Association of a Polymorphic Variant of the Adiponectin Gene with Insulin Resistance in African Americans

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Abstract

Hypertension, type 2 diabetes, and obesity are common complex disorders that contribute to cardiovascular (CV) disease. Insulin resistance increases CV risk and is present in these disorders. Adiponectin, a protein secreted by adipocytes with metabolic and vascular protective effects, is lower in obesity and insulin resistance. Several single nucleotide polymorphisms (SNP) have been identified in the adiponectin (*ADIPOQ*) gene. Associations of *ADIPOQ* polymorphisms with diabetes and obesity have been described in Caucasians and Asians. The purpose of this study was to determine if genetic variants of *ADIPOQ* are associated with insulin resistance and CV risk in African Americans. Metabolic traits (lipids, glucose, insulin, and insulin sensitivity) and blood pressure were measured in 273 African Americans. DNA was examined by DNA sequence analysis and SNPs of candidate genes including *ADIPOQ* were studied. Statistical analyses were performed by regression of the quantitative trait phenotypes on the groups defined by the SNP genotypes, adjusting for age, sex, and body mass index (BMI). SNP 712 (rs3774261) of the *ADIPOQ* gene showed significant association with insulin resistance ($p = 0.001$). Despite the relatively small sample, our results indicate that genes that regulate adipocyte function may have a regulatory role in the expression of metabolic traits in obesity-associated chronic disease.

Keywords: adiponectin, African Americans, genetics, insulin resistance

Introduction

Hypertension, obesity, and type 2 diabetes are common complex disorders that contribute to cardiovascular (CV) disease morbidity and mortality. The metabolic syndrome is a construct that encompasses major metabolic and hemodynamic CV disease risk factors, which cluster within individuals.¹ Components of the metabolic syndrome include obesity (usually central obesity), dyslipidemia, high blood pressure (BP), hyperinsulinemia, and impaired glucose tolerance.^{1,2} Indicators of vascular injury, including microalbuminuria and abnormalities in fibrinolysis and inflammation, may also be present,³ but are more likely biomarkers of the vascular injury process. The core abnormality in the metabolic syndrome is insulin resistance or impaired insulin action with compensatory hyperinsulinemia. Insulin resistance is strongly linked with CV and endothelial damage. Because insulin resistance is difficult to quantify clinically, the constellation of associated CV risk factors designated as the metabolic syndrome serves as a surrogate clinical strategy to optimize detection of insulin resistance as an underlying pathogenic condition.⁴

Adipose tissue secretes a variety of biologically active molecules. These adipocytokines have metabolic and immune functions that suggest that adipose tissue-derived cytokines contribute to the pathogenesis of cardiovascular disease that is associated with insulin resistance.⁵ Some of the adipokines include leptin, plasminogen activator inhibitor 1 (PAI-1), and the inflammatory factors tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6).^{6–10} Adiponectin is a recently discovered protein secreted by adipocytes. In contrast to the dramatic increase in several of the proinflammatory adipokines observed in obesity, the plasma levels of adiponectin appear to be reduced in obesity and insulin resistance.^{11,12} Adiponectin exhibits a binding ability to some matrix proteins such as collagen I, III, and V, but not collagen II and IV, laminin, or fibronectin.¹³ Experimental data suggest that adiponectin can inhibit vessel injury by blocking expression of vascular adhesion molecules (intracellular adhesion molecule-1,

vascular cell adhesion molecule-1, E-selectin) in endothelial cells in response to inflammatory stimuli such as TNF- α and suppress other cytokine production.^{13,14} In experimental studies, mice that lack adiponectin expression develop severe vascular injuries, and replacement of adiponectin attenuates the vessel injury.¹⁵

Recent investigations have focused on identifying genes that regulate fat cell production of cytokines. Several single nucleotide polymorphisms (SNP) have been identified in the genes coding for adiponectin and the other adipokines. For the adiponectin gene (*ADIPOQ*; previously designated APM1), at least 13 SNPs have been identified,¹⁶ and certain *ADIPOQ* SNPs have been reported to be associated with diabetes and components of the metabolic syndrome.¹⁷ Most of the literature on adiponectin and other cytokines produced by adipocytes is based on data obtained from Caucasian and Asian populations. There is very limited data on African Americans despite the excessive cardiovascular morbidity and mortality related to diabetes, hypertension, and obesity in this racial group. The purpose of this study was to examine the associations of genetic variants of *ADIPOQ* and other adipokine-regulating genes with insulin resistance and components of the metabolic syndrome in African Americans.

