Impact of adiponectin gene polymorphisms on plasma lipoprotein and adiponectin concentrations of viscerally obese men

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Abstract **The aim of this study was first to examine the relationships between adiponectin gene (*Apm1*) polymorphisms and anthropometric indices as well as plasma adiponectin and lipoprotein/lipid levels, and then to investigate whether the presence of visceral obesity or insulin resistance may modulate the impact of these polymorphisms on metabolic risk variables. Molecular screening of the *Apm1* gene was achieved, and a sample of 270 unrelated men recruited from the greater Quebec City area and selected to cover a wide range of body fatness values was genotyped. Sequencing of the *Apm1* gene revealed two previously reported polymorphisms (c.45T>G and c.276G>T) as well as two newly identified genetic variations (−13752delT and −13702G>C). Carriers of the c.276T allele had higher LDL-cholesterol and lower HDL-triglyceride concentrations than did 276G/G homozygotes ($P = 0.02$ and $P = 0.01$, respectively). Carriers of the c.45G allele exhibited higher plasma adiponectin concentrations than did 45T/T homozygotes ($P = 0.04$). After dividing each genotype group into subgroups for visceral AT, homozygotes for the normal allele at position −13752delT, carriers of the c.45G allele, and carriers of the c.276T allele had similar total apolipoprotein B (apoB) concentrations, whether they were viscerally obese or not. These results suggest that some *Apm1* gene polymorphisms influence plasma adiponectin concentrations and lipoprotein/lipid levels. In addition, the impact of these polymorphisms is modulated by the presence of visceral obesity.—** Berthier, M-T., A. Houde, M. Côté, A-M. Paradis, P. Mauriège, J. Bergeron, D. Gaudet, J-P. Després, and M-C. Vohl. **Impact of adiponectin gene polymorphisms on plasma lipoprotein and adiponectin concentrations of viscerally obese men. *J. Lipid Res.* 2005.** 46: **237–244.**

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Obesity is associated with increased morbidity and mortality (1). Obesity, specially the abdominal form, can promote the parallel progression of insulin resistance to type 2 diabetes and endothelial dysfunction to atherosclerosis (2). Metabolic complications associated with abdominal obesity are insulin resistance and hyperinsulinemia as well as a dyslipidemic state characterized by increased plasma triglyceride and apolipoprotein B (apoB) levels, decreased HDL-cholesterol concentrations, and a greater proportion of small, dense LDLs (3–5).

Adipose tissue (AT) is highly implicated in cell function and regulation through a complex network of endocrine, paracrine, and autocrine signals (6) and thus represents a good source of cytokines (7) named "adipocytokines" (8). Most serum adipocytokine levels have been shown to be increased in obese or insulin-resistant subjects or in subjects with high cardiovascular risk (9–12). However, low levels of adiponectin (13–15), one of the most abundant specific adipocytokines (8), are associated with insulin resistance, abdominal obesity (8, 15), and activation of vascular inflammation (16). Although the role of adiponectin is not yet totally elucidated, this protein is believed to provide a new pathway influencing the genesis of the metabolic syndrome (17).

Adiponectin is the gene product of *Apm1*, which has been mapped on chromosome 3q27 (18). A genome-wide scan performed for phenotypes related to the obesity/metabolic syndrome has detected significant linkage signals in this chromosome region (19). Mutations in exon 2 of the *Apm1* gene, c.45G>T and c.276T>G, have been associated with obesity and insulin resistance among Italian subjects (20). Other mutations have been reported in the

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promoter, in introns 1 and 2, in exon 3, and at the 3′ end of the gene (21, 22).

The aim of this study was first to identify genetic variations in the *Apm1* gene in subjects with high levels of visceral AT and then to verify the impact of these sequence variants on two anthropometric indices, plasma adiponectin concentrations and plasma lipoprotein/lipid levels. Finally, we wanted to investigate whether the presence of visceral obesity or insulin resistance may modulate the observed effect.

MATERIALS AND METHODS

Subjects

The *Apm1* gene was first sequenced in 5 subjects exhibiting low visceral AT (<130 cm^2) and 19 subjects with high visceral AT accumulation (≥ 130 cm^2). When a polymorphism was found, a sample of 270 unrelated men, from the greater Québec City area and selected to cover a wide range of body fatness values, was genotyped. These men were all nonsmokers and free from metabolic disorders such as arterial hypertension, type 2 diabetes, and coronary heart disease (23). None of them was using medications known to affect insulin action or plasma lipoprotein levels. All subjects gave their written consent to participate into this study, which was approved by the Ethics Committee of Laval University.

