検討では日本人のメタボリックシンドロームの頻度や既存の危険因子の関連、アディポネクチンをはじめとするアジポサイトカインとの関連、循環器疾患既往・発症などとの関連がなど検討されつつある。端野・壮瞥町研究でも 2006 年度に報告したこれらの検討を発展させ、日本人のメタボリックシンドロームの意義の理解を深める。さらに、腹部超音波法や

インピーダンス法を用いた方法により、より簡便に正確に内臓脂肪蓄積の評価を行い、これらの新しい測定法の妥当性を証明し臨床応用を図る。またアディポネクチンレベルなどのアジポサイトカインの測定により、病態解明はもとより、動脈硬化の進展のマーカーとしての臨床応用の可能性を追求する。以上の検討は過栄養、運動不足、肥満を背景とした現代日本人の循環器疾患の予防に極めて重要であると考えられ、各地の疫学研究と比較、協調して研究成果が応用できるように考慮する。

Incidence of Type 2 Diabetes in Individuals With Central Obesity in a Rural Japanese Population

The Tanno and Sobetsu Study

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According to the new International Diabetes Federation (IDF) definition of metabolic syndrome, for a person to be defined as having metabolic syndrome they must have central obesity defined by waist circumference (1). In the definition, there are some ethnic or country-specific differences in the cutoff points of waist circumference, and Japanese cutoff points have been separately established in the IDF definition (≥85 cm for men and ≥90 cm for women). The Japanese Society of Internal Medicine and eight related scientific societies have also jointly announced new Japanese criteria of metabolic syndrome using the same cutoff points of waist circumference (2). However, the impact of central obesity using the cutoff points as a risk of type 2 diabetes is not known.

In this study, we investigated the incidence of type 2 diabetes in citizens of two rural communities in Japan to determine the relationship between type 2 diabetes and central obesity, and we also investigated the independent effects of central obesity compared with those of overall obesity.

RESEARCH DESIGN AND METHODS — The subjects were 348 men and 523 women selected from 938 citizens who had undergone medical examinations in the towns of Tanno and Sobetsu, Hokkaido, both in 1994 and 2003 or 2004.

The following participants in medical examinations in 1994 were excluded: those with missing data on blood pressure or waist circumference and those with type 2 diabetes (fasting plasma glucose level ≥126 mg/dl and/or those who were on medication for diabetes).

Participants with central obesity were determined according to the new criteria announced by the IDF (1). Central obesity in Japanese is defined by the IDF as waist circumference ≥85 cm for men and ≥90 cm for women. Participants with overall obesity were defined as those with BMI ≥25.0 kg/m^2, which is the standard of the Japan Society of the Study of Obesity (3).

The participants were divided into two groups, a normal group and a central obesity group, and the measured items in the two groups were compared. We also compared the incidences of type 2 diabetes in normal and central obesity groups of subjects who were newly determined as having type 2 diabetes on the basis of data obtained from medical examinations conducted in 2003 or 2004. Moreover, we estimated the relative risk of type 2 diabetes in people with central obesity compared with those who did not have central obesity.

As another analysis, the participants were divided into two groups, a normal group and an overall obesity group, and the same assessments as those described above were made for these two groups.

The SPSS package (version 11.5J) was used for statistical analysis. The χ^2 test was used for frequency comparison. Multiple logistic regression analysis was used to estimate the relative risk for type 2 diabetes. The significance level of all analyses was set at $P < 0.05$.

RESULTS — Thirty-eight of the 654 individuals in the normal group and 27 of the 173 individuals in the central obesity group were newly defined as having type 2 diabetes in 2003 or 2004. The incidence of type 2 diabetes was significantly higher in the central obesity group than in the normal group (15.6 vs. 5.8%; $P < 0.0001$). Thirty-five of the 591 individuals in the normal group and 30 of the 236 individuals in the overall group were newly defined as having type 2 diabetes in 2003 or 2004. The incidence of type 2 diabetes was significantly higher in the overall obesity group than in the normal group (12.7 vs. 5.9%; $P < 0.0001$).

The results of logistic regression analysis showed that both central obesity and overall obesity were closely related to type 2 diabetes and that the relative risks of occurrence of type 2 diabetes adjusted for age, sex, systolic blood pressure, total cholesterol, and smoking were 2.59 for central obesity and 2.06 for overall obesity (model 2; Table 1). Central obesity maintained its significance when additionally adjusted for overall obesity, but overall obesity lost its significance when additionally adjusted for central obesity (model 3; Table 1).

CONCLUSIONS — Waist circumference is a better predictor of visceral fat (assessed using advanced techniques such as dual-energy X-ray absorptiometry and computed tomography) than BMI

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Received and accepted for publication 27 January 2006.
Abbreviations: IDF, International Diabetes Federation.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
DOI: 10.2337/dc06-0222

Table 1—*Comparison of the relative risks for type 2 diabetes in central obesity and overall obesity*

	Model 1 (Adjusted for age and sex)	Model 2 (model 1 + total cholesterol, systolic blood pressure, and smoking)	Model 3* (model 2 + overall obesity or central obesity)
Central obesity	2.84 (1.54–5.25)†	2.59 (1.39–4.81)†	2.07 (1.03–4.16)‡
Overall obesity	2.30 (1.37–3.85)†	2.06 (1.20–3.54)†	1.53 (0.83–2.83)

Data are relative risk (95% CI). *Relative risk of central obesity was adjusted for overall obesity (yes/no) and that of overall obesity was adjusted for central obesity (yes/no). The results of logistic regression analysis showed that both central obesity and overall obesity were closely related to type 2 diabetes (models 1 and 2). Central obesity maintained its significance when additionally adjusted for overall obesity, but overall obesity lost its significance when additionally adjusted for central obesity (model 3). †$P < 0.01$; ‡$P < 0.05$.

and waist-to-hip ratio (4–6). There is a strong association between waist circumference and risk of developing health conditions such as cardiovascular disease and type 2 diabetes (7–11). In our study, only central obesity remained a significant predictor of risk of type 2 diabetes when central obesity and overall obesity were included in the model simultaneously.

The IDF also announced a new definition of metabolic syndrome in 2005, and according to the new definition, for a person to be defined as having metabolic syndrome he or she must have central obesity assessed by waist circumference (1). Since there are some ethnic or country-specific differences in cutoff points of waist circumference, ethnic and country-specific cutoff points have been separately established in the IDF definition on the basis of results of various epidemiological studies. Japanese cutoff points have also been independently established in the IDF definition (waist circumference ≥85 cm for men and ≥90 cm for women). The reason for the selection of these cutoff points for Japanese subjects has been described in detail by Matsuzawa et al. (3).