Materials and Methods

Subjects

The participants were all self-identified young adult African American volunteers from urban Philadelphia who were enrolled in a study on blood pressure, insulin resistance, and cardiovascular risk between January 2001 and April 2006. Caribbean African Americans were not enrolled. The European admixture of this African American cohort has been previously analyzed and found to be between 12.7% and 13.6%.¹⁸ Known diabetic subjects were excluded from enrollment. All subjects provided written informed consent for the protocol on a consent form approved by

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DOI: 10.1111/j.1752-8062.2008.00055.x

the institutional review board of the Thomas Jefferson University at enrollment. All women were premenopausal at enrollment, and all procedures were conducted in the prefollicular phase of their menstrual cycles, estimated as 2–7 days following the onset of menses.

Procedures

Enrollment assessment consisted of anthropometric measurements (height, weight, and BP), a fasting blood sample, and an oral glucose tolerance test (OGTT) after a 12-hour fast. The buffy coat was removed from the fasting blood sample and stored at -80°C until extraction of DNA. Anthropometric measurements were used to calculate BMI (kg/m^2). BP was measured using a mercury column sphygmomanometer with the participant in a seated position after 10 minutes of rest. An average of two measurements for systolic BP (SBP) and diastolic BP (DBP) were determined. For the OGTT, an oral 75-g glucose solution (Glucola; Ames Laboratories, Elkhart, IN, USA) was ingested. Blood samples were drawn preglucose load (fasting), and at 30, 60, and 120 minutes postglucose load. All samples were assayed for plasma insulin (radioimmunoassay; Diagnostic Products Corporation, Los Angeles, CA, USA) and glucose concentrations after storage at -80°C . Plasma adiponectin was assayed from the fasting samples by ELISA (R&D, Minneapolis, MN, USA).

The euglycemic hyperinsulinemic clamp procedure was administered to assess insulin-stimulated glucose uptake.^{19,20} This procedure suppresses endogenous glucose production and then quantifies insulin-mediated glucose uptake by cells. The clamp procedure creates steady-state hyperinsulinemia and then measures the amount of exogenous glucose required to hold (or clamp) the plasma glucose at fasting level. The amount of exogenous glucose that is delivered to maintain plasma glucose at the fasting level is equivalent to the insulin-mediated glucose uptake by cells. The glucose infusion rate (M) is used to determine the amount of exogenous glucose that is required for an individual to maintain euglycemia during hyperinsulinemia and is a measure of how sensitive the individual is to insulin. A high glucose infusion rate indicates a greater sensitivity to insulin, whereas a low glucose infusion rate indicates a lower insulin sensitivity or relative insulin resistance. For the euglycemic clamp procedure, each participant returned to the clinic at 8 am after a 12-hour fast. The euglycemic clamp procedure was conducted according to methods previously described.^{19–21} In brief, two peripheral venous catheters were placed after the subject rested for at least 20 minutes. Three samples were withdrawn to determine fasting plasma glucose and insulin concentration. Euglycemic hyperinsulinemia was induced with a priming dose and infusion rate of insulin as previously described.²⁰ The infused insulin was administered at 1,000 mU/mL in normal saline (Novolin R; Eli Lilly, Indianapolis, IN, USA). Using this method, euglycemic hyperinsulinemia was maintained at 80–120 $\mu\text{U}/\text{mL}$ above fasting insulin concentration for 120 minutes. Glucose was infused as 20% (w/v) dextrose (Abbott Laboratory, Abbott Park, IL, USA) to maintain euglycemia. The fasting plasma glucose concentration, prior to beginning the insulin and glucose infusions, was used as the target glucose level for euglycemia during the clamp procedure. The glucose infusion rate was adjusted as a function of the plasma glucose concentrations sampled every 10 minutes, according to the negative feedback equation of DeFronzo et al.¹⁹ Insulin-stimulated glucose metabolism, designated as M, was quantified as the mean glucose infusion rate required to maintain