Anthropometric measurements

Waist circumference, body weight, and height were measured according to standardized procedures (24). Visceral AT area at the l_4l_5 level was measured by computed tomography on a Siemens Somatom DRH scanner (Erlangen, Germany), as previously described (25, 26).

Plasma lipoprotein and lipid measurements

Blood cholesterol and triglyceride concentrations were enzymatically measured on a Technicon RA-500 analyzer (Bayer Corp., Tarrytown, NY). VLDLs were isolated by ultracentrifugation ($d < 1.006$ g/ml), and the HDL fraction was obtained after precipitation of LDL in the infranatant ($d > 1.006$ g/ml) with heparin and $MnCl_2$ (27). ApoB concentrations were measured in plasma and infranatant (LDL-apoB; $d > 1.006$ g/ml) by the rocket immunoelectrophoretic method of Laurell (28), as previously described (27). Serum standards were prepared in our laboratory, calibrated against reference sera obtained from the Centers for Disease Control (Atlanta, GA), which had been lyophilized, and stored at −85°C until use.

Insulin measurements

After an overnight fast, blood samples were collected in EDTA-containing tubes (Miles Pharmaceuticals, Resedale, Ontario, Canada) through a venous catheter placed in an antecubital vein for the measurement of plasma insulin concentrations (29). Plasma insulin levels were assessed by radioimmunoassay with polyethylene glycol separation (30).

Plasma adiponectin concentrations

Fasting plasma adiponectin concentrations were determined using an enzyme-linked immunosorbent assay (B-Bridge International, Inc., San Jose, CA). The intra-assay and interassay variation coefficients were 3.3% and 7.4%, respectively. Plasma concentrations of adiponectin ranged from 1.26 to 28.84 μg/ml, with a median value of 8.86 μg/ml in the study sample.

PCR amplification and sequencing of *Apm1*

To design intronic primers for the amplification of each exon, genomic sequences were sought for the intron regions surrounding all *Apm1* exons. To do so, we compared mRNA sequences of each exon found at the Nucleotides website (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi/nucleotide) with a genomic DNA sequence (accession number gi:22045820). Intronic primers were designed using Primer 3.0 software (http://wwwgenome.wi.mit.edu/cgibin/primer/primer3.cgi). The primer sequences chosen are presented in **Table 1** and were located ~10–30 bp upstream and downstream of each exon. Between positions −14000 and +1 of the transcription site, two regions in the promoter (−13761 to −13362 and −13113 to −12426) were considered of interest because they contain ligand binding sites. These two regions were chosen to be sequenced and are designated promoter 1 and promoter 2 in Table 1.

Genomic DNA was isolated from white blood cells with the GenElute™ Blood Genomic DNA Miniprep Kit (Sigma). The promoter region and the three exons were amplified from genomic DNA by use of specific primers with adequate annealing temperatures (Table 1). PCR volume was 50 μl, and conditions were as follow: 0.2 mM deoxynucleoside triphosphates, primers at a final concentration of 1.26 μM, and 100 ng of genomic DNA.

TABLE 1. Primer sequencing, annealing temperature, and sequence variations in the *Apm1* gene

Exon	Name	Primer	Annealing Temperature	DNA	Protein	Restriction Enzyme	Frequency
			°C				
Promoter 1	AdiPr1F	5′-AAGTAGCTGGGACTACAGGTATGTG-3′	67	c.−13752del T[a]	—	—	7% delT
−13762 to −13365	AdiPr1R	5′-AGATGAGAGACATGAGAGACTTTCC-3′		c.−13702G>C[a]	—	—	3% G
Promoter 2	AdiPr2F	5′-CATCAGAATGTGTGGCTTGC-3′	65	—	—	—	—
−13113 to −12426	AdiPr2R	5′-TCTCCCCCTCCCATAGATTT-3′					
Exon 1	Adi1F	5′-CTGAGTTGGCCAATGGGAAAT-3′	57	—	—	—	—
	Adi1R	5′-TCCCTTATCCCCCTTAATCG-3′					
Exon 2/intron2	Adi2F	5′-TGGACGGAGTCCTTTGTAGG-3′	55	c.45T>G	p. G15G	*Sma*I	12% G
	Adi2R	5′-TCATCCTTGGAAGACCAACC-3′					
	Adi276R-M	5′-TCTAGGCCTTAGTTAATAAGAA**GG**-3′		c.276G>T	—	*Stu*I	30% T
Exon 3	Adi3F	5′-CCTCCATGTCTGTGGAGAT-3′	55	—	—	—	—
	Adi3R	5′-ATTGACTTTGGGGCTGTTTG-3′					

Allele frequency was calculated in a sample of 270 unrelated French-Canadian men. F, forward primer; R, reverse primer. For primer Adi276R-M, the nucleotide in boldface corresponds to a mismatch nucleotide. Promoter 1 and promoter 2 represent two regions of interest in the promoter. Primers Adi2F and Adi276R-M were used to sequence and genotype c.276G>T.

