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Effect of apoC-III gene polymorphisms on the lipoprotein-lipid profile of viscerally obese men

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Abstract **Abdominal visceral adipose tissue (AT) accumulation is associated with an atherogenic metabolic profile that includes increased plasma triglyceride (TG), low HDL cholesterol levels, and an insulin-resistant hyperinsulinemic state. Whereas the apolipoprotein (apo) C-III C3238G gene variant, often referred to as the *Sst*I polymorphism, has been related to variations in plasma TG concentrations, another variation within the insulin responsive element (C-482T) of the apoC-III gene has been associated with greater glucose and insulin responses to an oral glucose tolerance test (OGTT); however, these results were obtained in nonobese individuals. We therefore investigated the effects of three apoC-III gene polymorphisms, namely *Sst*I, C-482T, and T-455C, on fasting plasma lipoprotein-lipid levels and response to a 75 g OGTT in a sample of 122 viscerally obese men (abdominal visceral AT area ≥130 cm^2). Among the three gene variants that were examined, the *Sst*I variation was the only one found to be associated with hypertriglyceridemia. Indeed, S1/S2 heterozygotes (n = 24) were characterized by increased fasting plasma TG concentrations compared with S1/S1 homozygotes (n = 98) (mean ± SD: 3.03 ± 1.58 vs. 2.34 ± 0.95 mmol/l respectively, $P < 0.05$). The higher TG concentrations in S1/S2 were associated with the presence of smaller, denser LDL particles compared with S1/S1 subjects (LDL peak particle diameter: 24.8 ± 0.5 nm vs. 25.1 ± 0.5 nm respectively, $P < 0.05$). Furthermore, there was no association between the response to the OGTT and any of the apoC-III gene variants (*Sst*I, T-455C, or C-482T) examined. Results of the present study support the notion of a hypertriglyceridemic effect associated with the apoC-III *Sst*I polymorphism that could modulate the magnitude of the dyslipidemic state in abdominally obese patients.**—Couillard, C., M-C. Vohl, J. C. Engert, I. Lemieux, A. Houde, N. Alméras, D. Prud'homme, A. Nadeau, J-P. Després, and J. Bergeron. **Effect of apoC-III gene polymorphisms on the lipoprotein-lipid profile of viscerally obese men.** ***J. Lipid Res.*** **2003:** 44: **986–993.**

Supplementary key words oral glucose tolerance test • high density lipoprotein • very low density lipoprotein • triglycerides

Apolipoprotein (apo) C-III, a protein produced by the liver, is an essential constituent of VLDL and HDL (1). Considering the inhibitory effect of apoC-III on lipoprotein lipase (LPL) activity and hepatic uptake of lipoproteins (2, 3), apoC-III gene variants have been proposed as being potentially responsible for the occurrence of lipoprotein-lipid profile disturbances. Accordingly, numerous polymorphisms in the apoC-III gene have been identified (4). The first one reported involves the substitution of a cytosine to a guanine in the 3′ untranslated region of the gene, which alters a *Sst*I restriction site (5). The prevalence of the rare S2 allele in the white population has been estimated to vary between 0.08 and 0.30 (6–10). The *Sst*I apoC-III gene polymorphism has been associated with altered plasma triglyceride (TG) concentrations (4, 5, 7, 10–20). However, the physiological mechanisms by which this genetic variation leads to an impairment of TG metabolism remain unexplained.

In addition to the *Sst*I, other variations are noted in the promoter region of the apoC-III gene (**Fig. 1**). More specifically, the C-482T and T-455C variants are located within an insulin response element (IRE) of the apoC-III gene (21). These two apoC-III promoter polymorphisms have been shown to inactivate a negative IRE located between nucleotides −449 and −490 (21), and are in linkage disequilibrium with the apoC-III *Sst*I polymorphism (22). In addition, variation at the C-482T position has been

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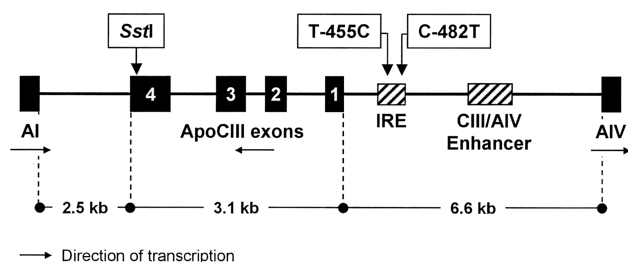

Fig. 1. The apolipoprotein (apo) AI-C-III-AIV gene region.

shown to influence the glucose and insulin response to an oral glucose tolerance test (OGTT), carriers of the C-482T rare allele having significantly elevated glucose and insulin concentrations following the glucose challenge (20).