euglycemia during the final 60 minutes (clamp period) of the clamp hyperinsulinemic procedure (M in mg/kg per min). The mean plasma glucose concentration during the final 60 minutes of the clamp procedure was within 5% of the fasting plasma glucose, and the coefficient of variation for the plasma glucose concentration during the final 60 minutes was less than 3.0%. Due to variations between subjects in the level of steady-state hyperinsulinemia, M was adjusted for the level of hyperinsulinemia during the clamp period by dividing M by the mean insulin concentration (I) $\times 1,000$ to derive an insulin sensitivity index of M/I .

The fasting blood sample from the enrollment assessment was analyzed for serum lipid concentrations (total cholesterol, high-density lipoprotein [HDL] cholesterol, and triglycerides) by a lipid research laboratory using standard enzymatic methods and an automated analyzer Hitachi 704 (Boehringer-Mannheim Diagnostics, Indianapolis, IN, USA). HDL was isolated using a method previously described.²² The Friedewald equation was used to calculate low-density lipoprotein (LDL) cholesterol.²³ The coefficients of variation for inter- and intraassay variability for the lipid assays and the above glucose and insulin assays were $<5\%$.

Genotyping

The candidate genes for genotyping included adiponectin (276G>T, 45T>G, 164T>I, 11391G>A, 712A>G, 2019 del/insA) and proinflammatory cytokines including IL-6 (–597G>A, –174G>C), TNF- α (308G>A), PAI-1 (–765 4G/5G, –844A>G), and calpain 10 (Cal10–44, Cal10–45, Cal10–63). Genomic DNA was isolated from peripheral blood leukocytes using the Puregene DNA isolation kit, as described by the manufacturer (Gentra Systems, Minneapolis, MN, USA). SNPs within IL-6, TNF- α , PAI-1, and calpain 10 were assessed by PCR of genomic DNA, as described previously,²⁴ followed by DNA sequence analysis using an ABI 3730 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA, USA).

The adiponectin 712 assay conditions are detailed since this variant was significantly associated with the variation in insulin sensitivity. PCR reactions (25 μL) contained 2 μL of 10 ng/ μL genomic DNA, 500 nM of forward primer 5'-AAGATGTCTAATGTGCAAGG-3', 500 nM of reverse primer 5'-CTCCTCTATTCTGCCTACCC-3' (Integrated DNA Technologies, Coralville, IA, USA), 0.2 mM of each dNTP, 0.625 U of AmpliTaq Gold (Applied Biosystems, Inc.), 1.5 mM MgCl_2 , and 1X PCR buffer. The samples were thermal cycled on an ABI 9700 using the following conditions: 35 cycles of 95°C for 1 minute, 57°C for 1 minute, 72°C for 1 minute, with a final extension at 72°C for 10 minutes and a 4°C hold. Following post-PCR purification, DNA sequence analysis was performed as recommended by the manufacturer (Applied Biosystems, Inc.) using BigDye v3.1 sequencing reaction mix. The sequences were analyzed using ABI DNA sequencing analysis software v3.2 (Applied Biosystems, Inc.) and aligned using Sequencher v4.2 (GeneCodes Corporation, Ann Arbor, MI, USA).

Statistical methods

Distributions of BMI, plasma adiponectin, systolic and diastolic BP, and metabolic traits including insulin sensitivity, glucose tolerance, and lipids were described by a sample mean and standard deviation. The pairwise Pearson's correlation coefficients were calculated and tested for significance using the Bonferroni correction. Logarithm or square-root transformations were applied when necessary in order to increase normality of phenotypes distribution.