Controversy remains regarding the cutoff points for waist circumference that should be used in clinical practice. The influence of abdominal fatness on health risks such as risk of type 2 diabetes is a continuous one, and any cutoff point is therefore arbitrary (12). Further epidemiological data must be obtained in each country to determine the appropriate country-specific cutoff points for assessing the risk of type 2 diabetes.

In conclusion, our study suggested that the current cutoff points of waist circumference for Japanese people in the IDF definition are useful for assessing the risk of type 2 diabetes and that central obesity may be more useful than overall obesity for evaluating the risk of type 2 diabetes.

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Original Article

Effects of Angiotensin II Type 1 Receptor Gene Polymorphisms on Insulin Resistance in a Japanese General Population: The Tanno-Sobetsu Study

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Although gene polymorphisms in the renin-angiotensin system (RAS) are predisposing factors for cardiovascular diseases, the precise mechanisms and interactions among confounding factors have not been clarified. We investigated whether genetic variants of RAS are involved in insulin sensitivity in a Japanese general population. During a medical checkup in 2001, participants (n=550) were recruited from among the residents of the towns of Tanno and Sobetsu, and written informed consent was obtained to participate in the genetic analysis and the epidemiological study. The insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme gene (*ACE*), the Met235Thr polymorphism of the angiotensinogen gene (*AGT*), and the A1166C polymorphism of the angiotensin II type 1 receptor gene (*AGTR1*) were determined by gel electrophoresis or the TaqMan PCR method. We assessed insulin sensitivity using the homeostasis model assessment insulin resistance (HOMA-IR). The RAS gene polymorphisms were not associated with log-transformed values of HOMA-IR, whereas borderline association (p=0.02) was found between the A1166C polymorphism and dichotomous categorization of insulin resistance (defined as HOMA-IR ≥1.73). Our results suggested that the A1166C polymorphism of *AGTR1* might affect insulin resistance by altering the responsiveness to angiotensin II signaling, though this mechanism is as yet inconclusive. Further study is required to confirm these findings in a larger, multi-ethnic population. (*Hypertens Res* 2006; 29: 961–967)

Key Words: **gene polymorphisms, renin-angiotensin system, insulin resistance, epidemiology**

Introduction

The metabolic syndrome, which includes visceral obesity, hypertension, glucose intolerance, and dyslipidemia, is known to be a risk factor for cardiovascular diseases (*1*). Using the criteria defined by the National Cholesterol Education Program–Adult Treatment Panel III (NCEP-ATP III), the prevalence of the metabolic syndrome is about 25% of the general populations of both Western countries (*2*) and Japan (*3*). Prospective epidemiological studies indicate that the metabolic syndrome increases the risk of cardiovascular disease 3- to 6-fold (*4*, *5*). Similarly, in our prospective epidemiological study the subjects with metabolic syndrome had a 2.2-times greater risk of developing cardiac disease than the subjects without metabolic syndrome (*6*). Therefore, intensive

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The present study was supported by Grants-in-Aid for Scientific Research (15220401, 17090101) from the Japanese Ministry of Health, Labor, and Welfare, Grants-in-Aid for Scientific Research (17650203, 16659224) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and research grants from the Salt Science Research Foundation, Takeda Science Foundation, and Japan Heart Foundation.

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Received June 13, 2005; Accepted in revised form August 11, 2006.

preventive measures must be implemented against this condition. Insulin resistance is closely linked to the metabolic syndrome (*7*), and the renin-angiotensin system (RAS) plays a central role in the regulation of insulin sensitivity (*8–10*), as well as in the regulation of blood pressure and sodium homeostasis (*11*). Many studies have examined the genetic involvement of homozygous deletion polymorphism (DD) in exon 16 of the angiotensin-converting enzyme gene (*ACE*) in insulin resistance, but their results have been controversial (*12–14*). The association between *ACE* insertion-deletion (I/D) polymorphism in intron 16 and cardiovascular phenotypes has also been studied, with the main result being that the D allele is a genetic risk for coronary artery disease or left ventricular hypertrophy (*15*, *16*).

Hypertension is one of the most common traits of the metabolic syndrome, and is also closely linked to insulin resistance. Numerous studies have reported an association between RAS gene polymorphisms and hypertension (*17–20*). One study has indicated that the RAS gene polymorphisms have a synergistic effect on the predisposition to myocardial infarction (*21*). In this study, we investigated the association between RAS gene polymorphisms and insulin resistance to detect a genetic risk for cardiovascular disease among the Japanese general population, and whether RAS polymorphisms synergistically affect insulin resistance.

Methods

Study Subjects

We recruited 550 subjects who had undergone medical checkups in the towns of Tanno and Sobetsu in Hokkaido, Japan in 2001 (the Tanno-Sobetsu Study). The detailed epidemiological findings have already been reported (*22–24*). Subjects completed a standard questionnaire regarding their medical history and smoking and drinking habits. We measured the systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), total cholesterol, triglyceride, high density lipoprotein (HDL) cholesterol, plasma glucose, immunoreactive insulin (IRI), and highly-sensitive C-reacting protein (hs-CRP) of all participants. The homeostasis model assessment insulin resistance (HOMA-IR) was used to determine insulin sensitivity, and was calculated as plasma glucose (mmol/l) × immunoreactive insulin (mU/l)/22.5. Blood samples were collected during fasting in the early morning.

Blood pressure was measured twice after 5 min of rest, with the subjects seated. Hypertension was defined as SBP ≥140 mmHg, DBP ≥90 mmHg, or the current use of antihypertensive agents. One hundred and fifty of the 550 subjects (27.3%) were taking antihypertensive agents, and these subjects were included in the study. The precise types of antihypertensive agents were unknown. Individuals in the acute phase of coronary heart disease (n=4) or of cerebrovascular disease (n=15), and individuals undergoing medical treatment for diabetes mellitus (n=44) were excluded from the study. Individuals receiving diet therapy or exercise therapy alone for diabetes were not counted among those receiving medical treatment for diabetes. All participants gave written informed consent to participate in the genetic analysis and in all other procedures associated with the study. The Ethics Committee of the Osaka University approved the study protocol. The final number of subjects participating in the genetic study was 550.