[a] Genotyping by direct sequencing.

Then, two different polymerases were used for PCR depending on the region to be amplified. To achieve the amplification of promoter exons 2 and 3, 2.5 units of Red Taq genomic DNA polymerase (Sigma Aldrich Co.) in 1.5 mM $MgCl_2$ was used. PCR amplification of exon 1 was performed using 2.5 units of Taq polymerase (New England Biolabs) in the buffer recommended by the manufacturer. PCR products were purified with the QIAQuick® 8 PCR Purification Kit (Qiagen, Inc., Mississauga, Ontario, Canada). Sequencing reactions were performed using BigDye™ Terminator version 3.0 cycle sequencing (ABI Prism, Applied Biosystem, Foster City, CA), and the products were analyzed on an ABI 3100 automated DNA sequencer (PE Applied Biosystems, Foster City, CA). The gel files were processed using ABI Prism® 3100 data collection software version 1.1 and ABI Prism® DNA sequencing analysis software (PE Applied Biosystems, Foster City, CA), then assembled and analyzed using the STADEN preGap4 and Gap4 programs.

Detection of sequence variations

A PCR-restriction fragment length polymorphism-based method was used to genotype sequence variations in the larger cohort of 270 unrelated men (Table 1). However, because the c.276G>T in exon 2 does not alter any restriction site, a mismatch PCR method was performed using the primers Adi2F and Adi276R-Mismatch (Table 1). Subsequently, restriction enzymes *Sma*I and *Stu*I were chosen to differentiate the presence or absence of the c.45T>G and c.276G>T mutations, respectively. Digestion products were size-separated on 4% agarose gels, stained with ethidium bromide, and pictured on an ultraviolet light box.

Because no restriction enzyme exists to detect the −13752delT variant in the cohort, genotyping by direct sequencing of the promoter 1 region was achieved, which also allowed the detection of the c.−13703G>C variation.

The terminology for each mutation described in Table 1 is in accordance with the mutation nomenclature of den Dunnen and Antonarakis (31). The polymorphisms located in the promoter were numbered from the translation initiation site.

Genotype determination of peroxisome proliferator-activated receptor γ P12A

DNA was amplified using polymerase chain reaction amplification. This reaction used 1.25 units of ampliTaq (Perkin-Elmer Cetus), 200 μg of genomic DNA, 40.0 mmol of deoxynucleoside triphosphate, 5% DMSO, and 62 pmol of each oligonucleotide (left primer, 5′-GCCAATTCAAGCCAGTC-3′; right primer, 5′-GATATGTTTGCAGACGTGTATATCAGTGAAGGAATCGCTTTCCG-3′). The annealing temperature of the PCR was 55°C after a hot start at 100°C. PCR products were digested with 12 units of ACI1 in the buffer recommended by the manufacturer. After digestion, fragments were size-separated on a 20% acrylamide gel and pictured on an ultraviolet light box.

Linkage disequilibrium coefficient of polymorphism sites of the *Apm1* gene

To establish possible linkage disequilibrium between two polymorphisms, we used the software programs EH and 2LD found at www.hgmp.mrc.ac.uk.

Statistical analyses

Before analyses, fasting adiponectin and HDL-triglyceride levels were $\log_{10}$-transformed to reduce the skewness of their distribution. After dividing the sample according to normal homozygotes and carriers of mutations, comparisons were performed using Student's *t*-test. Analyses of covariance were achieved to adjust variables for age, and the least square means (LSMEANS) procedure was used to detect significant differences between groups.

To verify whether visceral obesity modulates the effect of the *Apm1* genotype on metabolic parameters, subjects were first classified on the basis of visceral AT area and then on the basis of the genotype. The value of 130 cm^2 for visceral AT was used to identify subjects with low versus high visceral AT accumulation. As previously reported by Després and Lamarche (32), men with visceral AT accumulation greater than this value are frequently characterized by a cluster of metabolic abnormalities that increase the risk of cardiovascular disease, such as insulin resistance, glucose intolerance, and dyslipidemic state. Comparisons among subgroups were performed using ANOVA to quantify the effect of visceral AT on genotype. The Duncan multiple-range comparison test was used in cases in which a significant group effect was noted. Analysis of covariance was also performed to adjust variables for age or age and adiponectin concentrations, and differences between groups were determined by the LSMEANS procedure. A similar procedure was used to study the effect of insulin concentrations on adiponectin and lipoprotein/lipid levels. The cutoff point for plasma insulin levels used in the analysis was 80 pmol/l. This value was chosen not only because it was the median value of the sample studied but also because men with fasting insulin levels greater than 80 pmol/l were shown to be more at risk of coronary heart disease, especially of myocardial infarction, independent of other risk factors related to fasting insulin (33).