Abdominal obesity, especially in the presence of an excessive visceral adipose tissue (AT) accumulation, has been associated with numerous metabolic disturbances that are known to increase the risk of coronary heart disease (CHD) (23–25). For example, viscerally obese subjects are frequently characterized by high fasting TG and low HDL cholesterol concentrations, elevated apoB and insulin levels, as well as by small, dense LDL particles (25–27). This cluster of metabolic abnormalities has been described as the insulin resistance-dyslipidemic syndrome. However, little is known about the effect of apoC-III gene polymorphisms in the viscerally obese population. Therefore, the present study was undertaken in order to investigate the association between the apoC-III *Sst*I, T-455C, and C-482T polymorphisms and fasting metabolic profile variables, as well as response to an OGTT in a group of viscerally obese men.

MATERIALS AND METHODS

Subjects

One hundred and twenty two men (mean age ± SD: 44 ± 8 years) with an abdominal visceral AT area ⩾130 cm^2 were recruited through the media and gave their written consent to participate in the study, which was approved by the Medical Ethics Committee of Laval University. Subjects were all nonsmokers, and those with diabetes, endocrine disorders, or CHD were excluded from the study. None of the subjects was on medication known to affect insulin action or plasma lipoprotein levels.

Anthropometry, body composition, and fat distribution

Body weight and height, as well as waist and hip circumferences, were measured following standardized procedures (28), and the waist-to-hip ratio was calculated. Body density was measured by the hydrostatic weighing technique (29). The mean of six measurements was used in the calculation of percent body fat from body density using the equation of Siri (30). Fat mass was obtained by multiplying body weight by percent body fat. Abdominal visceral and subcutaneous AT accumulations were assessed by computed tomography, which was performed on a Siemens Somatom DRH scanner (Erlangen, Germany) using previously described procedures (31, 32).

DNA analysis

Peripheral venous blood was collected in tubes containing EDTA and kept frozen at −20°C until processing. Total DNA was extracted by a standardized protocol of digestion with proteinase K. A PCR-restriction fragment length polymorphism-based method adapted by Dammerman et al. (19) was used to genotype the apoC-III gene polymorphisms. PCR products were digested with 15 units of *Sst*I enzyme for a minimum of 3 h at 37°C. The resulting fragments were separated according to their size by electrophoresis on 8% nondenaturing polyacrylamide gel. The alleles lacking the restriction site were designated as S1, while those containing the *Sst*I site were designated as S2. C-482T and T-455C variations were determined by the allele-specific oligonucleotide hybridization assay as previously described (19).

Fasting plasma lipoprotein concentrations

Blood samples were obtained in the morning after a 12 h overnight fast. Cholesterol and TG levels were determined in plasma and in lipoprotein fractions by enzymatic methods (Randox Co., Crumlin, UK) using a RA-500 analyzer (Bayer Corporation Inc., Tarrytown, NY), as previously described (33). Plasma VLDLs (d < 1.006 g/ml) were isolated by ultracentrifugation, and the HDL fraction obtained after precipitation of LDL in the infranatant (d > 1.006 g/ml) with heparin and $MnCl_2$ (34). The cholesterol and TG contents of the infranatant fraction were measured before and after the precipitation step. The cholesterol content of HDL_2 and HDL_3 subfractions was also determined after further precipitation of HDL_2 with dextran sulfate (35). ApoB concentration was measured in plasma by the rocket immunoelectrophoretic method of Laurell (36), as previously described (37). The lyophilized serum standards for apoB measurements were prepared in our laboratory and calibrated with reference standards obtained from the Centers for Disease Control (Atlanta, GA). Plasma apoC-III concentrations were measured by nephelometry (BN-100, Dade-Behering, Marburg, Germany) using polyclonal antibodies against human apoC-III (Kamiya Biomedical Co., Seattle, WA). Intra- and interassay coefficients of variation for this measurement were both ⩽6.0%. LDL particle diameter was assessed by nondenaturing 2–16% PAGE as already described (27, 38).