Table 1. Correlation Coefficient between log-Transformed HOMA-IR and Clinical Parameters

Term	r	p
Age	0.071	0.096
Gender	0.013	0.75
BMI	0.47	<0.0001
Prevalence of hypertension	0.21	<0.0001
Total cholesterol	0.050	0.24
Triglyceride	0.36	<0.0001
HDL-cholesterol	−0.30	<0.0001
hs-CRP	0.16	0.0002

HOMA-IR, homeostasis model assessment of insulin resistance; BMI, body mass index; HDL-cholesterol, high density lipoprotein cholesterol; hs-CRP, highly-sensitive C-reacting protein.

In the Tanno-Sobetsu Study, Oimatsu *et al.* attempted to determine the optimal cut-off point of the HOMA-IR as a practical index for evaluating insulin resistance (*25*). The tests using the glucose clamp (GC) method and 75-g glucose tolerance tests (OGTTs) were carried out in 57 men and women with normotension or essential hypertension. The *M* value is the rate of infusion of glucose in the GC method and serves as an index of insulin resistance; we used an *M* value of 167.3 mg/m^2/min (the mean value minus 1 SD of the mean value) as a cut-off point to divide the subjects into an insulin resistance group and insulin non-resistance group, and compared the data between the two groups. Also, from data on temporal plasma glucose levels, insulin values and HOMA-IR values obtained from simultaneously performed OGTTs, the cut-off values for distinguishing insulin resistance, classified according to the results of the GC tests, were examined using the receiver operator characteristic (ROC) curve. As a consequence, HOMA-IR 1.73 was adopted. In consideration of the fact that the 75-g OGTT is sometimes difficult to perform for large numbers of people, the examinations were carried out using HOMA-IR. The sensitivity and specificity of HOMA-IR 1.73 were 64.7% and 78.9%, respectively. Therefore, in this study, dichotomous classification of insulin resistance was made at HOMA-IR ≥1.73, in addition to the evaluation of HOMA-IR as a continuous variable.

Genotyping

Genomic DNA was extracted from 200 μl of buffy coat using

Table 2. Comparison between Insulin Resistance Group (HOMA-IR ≥1.73) and Insulin Non-Resistance Group (HOMA-IR <1.73) on Clinical Characteristics

	Insulin resistance (−) (n=434)	Insulin resistance (+) (n=116)	p
Age (years)	63.2±0.5	65.2±0.9	0.04
Gender (male (%))	35.7	37.9	0.66
BMI (kg/m^2)	23.0±0.1	25.9±0.2	<0.0001
Prevalence of hypertension (%)	43.3	62.1	0.0003
Total cholesterol (mmol/l)	5.2±0.04	5.2±0.07	0.57
Triglyceride (mmol/l)	1.1±0.03	1.7±0.07	<0.0001
HDL-cholesterol (mmol/l)	1.4±0.02	1.2±0.04	<0.0001
hs-CRP (mg/l)	0.67±0.05	1.1±0.09	0.0003
IRI (mU/l)	4.2±0.1	10.8±0.3	<0.0001
HOMA-IR	0.97±0.0	2.85±0.1	<0.0001

Values are expressed as %, or means±SEM. HOMA-IR, homeostasis model assessment of insulin resistance; BMI, body mass index; HDL-cholesterol, high density lipoprotein cholesterol; hs-CRP, highly-sensitive C-reacting protein; IRI, immunoreactive insulin.

a QIAamp DNA Blood Kit (QIAGEN Inc., Hilden, Germany). The insertion-deletion polymorphisms in intron 16 of the angiotensin-converting enzyme gene (*ACE* I/D) were determined by gel electrophoresis. The Met→Thr transversion at codon 235 of the angiotensinogen gene (*AGT* Met235Thr) and the A→C transversion at nucleotide position 1166 of the angiotensin II type 1 receptor gene (*AGTR1* A1166C) were determined by the TaqMan polymerase chain reaction (PCR) method.

To amplify the intron 16 region where the I/D fragment of *ACE* is located, the following primer pairs were used: forward, 5′-CTGGAGACCACTCCCATCCTTTCT-3′; and reverse, 5′-GATGTGGCCATCACATTCGTCAGAT-3′. Genomic DNA was amplified for 45 cycles, each comprising denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. The products were separated by electrophoresis on 1.5% agarose gels and identified by ethidium bromide staining.

The *AGT* Met235Thr polymorphism was detected using the following primers and probes: forward, 5′-GCTGTGACA GGATGGAAGACT-3′ and reverse, 5′-AGTGGACGTAGG TGTTGAAAGC-3′; cytosine base (C) specific probe, 5′-FAM-CTGGCTCCCGTCAGG-MGB-3′ and thymine base (T) specific probe, 5′-VIC-CTGGCTCCCATCAGG-MGB-3′. The *AGTR1* A1166C polymorphism was detected using the following primers and probes: forward, 5′-CATTCCTCT GCAGCACTTCACT-3′ and reverse, 5′-CGGTTCAGTCCA CATAATGCAT-3′; adenine base (A) specific probe, 5′-FAM-CAAATGAGCATTAGCTAC-MGB-3′; cytosine base (C) specific probe, 5′-VIC-CAAATGAGCCTTAGCTACT-MGB-3′. PCR was conducted using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, USA). The PCR conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 60 s. The fluorescence level of PCR products measured using an ABI PRISM 7900HT Sequence Detector (Applied Biosystems) differentiated the three genotypes of the two polymorphisms.

Statistical Analysis

Associations between the polymorphisms and clinical variables were analyzed using one-way analysis of variance (ANOVA). Differences in genotype or allele distribution were examined by χ^2 analysis. In order to evaluate the association between gene polymorphisms and insulin resistance represented as HOMA-IR, we used the stepwise method as follows. First, a multiple-comparison procedure using ANOVA was done between genotypes and log-transformed HOMA-IR. Second, χ^2 analysis was done between genotypes and insulin resistance was defined using the dichotomous qualitative trait of HOMA-IR ≥1.73 or HOMA-IR <1.73. Third, stepwise analysis was done between genotypes and quintiles of log-transformed HOMA-IR to confirm whether or not the result of the second step was a chance effect. Multiple logistic regression analysis was used to assess the contribution of confounding factors. All numerical values are expressed as the mean±SEM. Values of $p<0.05$ were considered to indicate statistical significance. To adjust for multiple-testing of the three gene polymorphisms by Bonferroni's correction, we arbitrarily adopted $p<0.017$ as the level of statistical significance. All statistical analyses were conducted using JMP software version 5.0.1 for Windows (SAS Institute Inc., Cary, USA).