The Pearson correlation product coefficient was used to quantify associations between adiponectin concentrations and visceral AT within each genotype group after adjustment for age.

To determine a possible gene-gene interaction effect between the peroxisome proliferator-activated receptor γ (PPARγ) P12A polymorphism and variations of the *Apm1* gene on lipoprotein/lipid concentrations, analyses of variance and covariance were performed as described above.

It may be argued that Bonferroni correction would be appropriate to the thresholds of significance of associations of genotypes with the variety of variables tested. It is true that there is a chance of type 1 error attributable to multiple comparisons, based on the fact that the comparisons we made were not totally independent of each other. However, because of the observational design of this study, we preferred having false-positive to false-negative associations. That is why we believe that Bonferroni correction may be too conservative for this study.

All statistical analyses were achieved with the SAS statistical package (SAS Institute, Cary, NC), and a value of $P < 0.05$ was considered significant.

RESULTS

Molecular screening of the *Apm1* gene in 5 subjects with low visceral AT (<130 cm^2) and 19 subjects with high visceral AT (≥130 cm^2) revealed four sequence variations, and two of them, −13752delT and −13702G>C, were newly identified (Table 1). The frequency of each variant is shown in Table 1. The frequency of the −13702G>C allele was of only 3% in the study sample, and association studies were not further pursued with this single nucleotide polymorphism (SNP). The mutation −13752delT was in partial linkage disequilibrium with c.45T>G [linkage disequilibrium coefficinet (D′) = 0.45, $P < 0.001$]. Moreover, c.45T>G was also in linkage disequilibrium with c.276G>T (D′ = 0.64, $P = 0.002$).

Associations with *Apm1* polymorphisms

None of the *Apm1* gene polymorphisms were directly associated with anthropometric indices (**Table 2**). We also

TABLE 2. Anthropometric characteristics of subjects according to *Apm1* polymorphisms

Exon	Age	Body Mass Index	Waist Circumference	Visceral Adipose Tissue
	years	*kg/m²*	*cm*	*cm²*
−13752delT				
1	44.04 ± 7.93 (25)	29.14 ± 4.03 (25)	100.91 ± 10.64 (25)	158.09 ± 50.60 (25)
2	41.52 ± 8.09 (141)	28.59 ± 4.47 (140)	99.09 ± 12.18 (141)	152.53 ± 60.23 (137)
P	0.15	0.56	0.48	0.66
c.45T>G				
1	42.64 ± 6.24 (56)	29.49 ± 3.58 (56)	101.80 ± 10.70 (56)	164.93 ± 54.75 (56)
2	42.75 ± 8.26 (198)	29.42 ± 4.26 (197)	101.01 ± 10.98 (198)	161.18 ± 55.74 (196)
P	0.93	0.91	0.63	0.65
c.276G>T				
1	42.52 ± 7.95 (109)	29.14 ± 4.21 (109)	100.52 ± 11.23 (109)	158.51 ± 55.42 (108)
2	42.66 ± 7.93 (110)	29.05 ± 4.01 (109)	100.34 ± 11.20 (109)	159.72 ± 55.40 (108)
P	0.89	0.87	0.91	0.87

1 = carriers of the mutated allele; 2 = homozygotes for the normal allele. Numbers of subjects are shown in parentheses. *P* values are given without adjustment.

verified whether these genetic variations may affect plasma adiponectin and lipoprotein/lipid levels. Variables tested were cholesterol, triglyceride, HDL-cholesterol, HDL-triglyceride, LDL-cholesterol, LDL-triglyceride, VLDL-cholesterol, VLDL-triglyceride, fasting insulin, total apoB, and LDL-apoB concentrations. As presented in **Table 3**, plasma LDL-cholesterol, HDL-triglyceride, and adiponectin levels showed significant differences among genotype groups. Carriers of the c.276G allele exhibited higher LDL-cholesterol and HDL-triglyceride concentrations than their counterparts. Carriers of the c.45G allele had higher adiponectin concentrations than did 45T/T homozygotes ($P < 0.05$). The −13752delT polymorphism was not associated with any of these variables.