A chi-squared test was performed to assess whether the observed genotype frequencies were in Hardy-Weinberg equilibrium (HWE) in the sample. SNPs that were shown to be in HWE were included in the quantitative trait association analysis. The pairwise linkage disequilibrium (LD) among the SNPs was assessed by the correlation coefficient r^2 .

A multiple linear regression analysis was performed to test the null hypothesis that the phenotype means did not differ between SNP genotypes and to quantify the impact of the statistically significant genotypes. The regression models included age, sex, and BMI as covariates. The analysis of BP was also adjusted for the presence of a treatment for hypertension. The adjusted percentage of the phenotypic variance explained by each genotype was estimated by subtracting the adjusted R^2 coefficient for a model that includes the genotype from the R^2 for a model that excludes it.

A statistical analysis was performed using STATA 9.0 (<http://www.stata.com>) and its additional module for genetic association test (<http://www-gene.cimr.cam.ac.uk/clayton/software/stata/>). A two-sided p value <0.05 was considered significant.

Results

Data were obtained on a sample of 273 African American unrelated subjects. More than 65% were females, and the mean age of the participants was 39.8 years (range 28–52 years, SD = 3.5 years). Descriptive data on the participant sample are provided for BMI, BP, and metabolic traits including M/I, fasting glucose, insulin, lipids, and plasma adiponectin, separately for males and females, in Table 1.

The correlation coefficients among the measured clinical traits are shown in Table 2. As expected, significant correlations among the parameters of the metabolic syndrome are present. The M/I correlates significantly with BP and the metabolic variables, except glucose. The relationship of M/I with BP and triglycerides is negative, indicating that a lower M/I index (or more insulin resistance) is related to a higher BP and higher triglyceride levels.

SNPs within IL-6, TNF- α , PAI-1, and calpain 10 showed no statistically significant association. A statistically significant association was detected for genotypes of the variant ADIPOQ 712 of the adiponectin gene with insulin sensitivity (M/I). In Table 3, the mean values of all studied traits are provided for each SNP genotype. A statistically significant difference between the three genotypes was present for M/I ($p < 0.05$). From Table 3, it can be seen that the mean M/I is significantly lower in the GG genotype compared with the AA and AG genotypes. M/I was then compared between carriers and noncarriers of the A allele of ADIPOQ 712. The box plot in Figure 1 shows that M/I for carriers of the A allele (grouping the AA and AG genotypes) is higher than for noncarriers. This result was confirmed by a linear regression

analysis after adjustment for sex and BMI, which resulted in a significant coefficient for A allele carriers equal to 0.24 (p value = 0.001, $R^2 = 0.50$). BMI was the only significant covariate ($p < 0.001$) in the model. The adjusted percentage of the phenotypic variance explained by A carrier genotype was estimated to be equal to 3%. No statistically significant association was detected for plasma adiponectin concentration with any SNPs of the ADIPOQ gene.

Discussion

In this genetic association study on a sample of healthy young adult African Americans, a small but statistically significant association of a variant of the adiponectin gene (ADIPOQ 712) with insulin sensitivity was detected. Carriers of the A allele had a greater insulin sensitivity, as measured directly by an insulin clamp procedure, compared with noncarriers of the A allele for this adiponectin gene SNP. The variability in this SNP genotype contributed an estimated 3% to the variance of insulin sensitivity. Specifically, those without the A allele tended to be more insulin resistant. Insulin sensitivity, quantified by the insulin clamp, also correlated significantly with the other parameters of the metabolic syndrome. An association of plasma adiponectin concentration with variants of ADIPOQ 712 was not detected in this sample.