Results

The mean age among the 550 subjects was 63.6±0.4 years, and the mean body mass index (BMI) was 23.6±0.1 kg/m^2. The mean SBP and DBP were 133.5±0.8 mmHg and 77.8±0.4 mmHg, respectively. The mean plasma levels of total cholesterol, triglyceride, HDL-cholesterol, hs-CRP, glu-

Table 3. Multiple-Comparison between log-Transformed HOMA-IR and RAS Gene Polymorphisms

Gene polymorphism	Genotype	*n*	*p*
ACE I/D	II: 0.12±0.04	214	0.92
	ID: 0.12±0.04	267	
	DD: 0.15±0.07	69	
AGT Met235Thr	Met/Met: −0.01±0.16	14	0.55
	Met/Thr: 0.15±0.04	165	
	Thr/Thr: 0.12±0.03	335	
AGTR1 A1166C	AA: 0.13±0.03	464	0.46
	AC: 0.09±0.06	82	
	CC: −0.19±0.29	4	

Values are expressed as means±SEM. HOMA-IR, homeostasis model assessment of insulin resistance; *ACE* I/D, insertion-deletion polymorphisms in intron 16 of the angiotensin-converting enzyme gene; *AGT* Met235Thr, Met→Thr transversion at codon 235 of angiotensinogen gene; *AGTR1* A1166C A→C transversion at nucleotide position 1166 of angiotensin II type 1 receptor gene.

cose, and IRI were 5.2±0.03 mmol/l, 1.3±0.03 mmol/l, 1.4±0.02 mmol/l, 0.75±0.04 mg/l, 5.4±0.03 mmol/l, and 5.5±0.2 mU/l, respectively. The mean calculated HOMA-IR was 1.3±0.05; this HOMA-IR value did not satisfy normal distribution, but did satisfy log-normal distribution.

Table 1 shows the correlation coefficient between the log-transformed HOMA-IR and the clinical parameters. BMI, prevalence of hypertension, triglyceride, HDL-cholesterol, and hs-CRP were significantly correlated with log-transformed HOMA-IR.

Table 2 shows the clinical characteristics of the insulin resistant (n=116) and insulin non-resistant (n=434) groups when we defined insulin resistance as HOMA-IR ≥1.73. Age, BMI, prevalence of hypertension, triglyceride, HDL-cholesterol, and hs-CRP were significantly higher in the resistant than in the non-resistant group.

The genotype frequencies of the gene polymorphisms examined did not significantly differ from the values predicted by Hardy-Weinberg equilibrium. The frequencies of the II, ID, and DD genotypes of *ACE* were 38.9%, 48.5%, and 12.5%, respectively. The frequencies of the Met/Met, Met/Thr, and Thr/Thr genotypes of *AGT* were 2.7%, 32.1%, and 65.2%, respectively. The frequencies of the AA, AC, and CC genotypes of *AGTR1* were 84.4%, 14.9%, and 0.7%, respectively. Although a multiple-comparison procedure was done among the three genotypes of the three genes and log-transformed HOMA-IR, no significant correlation was found (Table 3). When insulin resistance was defined as HOMA-IR ≥1.73, the A allele of the *AGTR1* A1166C polymorphism was significantly associated with insulin resistance, but not the *ACE* I/D or *AGT* Met235Thr polymorphisms (Table 4). We examined three modes of inheritance: dominant, co-dominant (additive) and recessive, and found that only the recessive

Table 4. Allelic Frequency of RAS Gene Polymorphism

	Insulin resistance (−)	Insulin resistance (+)	*p*
ACE I/D			
Polymorphism (*n* (%))			0.92
II	167 (38.5)	47 (40.5)	
ID	212 (48.8)	55 (47.4)	
DD	55 (12.7)	14 (12.1)	
Total	434	116	
Allele (*n* (%))			0.71
I	546 (62.9)	149 (64.2)	
D	322 (37.1)	83 (35.8)	
Total	868	232	
AGT Met235Thr			
Polymorphism (*n* (%))			0.59
Met/Met	12 (3.0)	2 (1.8)	
Met/Thr	133 (32.8)	32 (29.4)	
Thr/Thr	260 (64.2)	75 (68.8)	
Total	405	109	
Allele (*n* (%))			0.32
Met	157 (19.4)	36 (16.5)	
Thr	653 (80.6)	182 (83.5)	
Total	810	218	
AGTR1 A1166C			
Polymorphism (*n* (%))			0.054
AA	358 (82.5)	106 (91.4)	
AC	72 (16.6)	10 (8.6)	
CC	4 (0.9)	0 (0.0)	
Total	434	116	
Allele (*n* (%))			0.015
A	788 (90.8)	222 (95.7)	
C	80 (9.2)	10 (4.3)	
Total	868	232	

Values are expressed as *n* (%). RAS, renin-angiotensin system; *ACE* I/D, insertion-deletion polymorphisms in intron 16 of the angiotensin-converting enzyme gene; *AGT* Met235Thr, Met→Thr transversion at codon 235 of angiotensinogen gene; *AGTR1* A1166C A→C transversion at nucleotide position 1166 of angiotensin II type 1 receptor gene.

model of *AGTR1* polymorphism (*i.e.*, AA *vs.* AC+CC) was associated with insulin resistance. The estimated odds ratio (OR) for insulin resistance in the subjects with AA was 2.25 (95% confidence interval [CI] 1.17–4.77, p=0.02) compared to those with AC or CC. Next, we examined the association between genotype or allelic frequencies and quintiles of log-transformed HOMA-IR, but no significant difference was found. Furthermore, no significant difference was found between genotype or allelic frequencies of the lowest and the highest quintile of log-transformed HOMA-IR.

Table 5 shows the results of multiple logistic regression

Table 5. Multiple Logistic Regression Analysis for Insulin Resistance

Term	β	SEM	p
Gender	−0.69	0.28	0.01
BMI	0.31	0.05	<0.0001
Prevalence of hypertension	0.60	0.25	0.02
Triglyceride	0.008	0.002	0.0002
HDL-cholesterol	−0.047	0.012	<0.0001
hs-CRP	2.29	1.0	0.03
AGTR1 AA *vs.* AC+CC	0.81	0.39	0.04

r^2=0.27 (n=550). BMI, body mass index; HDL-cholesterol, high density lipoprotein cholesterol; hs-CRP, highly-sensitive C-reacting protein.

analysis for the risk of insulin resistance. The confounding factors were selected by using the stepwise method. After adjusting for confounding factors such as gender, BMI, prevalence of hypertension, triglyceride, HDL-cholesterol, and hs-CRP, the *AGTR1* AA genotype was independently associated with insulin resistance (OR 2.25; 95% CI 1.04–4.84).