Modulation of lipoprotein/lipid profile by visceral obesity

We also verified whether the presence of visceral obesity can modulate the association between some *Apm1* gene polymorphisms and lipoprotein/lipid levels. Each genotype group was divided into two subgroups using a cutoff point of 130 cm² for visceral AT (**Fig. 1**). Significantly higher total apoB concentrations were found in viscerally obese homozygotes without deletion at position −13752 (Fig. 1A), carriers of the c.45G allele (Fig. 1B), and carriers of the c.276T allele ($P = 0.0015$) compared with lean −13752 homozygotes without deletion, carriers of the c.45G allele, and carriers of the c.276T allele, respectively. However, total apoB concentrations remained similar among carriers of the −13752delT allele, 45T/T homozygotes, and 276G/G homozygotes independent of the accumulation of visceral obesity. After adjustment for age and plasma adiponectin concentrations, differences between genotypes observed for total apoB levels were no longer significant. However, correlation analyses revealed that plasma adiponectin concentrations were negatively associated with visceral AT, but only in carriers of the 45G allele ($r^2 = -0.68$, $P = 0.0002$ vs. $r^2 = -0.11$, $P = 0.23$ in 45T/T homozygotes). No difference in the association between adiponectin concentrations and visceral AT was observed with the −13752delT genotype. Moreover, the highest levels of plasma adiponectin concentrations were found in lean carriers of the c.45G allele compared with the other groups (Fig. 1C).

TABLE 3. Impact of *Apm1* genotypes on adiponectin concentrations and biochemical measures

Exon	LDL-Cholesterol	HDL-Triglyceride	Adiponectin
	mmol/l		*μg/ml*
−13752delT			
1	3.41 ± 0.41 (25)	0.23 ± 0.05 (25)	10.83 ± 5.71 (14)
2	3.37 ± 0.78 (138)	0.24 ± 0.07 (136)	10.38 ± 5.00 (68)
P	0.91	0.52[a]	0.92[a]
c.45T>G			
1	3.42 ± 0.62 (56)	0.24 ± 0.06 (52)	11.76 ± 5.96 (26)
2	3.43 ± 0.76 (193)	0.24 ± 0.07 (193)	9.46 ± 4.50 (117)
P	0.89	0.99[a]	0.04[a]
c.276G>T			
1	3.52 ± 0.71 (107)	0.22 ± 0.05 (102)	10.02 ± 5.09 (63)
2	3.28 ± 0.77 (109)	0.25 ± 0.07 (103)	9.33 ± 4.16 (60)
P	0.02	0.01[a]	0.53[a]

1 = carriers of the mutated allele; 2 = homozygotes for the normal allele. Numbers of subjects are shown in parentheses. *P* values are given adjusted for age.

[a] Variable was $\log_{10}$-transformed before analysis.

Modulation of adiponectin concentrations by fasting insulin

Because insulin resistance was a strong negative predictor of *Apm1* gene expression (34), each genotype group was divided into subgroups using a cutoff point of 80 pmol/l for fasting insulin concentrations to separate potential insulin-resistant subjects from men with a normal insulin sensitivity. Carriers of the allele −13752delT with concomitant high fasting insulin levels had lower adiponectin concentrations than subjects with low fasting insulin levels. In contrast, similar adiponectin concentrations were observed for homozygotes without the deletion at position −13752 characterized by either high or low insulin level (**Fig. 2**).

Interaction between *Apm1* polymorphisms and PPARγ P12A polymorphisms

The adiponectin gene has a functional PPAR-responsive element that could reduce basal transcriptional activity (35). Thus, we investigated whether an interaction be-