Trait	Males		Females	
	No. of Observations	Mean $\pm$ SD	No. of Observations	Mean $\pm$ SD
BMI (kg/m ²)	94	29.5 $\pm$ 6.6	179	33.5 $\pm$ 8.1
Systolic BP (mmHg)	94	129.1 $\pm$ 17.0	179	125.8 $\pm$ 20.0
Diastolic BP (mmHg)	94	76.4 $\pm$ 13.2	179	73.4 $\pm$ 12.6
Glucose (mg/dL)	94	108.0 $\pm$ 44.6	179	105.4 $\pm$ 41.9
HDL cholesterol (mg/dL)	89	47.5 $\pm$ 17.9	175	49.3 $\pm$ 13.6
Triglycerides (mg/dL)	89	93.7 $\pm$ 70.9	175	86.5 $\pm$ 48.2
M/I (mg/kg-min/l)	85	10.7 $\pm$ 7.0	155	7.8 $\pm$ 5.2
Plasma adiponectin (μ g/mL)	56	12.9 $\pm$ 6.5	118	14.3 $\pm$ 8.7

M/I, insulin sensitivity index.

Table 1. Mean values for quantitative traits in African American males and females.

	BMI	Systolic BP	Diastolic BP	Glucose	HDL Cholesterol	Triglycerides	M/I
Systolic BP	0.21*						
Diastolic BP	0.17	0.76*					
Glucose	0.07	0.06	0.09				
HDL Cholesterol	−0.21*	−0.13	−0.17	−0.03			
Triglycerides	0.13	0.20*	0.27*	0.12	−0.24*		
M/I	−0.61*	−0.23*	−0.22*	−0.12	0.22*	−0.26*	
Adiponectin	−0.17	−0.001	0.04	−0.07	0.16	−0.21	0.18

*Significant ($p < 0.05$) after the Bonferroni correction.
M/I, insulin sensitivity index.

Table 2. Correlation coefficients among BP and metabolic traits.

Trait	AA		AG		GG	
	No. of Observations	Mean $\pm$ SD	No. of Observations	Mean $\pm$ SD	No. of Observations	Mean $\pm$ SD
BMI (kg/m ²)	61	31.2 $\pm$ 6.8	87	33.2 $\pm$ 8.5	44	33.6 $\pm$ 8.5
Systolic BP (mmHg)	61	125.4 $\pm$ 16.4	87	127.2 $\pm$ 21.1	44	129.5 $\pm$ 16.4
Diastolic BP (mmHg)	61	74.1 $\pm$ 12.2	87	74.7 $\pm$ 13.3	44	74.6 $\pm$ 12.6
Glucose (mg/dL)	61	110.0 $\pm$ 49.1	87	97.9 $\pm$ 13.0	44	116.3 $\pm$ 65.1
HDL cholesterol (mg/dL)	57	49.8 $\pm$ 14.1	86	49.8 $\pm$ 18.3	42	49.9 $\pm$ 15.6
Triglycerides (mg/dL)	57	88.1 $\pm$ 47.7	86	90.5 $\pm$ 54.8	42	106.7 $\pm$ 83.2
*M/I (mg/kg-min/l)	56	8.8 $\pm$ 6.6	79	9.5 $\pm$ 6.2	38	6.8 $\pm$ 4.5
Adiponectin (μ g/mL)	40	14.9 $\pm$ 9.3	51	14.2 $\pm$ 9.4	38	13.9 $\pm$ 7.1

* $p < 0.05$ after the Bonferroni correction.

M/I, insulin sensitivity index.

Table 3. Associations between the genotypes for SNP 712 of the *ADIPOQ* gene and the quantitative traits.

Figure 1. Box plot of the insulin sensitivity index for carriers and noncarriers of the A allele for the *ADIPOQ* 712 SNP. Boxes are bordered at the 25th and 75th percentiles of the trait and with a central line at the median (50th percentile). Whiskers extend to the largest and smallest data values that are, respectively, less than the third quartile plus $1.5 \times$ interquartile range (IQR) and greater than the first quartile minus $1.5 \times$ IQR. Values exceeding these thresholds are called outside values and are displayed as markers.