Since the *ACE* I/D and *AGTR1* A1166C polymorphisms have been reported to have a synergistic effect on determining the risk of myocardial infarction, we examined whether pairs of polymorphisms would have a similar synergistic effect on insulin resistance. However, none of the RAS polymorphisms exerted a synergistic effect on insulin resistance (data not shown).

Discussion

Angiotensin II plays a pivotal role in the pathogenesis of hypertension, vascular remodeling and insulin resistance. Our previous investigations revealed that RAS polymorphisms are genetically predisposing factors for cardiovascular diseases (*26–28*). Although many studies have examined the association between RAS polymorphisms and hypertension, the relationship between RAS polymorphisms and insulin resistance has not yet been clarified. Numerous studies have examined candidate genes for insulin resistance, such as genes of the angiotensin-converting enzyme (ACE) (*12*, *14*), β-3 adrenergic receptor (*29*), uncoupling protein (UCP) 2 (*30*), lamin A/C (LMNA) (*31*), adiponectin (*32*), and peroxisome proliferator–activated receptor (PPAR)-γ2 (*33*), but the findings of these studies were controversial.

A recent topic in clinical trials is that inhibitors of RAS, such as ACE inhibitors and angiotensin II type 1 (AT1) receptor blockers (ARBs), may reduce the incidence of new-onset diabetes in patients with or without hypertension and at high risk of developing diabetes (*34*). Since the insulin resistance is associated with upregulation of the AT1 receptor and an increase in oxygen free radicals in endothelial tissue caused by activation of NADPH oxidase (*35*), therapy using ACE inhibitors or ARBs may normalize oxidase stress and improve endothelial function. Angiotensin II activates various intracellular protein kinases, such as receptor or non-receptor tyrosine kinases and serine/threonine kinases (*36*), and these AT1-activated kinases are involved in vascular remodeling, vascular contractility, endothelial dysfunction and insulin resistance. Furthermore, the AT1 receptor undergoes rapid phosphorylation, desensitization, and internalization upon angiotensin II stimulation. Recent studies with site-directed mutagenesis of the AT1 receptor also demonstrated a structural requirement of the receptor for downstream signal transduction, suggesting that AT1 mutants provide an excellent means for examining the mechanisms of signal transduction and their significance in mediating angiotensin II function (*37*).

We identified a borderline significant association between insulin resistance and the *AGTR1*/AA genotype and considered the mechanisms of this relationship. van Geel *et al.* reported that the *AGTR1* polymorphism is associated with an increased response to angiotensin II and not with increased *AGTR1* expression (*38*). A hemodynamic study by Miller *et al.* found that normotensive individuals with the *AGTR1*/AA genotype have a higher glomerular filtration rate (GFR) than those with the *AGTR1*/AC or CC genotype at baseline, whereas the AA genotype is more responsive to an infusion of angiotensin II in the sodium-replete state, and the response to losartan is blunt (*39*). We therefore postulate that individuals with the *AGTR1*/AA genotype are more responsive to angiotensin II, which together with increased RAS activity, blunts insulin sensitivity. Recent studies have revealed that a RAS blockade increases insulin sensitivity and improves impaired insulin signaling due to angiotensin II (*40*), thereby activating the glucose transporter *via* translocation from the intracellular membrane compartment to the plasma membrane fraction (*9*, *41*).

There were several limitations to this study. First, the significance of association with insulin resistance was only observed for the recessive model and for allele frequency. Even though the p value (0.015) of the genetic predisposition to insulin resistance in the subjects with an A allele was less than 0.017, which was estimated as the level of significance from Bonferroni's correction, we have to keep in mind that the significance was borderline. In addition, the *AGTR1* polymorphism was significantly associated with dichotomous categorization of HOMA-IR as a qualitative trait, not as a continuous trait, suggesting that the polymorphism only affects insulin resistance in the advanced state. Furthermore, we did not find a significant association between quintiles of the log-transformed HOMA-IR and genotype or allelic frequencies. This suggested that the result of a significant association between gene polymorphism and insulin resistance as a qualitative trait might be a chance effect. To clarify this matter, the genetic involvement of *AGTR1* should be examined in larger epidemiological studies.

In conclusion, like other investigations into the risk of hypertension, the present study indicates the possibility that

AGTR1 gene polymorphism affects the risk of insulin resistance. The AA genotype of *AGTR1* in the general Japanese population might be an independent risk for insulin resistance. Further investigation is required to confirm these findings in a larger, multiethnic population, and to confirm the risk of RAS gene polymorphism for cardiovascular diseases in a longitudinal prospective study.

Acknowledgements

We would like to express our gratitude to Mr. Masafumi Kuremura and Ms. Kazuko Iwasa for their continuous support of our investigations.

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REVIEW

T. ISOBE, ET AL. [2006] MED HYPOTHESES RES 3: 751-760.

RELATIONSHIP BETWEEN SERUM ADIPONECTIN LEVELS AND METABOLIC SYNDROME DIAGNOSED BY USING THE NEW CRITERIA FOR METABOLIC SYNDROME FOR JAPANESE: THE TANNO AND SOBETSU STUDY

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ABSTRACT. THE AIM OF THIS REVIEW STUDY is to determine the relationship between adiponectin level and metabolic syndrome (MS), based on a new clinical definition made for Japanese. A total of 1067 participants in mass-screening tests for residents of two rural communities in Japan in 2002 and 2003 were selected after exclusion of patients undergoing treatment for hypertension or diabetes. MS was defined on the basis of visceral fat accumulation, which is defined as waist circumference (WC) ≥85 cm for males and ≥90 cm for females, plus any two of the following three factors: 1. elevated triglyceride levels (≥150 mg/dL) or specific treatment for this lipid abnormality and/or reduced high-density lipoprotein (< 40 mg/dL) or specific treatment for this lipid abnormality; 2. elevated blood pressure (BP; systolic BP ≥130 and/or diastolic BP ≥85 mmHg); 3. elevated fasting plasma glucose (≥110 mg/dL). In multiple regression analysis with adiponectin as a dependent variable, the body mass index (BMI) and WC were selected as independent variables, and so as the sex differences, age, and fasting plasma insulin levels. Furthermore, adiponectin showed tighter, negative standardized regression coefficients with WC than with BMI (−0.268 versus −0.160). Adiponectin levels were significantly lower in subjects with MS than in subjects without MS in both males and females, and were still significantly lower after adjustment for age differences. Plasma levels of adiponectin, an adipocyte-derived antiatherogenic protein, were low in subjects with MS diagnosed by the new criteria for Japanese.