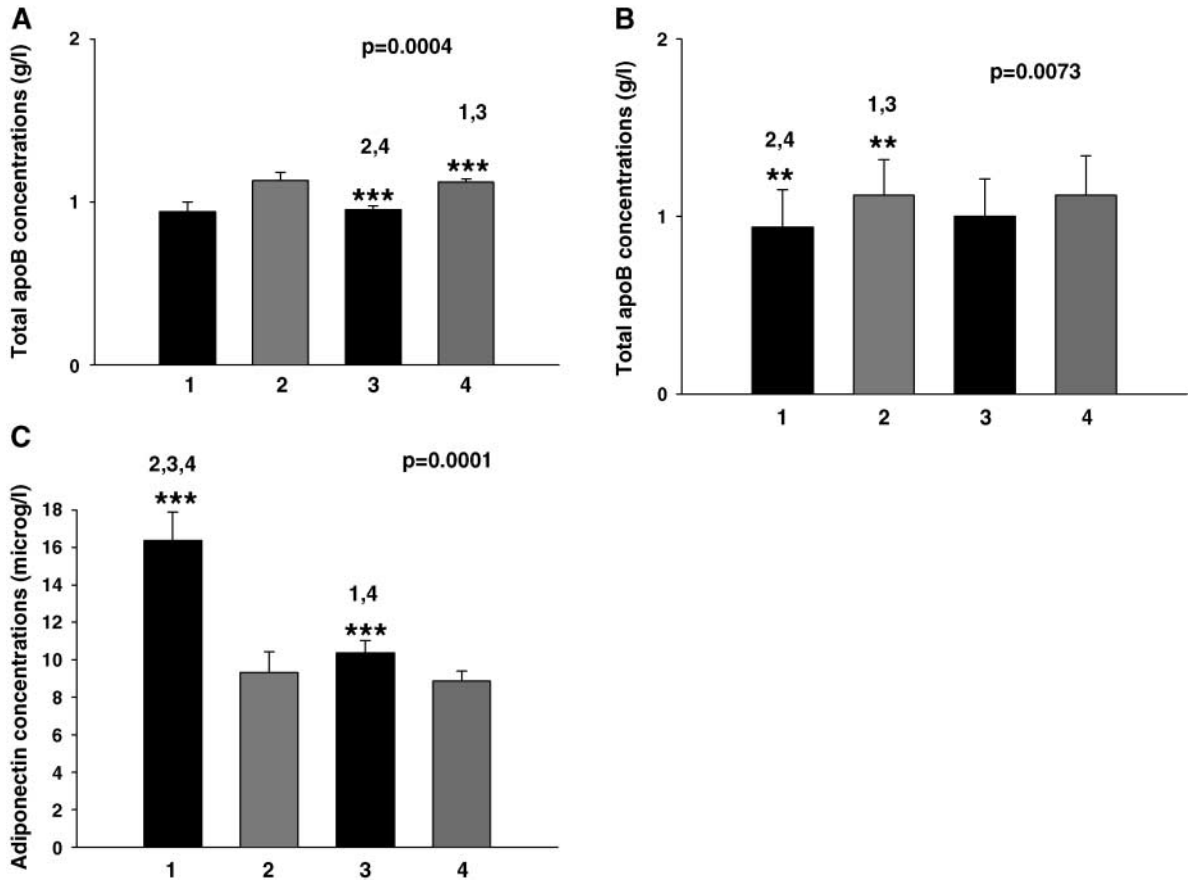

Fig. 1. Combined effect of visceral obesity and *Apm1* genotypes on adiponectin and lipoprotein/lipid concentrations. A: Bar graphs comparing total apolipoprotein B (apoB) concentrations within each −13752delT genotype in men with low (<130 cm^2) or high (≥130 cm^2) visceral adipose tissue (AT) after adjustment for age. The number below each bar identifies the subgroup [1 = carriers of the deletion and visceral AT < 130 cm^2 (n = 9), 2 = carriers of the deletion and visceral AT ≥ 130 cm^2 (n = 6), 3 = homozygotes without the deletion and visceral AT < 130 cm^2 (n = 55), and 4 = homozygotes without the deletion and visceral AT ≥ 130 cm^2 (n = 81)]. Numbers above the bars indicate groups that differ significantly. *P* values are given after adjustment for age. B: Bar graphs comparing total apoB concentrations within each c.45T>G genotype in men with low (<130 cm^2) or high (≥130 cm^2) visceral AT after adjustment for age. The number below each bar identifies the subgroup [1 = carriers of the c.45G allele and visceral AT < 130 cm^2 (n = 9), 2 = carriers of the c.45G allele and visceral AT ≥ 130 cm^2 (n = 17), 3 = 45T/T homozygotes without the deletion and visceral AT < 130 cm^2 (n = 47), and 4 = 45T/T homozygotes and visceral AT ≥ 130 cm^2 (n = 70)]. Numbers above the bars indicate groups that differ significantly. *P* values are given after adjustment for age. C: Bar graphs comparing adiponectin concentrations within each c.45T>G genotype in men with low (<130 cm^2) or high (≥130 cm^2) visceral AT after adjustment for age. The number below each bar identifies the subgroup [1 = carriers of the c.45G allele and visceral AT < 130 cm^2 (n = 9), 2 = carriers of the c.45G allele and visceral AT ≥ 130 cm^2 (n = 17), 3 = 45T/T homozygotes and visceral AT < 130 cm^2 (n = 47), and 4 = 45T/T homozygotes and visceral AT ≥ 130 cm^2 (n = 70)]. Numbers above the bars indicate groups that differ significantly. *P* values are given after adjustment for age. *** $P < 0.001$, ** $P < 0.01$. Error bars represent the value of standard error.

tween the PPARγ P12A polymorphism and adiponectin polymorphisms could modulate lipoprotein/lipid concentrations and anthropometric indices. No significant interaction effect was noted (data not shown).