ADIPOQ 712 (rs3774261) is an A to G SNP located in intron 2 of the gene. Two nonsynonymous SNPs (rs13061862 and rs17366743) are located in exons 2 and 3, respectively, of *ADIPOQ*. Based on the HapMap data, *ADIPOQ* 712 is included in a block of high LD that contains intron 2, exon 3, and part of the 3'-UTR of the gene, and is in complete LD with rs17366743 in the Caucasian Central European population. SNPs rs13061862 and rs17366743 are not polymorphic in the HapMap Yoruba African population, nor is rs13061862 in the Central European population.

for the SNPs -11426(A/G) and -11377(G/C) in the proximal promoter region of the *ADIPOQ* gene were different in type 2 diabetic subjects compared with controls. Based on an analysis of 53 SNPs in the *ADIPOQ* gene in 1,727 healthy Caucasians, Heid et al.²⁶ reported strong associations of SNPs and haplotypes of *ADIPOQ* with plasma adiponectin concentrations. There were also significant associations between adiponectin concentrations and the parameters of the metabolic syndrome. However, these investigators detected no association of the *ADIPOQ*

Previous genetic association studies have reported positive associations of other adiponectin SNPs with CV risk factors and diabetes. Iwashima et al.²⁵ reported that among subjects carrying the TC genotype of the I164T polymorphism, plasma adiponectin concentration was lower, and most had hypertension. In a report by Menzaghi et al.,¹⁷ two other SNPs of the adiponectin gene, 45T>G and 276G>T, were associated with obesity and other features of the metabolic syndrome in a Caucasian population. These two polymorphisms of the *ADIPOQ* gene were also reported to be associated with the risk for type 2 diabetes in a study by Hara et al.²⁴ Subjects with the G/G genotype at position 45 or the G/G genotype at position 276 had a significantly increased risk of type 2 diabetes compared with those having the T/T genotype at positions 45 and 276. Alternatively, Gu et al.¹⁶ found that allele frequencies

gene with the parameters of the metabolic syndrome in this healthy population. None of the above investigations quantified insulin sensitivity (or insulin resistance) by the insulin clamp procedures.

In another study, Hu et al.²⁷ performed a genetic association analysis of five adiponectin SNPs and risk for diabetes in the Nurses Health Study cohort. No significant associations were detected, but the investigators did find that the 276 genotypes associated significantly with diabetes risk only among subjects with the 12 Ala allele of the peroxisome proliferator-activated receptor γ (PPAR- γ) gene. Although the investigators considered their findings exploratory, they suggest a potential interaction between the adiponectin genotype and PPAR- γ genotype. The small but statistically significant association of the adiponectin SNP 712 with insulin sensitivity that was detected in our sample of relatively young healthy African Americans could reflect an underlying gene–gene interaction or possibly an undetected gene–environmental interaction effect.²⁸

In experimental studies, adiponectin is an adipocyte-produced protein that appears to have vascular protective properties by blocking expression of vascular adhesion molecules in endothelial cells in response to inflammatory stimuli.^{13,14} Mice that lack adiponectin expression develop severe vascular injuries, and replacement of adiponectin in this model attenuates the vessel injury.¹⁵ In humans, plasma adiponectin is lower in obese than in nonobese individuals²⁹ and correlates negatively with insulin and triglyceride.³⁰ Race and gender differences have also been described. Plasma adiponectin levels are lower in healthy lean adolescent boys compared with girls, with adiponectin concentration in boys inversely related to testosterone and dehydroepiandrosterone sulfate.³¹ A lower plasma adiponectin concentration in African American males compared with Caucasian males has also been reported.³² Data from our sample of African Americans did not detect a significant association of plasma adiponectin concentration with any variants of the *ADIPOQ* gene. An analysis of our data also did not identify a significant relationship of plasma adiponectin with insulin sensitivity. Our sample included more women than men, with more obesity among the women than the men. It is possible that the combination of the testosterone-lowering effect on adiponectin in men and the obesity-lowering effect on adiponectin in women extinguished the detection of a relationship of plasma adiponectin with insulin sensitivity in this sample. It is also possible that the association of *ADIPOQ* 712 with insulin sensitivity is functionally related to the specific molecular weight structure of adiponectin. Adiponectin circulates in serum as a hexamer of relatively low molecular weight (LMW) and a larger multimeric structure of high molecular weight (HMW).³³ In rodent models, Xu et al.³⁴ demonstrated that testosterone lowers the HMW form of adiponectin by inhibiting its secretion from adipocytes. It has also been reported that, in rodents, insulin sensitivity is related to the HMW form of adiponectin rather than total circulating adiponectin. We are not able to address this question because sufficient plasma samples from study participants were not available to measure LMW or HMW adiponectin.