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1. INTRODUCTION

Multiple risk factor syndrome, in which there is clustering of high blood pressure, lipid abnormality and glucose intolerance, is one of the important risk factors of atherosclerotic disease. Multiple risk factor syndrome has also been called visceral fat syndrome, syndrome X, deadly quartet, syndrome of insulin resistance, and recently metabolic syndrome (MS) [1-5]. A new worldwide definition of MS, emphasizing the importance of central obesity with modifications according to ethnic group, was announced by the International Diabetes Federation in 2005 [6]. In the same year, the Committee on Criteria for Metabolic Syndrome in Japan provided a definition of MS for Japanese [7] (TABLE 1).

Adiponectin is a plasma protein (composed of 244 amino acid residues), and was identified from a gene, apM1, that is specifically expressed in fat tissue [8]. Adiponectin has been shown to circulate as a trimer, hexamer, or higher-molecular-weight form in the blood of healthy subjects and to be present at a high level of 5–10 μg/mL [9-13]. It has been shown that the ratios among these forms determine their activity [14-16]. There are also significant sex differences in the circulating concentrations of adiponectin and in the ratios of their subunits [14,17]. It has been reported that adiponectin is closely associated with visceral fat accumulation [10] and insulin resistance [18-22] and that low levels of adiponectin are linked to components of MS. Adiponectin level is low in subjects carrying excessive organ fat and it increases with a reduction in body weight [10]. It has also been shown to be correlated negatively with blood pressure, triglyceride level, fasting plasma glucose level, plasma glucose level 2 h after a meal and fasting insulin concentration and to be correlated positively with high-density lipoprotein level [23-27].

We have shown that adiponectin is low in subjects having MS diagnosed by modified NCEP-ATP III criteria for Japanese [28]. In this review study, we examined the association between MS and adiponectin level by using a new definition of MS for Japanese in participants in mass-screening tests for residents in a region of Hokkaido, Japan.

2. SUBJECTS AND METHODS

Of 1,555 participants in mass-screening tests for the residents of Tanno Town and Sobetsu Town in Hokkaido, Japan in 2002 and 2003, 1,067 males and females with an average age of 59.9 ± 12.3 years (364 males with an average age of 62.9 ± 12.3 years and 703 females with an average age of 58.4 ± 12.0 years) were selected after exclusion of patients undergoing treatment for hypertension or diabetes.

The mass-screening tests were carried out between 0600 h and 0800 h in the morning. Height and body weight were measured before blood pressure measurement, and blood was collected from the subjects under fasting conditions before breakfast. Blood pressure was measured more than once from the right arm after resting for several minutes in a sitting position, and average blood pressure was calculated. Blood was collected from the median cubital vein in a sitting position with a vacuum tube. The items measured were systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), waist circumference (WC), and concentrations of fasting plasma glucose (FPG), fasting plasma insulin (F-IRI), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and serum adiponectin. Biochemical data were assayed as follows: FPG, the glucose-oxidase electrode method; F-IRI, enzyme immunoassay (ST AIA-PACK IRI, TOSOH, Tokyo, Japan); TC, the cholesterol oxidase enzymatic assay method; TG, the enzymatic colorimetric method; HDL, the direct liquid-stable assay; adiponectin, the sandwich enzyme-linked immunosorbent assay method (human adiponectin ELISA kit, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The insulin resistance index determined by homeostasis model assessment (HOMA-IR) was calculated by the formula HOMA-IR = FPG (mg/dL) × F-IRI (μU/mL)/405.

According to the new definition for Japanese in 2005 [7] (TABLE 1), MS was diagnosed as visceral fat accumulation (defined as WC ≥85 cm for males and ≥90 cm for females) plus any two of the following three factors: (1) raised TG level (≥150 mg/dL) or specific treatment for this lipid abnormality and/or reduced HDL (<40 mg/dL) or spe

TABLE 1. DEFINITION OF METABOLIC SYNDROME ACCORDING TO A JAPANESE CRITERIA PUBLISHED IN 2005.

According to the Japanese definition, for a person to be defined as having metabolic syndrome, one must have visceral fat accumulation (defined as waist circumference ≥ 85 cm for men and ≥ 90 cm for women), plus any two of the following three factors:
• raised TG level (≥ 150 mg/dL) or specific treatment for this lipid abnormality, and/or, reduced HDL (< 40 mg/dL) or specific treatment for this lipid abnormality both in males and females.
• raised BP: systolic BP ≥ 130 and / or diastolic BP ≥ 85 mmHg, or treatment of previously diagnosed hypertension.
• raised FPG ≥ 110 mg/dL, or treatment of previously diagnosed type 2 diabetes.
NOTE: Subjects receiving specific treatment for type 2 diabetes or for hypertension were excluded in this study. TG, triglyceride; HDL, high-density lipoprotein; BP, blood pressure; FPG, fasting plasma glucose. Converting factors: TG, mM = mg/dL × 0.01129; HDL, mM = mg/dL × 0.02586; FPG, mM = mg/dL × 0.05551.

TABLE 2. BASELINE CHARACTERISTICS (MEAN VALUES AND CORRELATIONS TO LNADIPONECTIN).

	Males	*r*	*P* value	Females	*r*	*P* value
	N = 364			N = 703		
Age (years)	62.9 ± 12.3†	0.353	<0.001	58.4 ± 12.0	0.123	0.001
BMI (kg/m^2)	23.8 ± 3.3†	–0.311	<0.001	23.1 ± 3.2	–0.181	<0.001
WC (cm)	84.8 ± 9.3†	–0.342	<0.001	79.8 ± 10.3	–0.239	<0.001
SBP (mmHg)	133.3 ± 21.0†	0.010	0.842	129.2 ± 21.2	0.005	0.890
DBP (mmHg)	75.8 ± 11.8†	–0.135	0.010	73.3 ± 11.9	–0.051	0.176
FPG (mg/dL)	97.0 ± 16.1†	–0.115	0.028	91.2 ± 11.2	–0.106	0.005
F-IRI (μU/mL)	4.6 ± 4.7	–0.286	<0.001	4.6 ± 2.8	–0.251	<0.001
HOMA-IR	1.1 ± 1.4	–0.262	<0.001	1.1 ± 0.8	–0.213	<0.001
TC (mg/dL)	193.2 ± 33.1†	–0.153	0.003	207.5 ± 33.8	–0.039	0.297
TG (mg/dL)	115.1 ± 74.6†	–0.333	<0.001	89.1 ± 42.0	–0.237	<0.001
HDL (mg/dL)	51.3 ± 11.6†	0.281	<0.001	54.3 ± 12.1	0.260	<0.001
Adipo (μg/mL)	6.0 ± 3.3†			7.2 ± 4.2		

Values are means ± standard deviations.
BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure.
FPG, fasting plasma glucose; F-IRI, fasting plasma insulin.
HOMA-IR, homeostasis model assessment.
TC, total cholesterol, TG, triglyceride; HDL, high-density lipoprotein; Adipo, adiponectin.
r, versus LnAdiponectin, Peason's correlation coefficient; † $P < 0.05$, versus females, unpaired *t*-test.
Conversion factors: FPG, mM = mg/dL × 0.05551; F-IRI, pM = μU/mL × 6.0; TC, mM = mg/dL × 0.02586; TG, mg/dL × 0.01129; HDL, mM = mg/dL × 0.02586.
Data are from ref. [28] and amended in part.