DISCUSSION

The recent discovery of the protein adiponectin and of its gene *Apm1* is important in the understanding of the pathophysiology of obesity (36), type 2 diabetes (15, 37, 38), and related cardiovascular risk (37, 39). The first objective of this study was to identify polymorphisms in the *Apm1* gene in a sample of 24 French-Canadian men chosen for their low or high visceral AT accumulation. Four sequence variants in the *Apm1* gene were identified. Frequencies of c.45T>G and c.276G>T variants were similar to those previously reported in other Caucasian populations (20, 22). However, the frequency of the c.276G>T mutation was slightly higher in a Japanese population (21), as noted by Vasseur et al. (22). This difference may be attributable to the different ethnic origins of the populations studied. In the present study, we report some significant associations of *Apm1* polymorphisms with obesity phenotypes.

First, higher LDL-cholesterol and lower HDL-triglyceride levels were observed in carriers of the 276T allele (Table 3). These data suggest an impaired transport of triglycerides and/or cholesterol. Thus, the c.276G allele may be more protective from a cardiovascular standpoint than

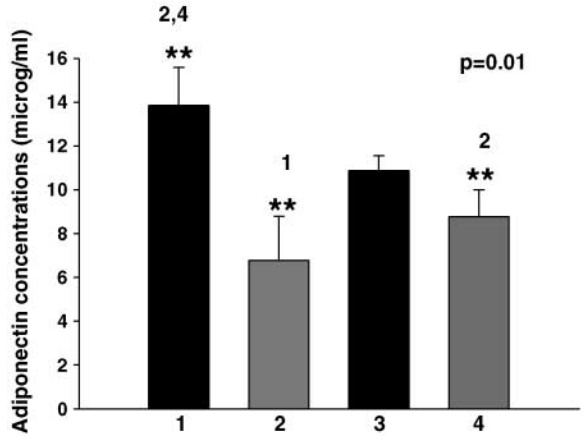

Fig. 2. Modulation of the effect of the −13752delT genotype on plasma adiponectin concentrations by the presence of insulin resistance. Bar graphs comparing the adiponectin concentrations within each −13752delT genotype in men with low (<80 pmol/l) or high (≥80 pmol/l) fasting insulin after adjustment for age. The number below each bar identifies the subgroup [1 = −13752delT carriers and fasting insulin < 80 pmol/l (n = 8), 2 = −13752delT carriers and fasting insulin ≥ 80 pmol/l (n = 6), 3 = −13752 normal homozygotes and fasting insulin < 80 pmol/l (n = 52), and 4 = −13752 normal homozygotes and fasting insulin ≥ 80 pmol/l (n = 16)]. Numbers above the bars indicate groups that differ significantly. *P* values are given after adjustment for age. *** $P < 0.001$, ** $P < 0.01$. Error bars represent the value of standard error.

the c.276T allele. At present, it is unclear how a silent polymorphism could have the observed impact on lipoprotein/lipid concentrations. This polymorphism may be in linkage disequilibrium with polymorphisms in introns that would destabilize pre-mRNA and would result in reduced mRNA levels (40), or it could be in linkage disequilibrium with a neighboring gene that influences plasma lipoprotein lipid levels.

Low plasma adiponectin levels have been reported in patients at high risk of cardiovascular events (41). An association between the 276G>T genotype and adiponectin levels has been observed among obese Japanese subjects (21). We did not observe such a relationship in our study. This discrepancy may be attributable to the different origin of the present study sample. Similar to the observation of Vasseur et al. (22) in a French population, 45T/T homozygotes had lower plasma adiponectin levels compared with carriers of the 45G allele. A dysregulated secretion of adiponectin has also been associated with a high risk of vascular disease in obesity (8, 42).