Postheparin plasma lipase activity

Postheparin LPL and hepatic lipase (HL) activities were also measured on one occasion in subjects after a 12 h overnight fast,

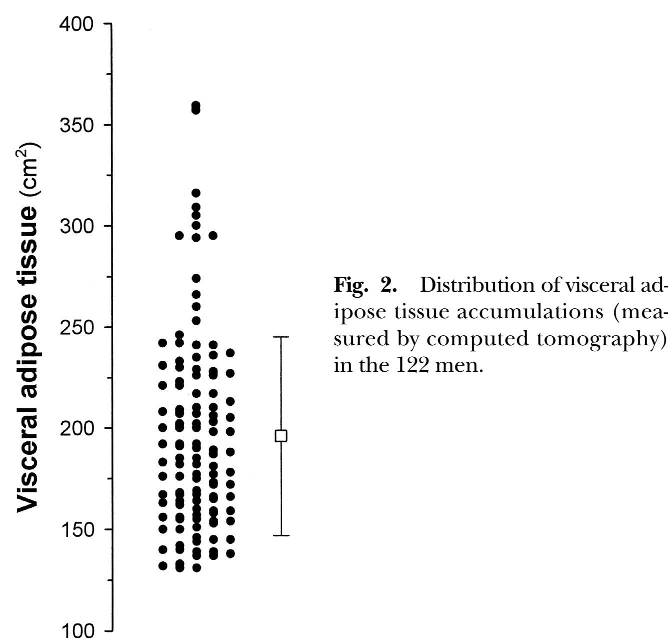

Fig. 2. Distribution of visceral adipose tissue accumulations (measured by computed tomography) in the 122 men.

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TABLE 1. Fasting plasma lipid concentrations of the subjects according to the different apoC-III gene polymorphisms

Variables	ApoC-III Gene Variant			P
*Sst*I	S1/S1	S1/S2	S2/S2	Carriers versus Noncarriers
		mmol/l		
Number of subjects	98	24		
Cholesterol	5.43 ± 0.77	5.38 ± 0.66		0.80
Triglycerides	2.34 ± 0.95	3.03 ± 1.58		<0.05
LDL cholesterol	3.71 ± 0.72	3.45 ± 0.66		<0.05
LDL triglycerides	0.32 ± 0.11	0.38 ± 0.15		<0.05
HDL cholesterol	0.88 ± 0.16	0.84 ± 0.16		0.20
HDL_2 cholesterol	0.21 ± 0.12	0.23 ± 0.12		0.24
HDL_3 cholesterol	0.68 ± 0.10	0.60 ± 0.10		<0.001
HDL triglycerides	0.24 ± 0.07	0.28 ± 0.08		<0.05
Total/HDL cholesterol	6.3 ± 1.3	6.7 ± 1.5		0.27
T-455C	T/T	T/C	C/C	Carriers versus Noncarriers
Number of subjects	41	53	9	
Cholesterol	5.43 ± 0.79	5.44 ± 0.72	5.62 ± 0.90	0.81
Triglycerides	2.39 ± 0.91	2.67 ± 1.19	1.85 ± 0.76	0.45
LDL cholesterol	3.74 ± 0.68	3.60 ± 0.73	4.04 ± 0.86	0.59
LDL triglycerides	0.31 ± 0.11	0.33 ± 0.12	0.38 ± 0.13	0.30
HDL cholesterol	0.85 ± 0.17	0.86 ± 0.14	0.94 ± 0.16	0.54
HDL_2 cholesterol	0.19 ± 0.10	0.19 ± 0.09	0.29 ± 0.21	0.53
HDL_3 cholesterol	0.66 ± 0.11	0.67 ± 0.10	0.65 ± 0.09	0.77
HDL triglycerides	0.24 ± 0.08	0.25 ± 0.07	0.24 ± 0.05	0.42
Total/HDL cholesterol	6.58 ± 1.34	6.48 ± 1.33	6.18 ± 1.37	0.59
C-482T	C/C	C/T	T/T	Carriers versus Noncarriers
Number of subjects	60	44	7	
Cholesterol	5.46 ± 0.72	5.36 ± 0.71	5.83 ± 1.06	0.69
Triglycerides	2.43 ± 0.91	2.73 ± 1.30	2.07 ± 1.03	0.20
LDL cholesterol	3.74 ± 0.66	3.49 ± 0.72	4.08 ± 1.04	0.15
LDL triglycerides	0.32 ± 0.11	0.34 ± 0.14	0.38 ± 0.12	0.25
HDL cholesterol	0.87 ± 0.17	0.84 ± 0.13	0.98 ± 0.20	0.74
HDL_2 cholesterol	0.19 ± 0.10	0.20 ± 0.09	0.31 ± 0.25	0.20
HDL_3 cholesterol	0.68 ± 0.11	0.64 ± 0.09	0.67 ± 0.11	0.06
HDL triglycerides	0.24 ± 0.07	0.25 ± 0.08	0.25 ± 0.07	0.12
Total/HDL cholesterol	6.46 ± 1.26	6.52 ± 1.33	6.23 ± 1.79	0.97

Values are presented as mean ± SD. Triglyceride values were $\log_{10}$ transformed.