TABLE 3. MULTIPLE REGRESSION ANALYSIS RELATED TO LNADIPONECTIN.

	β	*t* value	*P* value		β	*t* value	*P* value
Sex	0.117	3.922	<0.001	Sex	0.079	2.652	0.008
Age	0.181	6.071	<0.001	Age	0.226	7.546	<0.001
BMI	–0.160	–4.937	<0.001	WC	–0.268	–8.138	<0.001
F-IRI	–0.167	–5.183	<0.001	F-IRI	–0.125	–3.950	<0.001

β, standardized regression coefficients.
Sex, male = 0, female =1; BMI, body mass index; WC, waist circumference; F-IRI, fasting plasma insulin.

TABLE 4. RATIO OF FACTORS COMPRISING METABOLIC SYNDROME, AND ADIPONECTIN LEVELS IN SUBJECTS POSITIVE FOR VISCERAL FAT ACCUMULATION (VFA), LIPID ABNORMALITY (LA), HIGH LEVEL OF BLOOD PRESSURE (H-BP) OR HIGH LEVEL OF FASTING PLASMA GLUCOSE (H-FPG) AND IN SUBJECTS NEGATIVE FOR VFA, LA, H-BP OR H-FPG IN MALES AND FEMALES.

	Males (N = 364)			Females (N = 703)		
	%	Adiponectin (μg/mL)		%	Adiponectin (μg/mL)	
		+	–		+	–
VFA	53.8	5.0 ± 2.6†	7.2 ± 3.7	18.1	6.0 ± 3.7†	7.4 ± 4.2
LA	25.8	4.6 ± 2.3†	6.5 ± 3.5	19.6	5.8 ± 3.5†	7.5 ± 4.2
H-BP	53.6	6.0 ± 3.3	6.1 ± 3.4	44.5	7.1 ± 4.1	7.2 ± 4.2
H-FPG	9.6	5.0 ± 2.8	6.1 ± 3.4	4.6	6.6 ± 4.7	7.2 ± 4.1

Values are means ± standard deviations.
%, ratio of factors comprising metabolic syndrome.
+, subjects positive for visceral fat accumulation or lipid abnormality or high level of blood pressure or high level of fasting plasma glucose criteria.
–, subjects negative for visceral fat accumulation or lipid abnormality or high level of blood pressure or high level of fasting plasma glucose criteria.
VFA, visceral fat accumulation: waist circumference ≥85 cm for males or ≥90 cm for females.
LA, lipid abnormality: hypertriglyceridemia: triglyceride ≥150 mg/dL and/or low level of high-density lipoprotein: high-density lipoprotein <40 mg/dL and/or specific treatment for these lipid abnormality.
H-BP, high level of blood pressure: systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg.
H-FPG, high level of fasting plasma glucose: fasting plasma glucose ≥110 mg/dL.
† $P < 0.05$ versus subjects in the "–" group, unpaired *t*-test.
Conversion factors: triglyceride, mM = mg/dL × 0.01129.
high-density lipoprotein, mM = mg/dL × 0.02586.
fasting plasma glucose, mM = mg/dL × 0.05551.
Data are from ref. [28] and amended in part.

cific treatment for this lipid abnormality; (2) raised blood pressure (BP; systolic BP ≥130 and/or diastolic BP ≥85 mmHg); (3) raised FPG (≥110 mg/dL).

Multiple regression analysis was performed using adiponectin as a dependent variable and using sex differences, age, BMI, WC and F-IRI as dependent variables. The ratio of factors comprising MS was calculated, and adiponectin levels were compared among these factors. Since the female subjects diagnosed as having MS were significantly older than those not diagnosed as having MS (non-MS), adiponectin levels adjusted for each average age (males, 63 years of age; females, 58 years of age) were compared between MS and non-MS groups in males and females.

The present study was carried out in accordance with the Declaration of Helsinki (1981) of the World Medical Association, and the study protocol was approved by the Research Committee of Sapporo Medical University, Sapporo. Written informed consent was obtained from each subject after a full explanation of the purpose, nature, and risk of all procedures used.

Statistical analysis was performed with Windows SPSS version 12.0 in Japanese (SPSS Japan Inc.). Since adiponectin showed an F-distribution, natural logarithmic-transformed values (LnAdipo) were used, and each value is presented as mean ± standard deviation (SD). The unpaired *t*-test was used to compare data between two groups. A P value less than 0.05 was considered statistically significant.

3. RESULTS

Clinical characteristics of the study subjects are shown in TABLE 2. Adiponectin concentrations

TABLE 5. UNPAIRED *T*-TESTS BETWEEN METABOLIC SYNDROME AND NON-METABOLIC SYNDROME IN MALES AND FEMALES (MEAN VALUES).

	Males		Females	
	MS	Non-MS	MS	Non-MS
	N = 47	N = 317	N = 40	N = 663
Age (years)	63.7 ± 11.3	62.8 ± 12.5	64.3 ± 10.0†	58.0 ± 12.0
BMI (kg/m^2)	26.2 ± 3.5†	23.4 ± 3.1	27.1 ± 3.2†	22.9 ± 3.0
WC (cm)	91.2 ± 6.4†	83.8 ± 9.3	95.5 ± 4.9†	78.9 ± 9.7
SBP (mmHg)	147.6 ± 21.0†	131.1 ± 20.1	143.4 ± 12.5†	128.3 ± 21.4
DBP (mmHg)	82.0 ± 11.9†	74.9 ± 11.5	79.8 ± 11.2†	72.9 ± 11.8
FPG (mg/dL)	109.9 ± 31.0†	95.1 ± 11.4	100.3 ± 13.5†	90.6 ± 10.8
TC (mg/dL)	198.9 ± 37.3	192.4 ± 32.5	212.3 ± 39.9	207.2 ± 33.4
TG (mg/dL)	190.1 ± 115.8†	104.0 ± 59.0	131.9 ± 48.0†	86.5 ± 40.2
HDL (mg/dL)	44.5 ± 10.0†	52.3 ± 11.5	42.1 ± 9.5†	55.0 ± 11.9
Adipo (μg/mL)	4.4 ± 2.3†	6.3 ± 3.4	5.4 ± 3.4†	7.3 ± 4.2