It has been previously reported that plasma concentrations of adiponectin are significantly lower among obese than nonobese subjects (13, 43). After dividing the two genotype groups into subgroups using 130 cm^2 as a cutoff point for visceral AT, a combined effect of obesity and genotype c.45T>G was observed for plasma adiponectin concentrations (Fig. 1C). In addition, a trend ($P = 0.06$) toward increased plasma adiponectin levels in lean carriers of c.276T was noted compared with their obese counterparts. Because the presence of obesity was associated with reduced adiponectin concentrations (13, 36, 44), the 45G and 276T alleles seemed to be more protective than the 45T and 276G alleles, respectively. Hara et al. (21) observed a similar "effect" of the c.276G>T genotype on plasma adiponectin concentrations after dividing their study sample of 864 diabetic and normal glucose-tolerant men and women on the basis of median, tertiles, and quartiles of body mass index. Ukkola et al. (45) also found that obese Swedish women carrying the 45G allele were more protected against higher plasma cholesterol and waist circumference than obese women homozygous for 45T/T.

The presence of visceral AT also modulated the contribution of −13752delT, c.45T>G, and c.276G>T gene variants to individual differences in plasma total apoB levels. Among carriers of the c.45G allele, carriers of c.276T allele and homozygous for normal allele at position −1753, total apoB concentrations were more elevated in obese compared to lean men. However, apoB concentrations remained similar in carriers of c.−1753delT allele, 45T/T homozygotes as well as 276G/G homozygotes independently of their amount of visceral AT (Fig. 1A, B). The fact that −13752delT and c.45T>G as well as c.45T>G and c.276G>T were in partial linkage disequilibrium may explain the similar results observed for these genetic variations (Table 1).

To validate the potential interaction effect between c.45T>G and abdominal obesity, we tried to replicate this finding in an independent study sample of 500 unrelated French-Canadians originating from the Saguenay-Lac-St-Jean (SLSJ) region located in the northeastern part of the province of Quebec (46). The frequency of the 45G allele (14%) in the replication sample was similar to the frequency observed in the sample of men from the greater Quebec area (12%). Moreover, because visceral AT assessed by computed tomography was not available for subjects from SLSJ, we subdivided the subjects in both samples on the basis of waist circumference first and subsequently on the basis of genotype and compared plasma apoB concentrations. Similar results were observed in both samples, with the lowest total apoB concentrations observed in carriers of the 45G allele.

After adjustment for age and adiponectin concentrations, differences observed for total apoB levels were no longer significant for the −13752delT and c.45T>G genotypes ($P = 0.12$ and $P = 0.21$, respectively). Cnop et al. (47) failed to detect a relationship between adiponectin concentrations and apoB levels. Plasma adiponectin concentrations in Japanese patients with coronary artery disease were lower than in control subjects and negatively correlated with BMI (48). Kazumi et al. (43) found an association between adiponectin and apoB after adjustment for BMI in young healthy men. However, plasma adiponectin concentrations were correlated with visceral AT, but only among carriers of the 45G allele ($r^2 = -0.68$, $P = 0.0002$ vs. $r^2 = -0.11$, $P = 0.23$ among 45T/T homozygotes). In consequence, the presence of the c.45T>G genotype modified the impact of obesity on total apoB levels. This impact may be mediated by adiponectin concentrations in carriers of the 45G allele. These results warrant replication in other populations.

Because plasma adiponectin concentrations have been associated with insulin sensitivity (15, 49), we next verified whether fasting insulin plasma levels could modulate the effect of each polymorphism on plasma adiponectin concentrations. The normoinsulinemic (fasting insulin levels < 80 pmol/l) carriers of the −13752delT allele presented the highest concentrations of adiponectin compared with the hyperinsulinemic (fasting insulin levels ≥ 80 pmol/l) −13752delT carriers.

The absence of deletion in subjects may be seen as protective, in that adiponectin concentrations were unaffected by the variation in insulin levels (Fig. 2). Although this mutation was in partial linkage disequilibrium with the c.45T>G allele, we failed to show any difference in adiponectin concentrations for this last polymorphism. These results must be confirmed by other studies.

Finally, the adiponectin gene contains a PPAR-responsive element for PPARγ. Therefore, we verified the effect of the combination of the PPARγ P12A genotype and adiponectin polymorphisms on anthropometric and biochemical variables. Although PPARγ P12A did modulate adiponectin concentrations in a Japanese population (50), Thamer et al. (51) showed no influence of the PPARγ P12A polymorphism on serum adiponectin concentrations in healthy Europeans, similar to our findings in the present study (data not shown). We failed to show any gene-gene interaction effect on plasma adiponectin, lipoprotein/lipid levels, and anthropometric indicators.

In conclusion, some polymorphisms of the *Apm1* gene found in this study may contribute to the individual variations in plasma lipoprotein profiles and adiponectin concentrations among French-Canadian men. The presence of visceral obesity or insulin resistance may modulate these associations. These results warrant replication in larger cohorts and different populations.

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