A limitation of this study is that the sample of 274 unrelated participants is relatively small for a genetic association study. Despite the small sample, we used the euglycemic hyperinsulinemic insulin clamp procedure to quantify insulin sensitivity. This method is a direct measure of insulin-mediated glucose uptake and is considered to be highly rigorous.

Conclusion

The results of this investigation on a cohort of healthy relatively young African Americans detected a small but statistically significant association of insulin sensitivity with the 712 SNP of the *ADIPOQ* gene. These data are the first to be reported on genetic variants of the *ADIPOQ* gene and insulin sensitivity in African Americans.

Acknowledgments

This project was funded by a grant from the W.W. Smith Charitable Trust and, in part, by grants from the National Institutes of Health (HL51547 and DK46107).

References

- Reaven G. Role of insulin resistance in human disease. *Diabetes*. 1988; 37: 1595–1607.
- Meigs JB. Invited commentary: Insulin resistance syndrome? Syndrome X? Multiple metabolic syndrome? A syndrome at all? Factor analysis reveals patterns in the fabric of correlated metabolic risk factors. *Am J Epidemiol*. 2000; 152: 908–911.
- Yip J, Facchini FS, Reaven GM. Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. *J Clin Endocrinol Metab*. 1998; 83: 2773–2776.
- Reaven GM. The metabolic syndrome: is this diagnosis necessary? *Am J Clin Nutr*. 2006; 83: 1237–1247.
- Pittas AG, Joseph NA, Greenberg AS. Adipocytokines and insulin resistance. *J Clin Endocrinol Metab*. 2004; 89: 447–452.
- Pickup JC, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumor necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci*. 2000; 67: 291–300.
- Haffner SM, Mykkanen L, Rainwater DL, Karhapa P, Laakso M. Is leptin concentration associated with the insulin resistance syndrome in nondiabetic men? *Obes Res*. 1999; 7: 164–169.
- Festa A, D'Agostino R Jr, Tracy RP, Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes*. 2002; 51: 1131–1137.
- Winkler G, Salamon F, Salamon D, Speer G, Simon K, Cseh K. Elevated serum tumour necrosis factor-alpha levels can contribute to the insulin resistance in type II (non-insulin-dependent) diabetes and in obesity. *Diabetologia*. 1998; 41: 860–861.
- Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Takaranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab*. 2001; 86: 1930–1935.
- Arita Y, Kihara S, Ouchi N, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoaka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun*. 1999; 257: 79–83.
- Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein, adiponectin, suppress lipid accumulation and class A receptor expression in human monocyte-derived macrophages. *Circulation*. 2001; 103: 1057–1063.
- Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, Igura T, Inui Y, Kihara S, Nakamura T, Yamashita S, Miyagawa J, Funahashi T, Matsuzawa Y. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm Metab Res*. 2000; 32: 47–50.
- Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*. 1999; 100: 2473–2476.
- Goldstein BJ, Scalia R. Adiponectin: a novel adipokine linking adipocytes and vascular function. *J Clin Endocrinol Metab*. 2004; 89: 2563–2568.
- Gu HF, Abulaiti A, Ostenson C-G, Humphreys K, Wahlestedt C, Brookes AJ, Efendic S. Single nucleotide polymorphisms in the proximal promoter region of the adiponectin (APM1) gene are associated with type 2 diabetes in Swedish Caucasians. *Diabetes*. 2004; 53(Suppl 1): S31–S35.
- Menzaghi C, Ercolino T, Di Paola R, Berg AH, Warram JH, Scherer PE, Trischitta V, Doria A. A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes*. 2002; 51: 2306–2312.
- Parra EJ, Marcini A, Akey J, Martinson J, Batzer MA, Cooper R, Forrester T, Allison DB, Deka R, Ferrell RE, Shriver MD. Estimating African American admixture proportions by use of population-specific alleles. *Am J Hum Genet*. 1998; 63: 1839–1851.
- DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979; 237: E214–E223.
- Rizza RA, Mandarino LJ, Gerich JE. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol*. 1981; 240: E630–E639.
- Falkner B, Kushner H, Tulenko T, Sumner AE, Marsh JB. Insulin sensitivity, lipids, and blood pressure in young African blacks. *Arterioscler Thromb Vasc Biol*. 1995; 15: 1798–1804.