Values are means ± standard deviations.
MS, subjects diagnosed as having metabolic syndrome.
Non-MS, subjects not diagnosed as having metabolic syndrome.
BMI, body mass index.
WC, waist circumference.
SBP, systolic blood pressure.
DBP, diastolic blood pressure.
FPG, fasting plasma glucose.
TC, total cholesterol.
TG, triglyceride.
HDL, high-density lipoprotein.
Adipo, adiponectin.
†$P < 0.05$ versus Non-MS group, unpaired *t*-test.
Conversion factors: FPG, mM = mg/dL × 0.05551.
TC, mM = mg/dL × 0.02586.
TG, mM = mg/dL × 0.01129.
HDL, mM = mg/dL × 0.02586.
Data are from ref. [28] and amended in part.

were 6.0 ± 3.3 μg/mL in males and 7.2 ± 4.2 μg/mL in females, the concentration being significantly higher in females than in males. LnAdipo correlated positively with age and HDL and negatively with BMI, WC, DBP, FPG, F-IRI, HOMA-IR, TC and TG in males and correlated positively with age and HDL and negatively with BMI, WC, FPG F-IRI, HOMA-IR and TG in females. Age, BMI, WC, SBP, DBP, FPG and TG were significantly higher in males than in females, and TC and HDL were significantly lower in males than in females (TABLE 2).

In multiple regression analysis of sex differences, age, BMI, WC and F-IRI with LnAdipo as a dependent variable, BMI and WC were selected as significant independent variables as well as sex differences, age and F-IRI (TABLE 3). Furthermore, adiponectin showed tighter, negative standardized regression coefficients with WC than with BMI (–0.268 versus –0.160).

The ratio of factors comprising MS and the adiponectin level in each factor are shown in TABLE 4. Ratios of subjects positive for visceral fat accumulation (VFA) were 53.8% in males and 18.1% in females. Ratios of subjects positive for lipid abnormality (LA) were 25.8% in males and

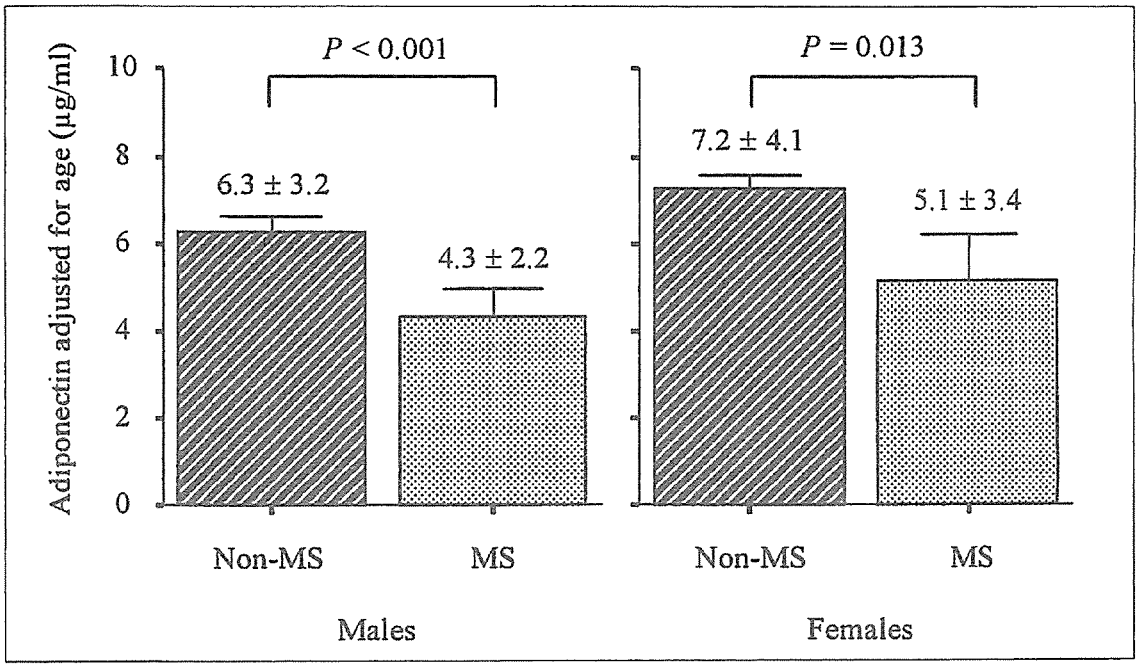

FIGURE 1. AGE-ADJUSTED PLASMA ADIPONECTIN LEVELS IN MALE AND FEMALE SUBJECTS WITH AND WITHOUT METABOLIC SYNDROME. Adiponectin level was adjusted for 63 years of age in males and was adjusted for 58 years of age in females. Non-MS, subjects not diagnosed as having metabolic syndrome; MS, subjects diagnosed as having metabolic syndrome. Values are means ± SDs. Data are from ref. [28] and amended in part.

19.6% in females. Ratios of subjects positive for high levels of blood pressure (H-BP) were 53.6% in males and 44.5% in females. Ratios of subjects positive for high levels of fasting plasma glucose (H-FPG) were 9.6% in males and 4.6% in females. Adiponectin concentrations were statistically lower in subjects positive for VFA or for LA than in subjects negative for VFA or for LA both in males and females.

The results of unpaired *t*-tests between the MS and non-MS groups are shown in TABLE 5. Adiponectin concentrations were significantly lower in the MS group than in the non-MS group both in males and females. Though there was no statistically significant difference in ages of males, females in the MS group were significantly older than females in the non-MS group. Adiponectin levels after adjustment for age were still significantly lower in the MS group than in the non-MS group both in males and females (FIG. 1).

4. DISCUSSION

In this study, adiponectin showed positive correlations with age and HDL and showed negative correlations with BMI, WC, FPG, F-IRI, HOMA-IR and TG both in males and females. In multiple regression analysis related to LnAdipo, BMI and WC were selected as significant predictor variables as well as sex differences, age and F-IRI. Furthermore, WC showed tighter, inverse standardized regression coefficients with adiponectin than BMI did. Adiponectin was also significantly lower in the MS group than in the non-MS group both in males and females and was still significantly lower after adjustment for age. These results suggest that WC