22. Bachorik PS, Walker RE, Virgil DG. High-density-lipoprotein cholesterol in heparin-MnCl₂ supernates determined with the Dow enzymic method after precipitation of Mn²⁺ with HCO₃⁻. *Clin Chem.* 1984; 30: 839–842.
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972; 18: 499–502.
24. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Kimura S, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 2002; 51: 536–540.
25. Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, Fu Y, Motone M, Yamamoto K, Matsuo A, Ohashi K, Kihara S, Funahashi T, Rakugi H, Matsuzawa Y, Ogihara T. Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension.* 2004; 43: 1318–1323.
26. Heid IM, Wagner SA, Gohlke H, Iglseider B, Mueller JC, Cip P, Ladurner G, Reiter R, Stadlmayr A, Mackevics V, Illig T, Kronenberg F, Paulweber B. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. *Diabetes.* 2006; 55: 375–384.
27. Hu FB, Doria A, Li T, Meigs JB, Liu S, Memisoglu A, Hunter D, Manson JE. Genetic variation at the adiponectin locus and risk of type 2 diabetes in women. *Diabetes.* 2004; 53: 209–213.
28. Brown NJ, Murphey LJ, Srikuma N, Koschachuhanan N, Williams GH, Vaughan DE. Interactive effect of PAI-1, 4G/5G genotype and salt intake on PAI-1 antigen. *Arterioscler Thromb Vasc Biol.* 2001; 21: 1071–1077.
29. Weiss R, Dufour S, Groszmann A, Petersen K, Dziura J, Taksali SE, Shulman G, Caprio S. Low adiponectin levels in adolescent obesity: a marker of increased intramyocellular lipid accumulation. *J Clin Endocrinol Metab.* 2003; 88: 2014–2018.
30. Yamamoto Y, Hirose H, Saito I, Tomita M, Taniyama M, Matsubara K, Okazaki Y, Ishii T, Nishikai K, Saruta T. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. *Clin Sci (Lond).* 2002; 103: 137–142.
31. Bottner A, Kratzsch J, Muller G, Kapellen TM, Bluher S, Keller E, Bluher M, Kiess W. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. *J Clin Endocrinol Metab.* 2004; 89: 4053–4061.
32. Degawa-Yamauchi M, Dilts JR, Bovenkerk JE, Saha C, Pratt JH, Considine RV. Lower serum adiponectin levels in African-American boys. *Obes Res.* 2003; 11: 1384–1390.
33. Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchannan TA, Scherer PE. Complex distribution, not absolute amount of adiponectin correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem.* 2004; 279: 12152–12163.
34. Xu A, Chan KW, Hoo RLC, Wang Y, Tan KCB, Zhang J, Chen B, Lam MC, Tse C, Cooper GJS, Lam KSL. Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *J Biol Chem.* 2005; 280: 18073–18080.