10 min after an intravenous injection of heparin (60 IU/kg body mass). The activities were measured using a modification of the method of Nilsson-Ehle and Ekman (39), as previously described (40), and expressed as nmoles of oleic acid released per ml of plasma per min.

OGTT

A 75 g OGTT was performed in the morning after an overnight fast. Blood samples were collected under EDTA through a venous catheter from an antecubital vein at −15 min, 0 min, 15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 150 min, and 180 min for determination of plasma glucose and insulin levels. Plasma glucose was measured enzymatically (41), whereas plasma insulin was measured by radioimmunoassay with polyethylene glycol separation (42).

Statistical analyses

Whereas the *Sst*I polymorphism was determined in all subjects, the T-455C and C-482T variants were determined in 103 and 111 men, respectively. Overall, 102 subjects had information for all three apoC-III gene polymorphisms. Differences between groups were tested for significance by using ANOVA with post-hoc Duncan multiple range tests. All comparisons were adjusted for age. Spearman correlation coefficients (nonparametric variables) were computed in order to study associations between variables. Areas under the curve of glucose and insulin were determined by the trapezoid method. Multiple regression analyses were also performed to estimate the independent contributions of the different apoC-III gene polymorphisms, as well as adiposity, fat distribution, and metabolic variables to the variation in fasting plasma TG concentrations. For statistical purposes and when necessary, variables were $\log_{10}$ transformed, but raw data are presented in tables and figures. In all analyses, a P value of <0.05 was considered statistically significant. All analyses were conducted with the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

On average, the 122 subjects were obese as suggested by a mean BMI of 31.2 ± 3.6 kg/m^2. Men also had high abdominal and visceral fat accumulation as indicated by a waist circumference of 104.4 ± 8.3 cm and an abdominal visceral fat area of 196 ± 49 cm^2, respectively. **Figure 2** shows the distribution of visceral AT accumulations in the 122 men. Allele frequencies of the minor *Sst*I, T-455C, and C-482T alleles in the present group of abdominally obese men were 9.8%, 34.5%, and 26.1%, respectively. Furthermore, distribution of genotypes for all variants in our group of men was not significantly different from the one expected by the Hardy-Weinberg equilibrium.

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TABLE 2. Fasting metabolic characteristics of the subjects according to the different apoC-III gene polymorphisms

Variables	ApoC-III Gene Variant			*P*
*Sst*I	S1/S1	S1/S2	S2/S2	Carriers versus Noncarriers
Number of subjects	98	24		
LDL size, nm	25.1 ± 0.51	24.8 ± 0.49		<0.05
ApoB, g/l	1.15 ± 0.22	1.11 ± 0.19		0.43
ApoC-III, mg/l	144 ± 36	156 ± 29		0.18
LPL activity, nmol/ml/min	34.5 ± 17.1	35.0 ± 17.0		0.91
HL activity, nmol/ml/min	221.5 ± 82.1	172.5 ± 85.1		<0.05
Insulin, pmol/l	127 ± 91	119 ± 72		0.71
Glucose, mmol/l	5.46 ± 0.52	5.52 ± 0.63		0.61
T-455C	T/T	T/C	C/C	Carriers versus Noncarriers
Number of subjects	41	53	9	
LDL size, nm	24.9 ± 4.8	25.1 ± 5.3	25.4 ± 0.4	0.06
ApoB, g/l	1.16 ± 0.20	1.15 ± 0.21	1.13 ± 0.33	0.83
ApoC-III, mg/l	150 ± 035	150 ± 33	123 ± 20	0.64
LPL activity, nmol/ml/min	33.6 ± 16.6	35.0 ± 18.3	31.3 ± 9.8	0.80
HL activity, nmol/ml/min	237.7 ± 83.2	211.1 ± 82.1	191.3 ± 81.7	0.14
Insulin, pmol/l	139 ± 108	119 ± 63	130 ± 90	0.30
Glucose, mmol/l	5.49 ± 0.61	5.50 ± 0.48	5.43 ± 0.63	0.97
C-482T	C/C	C/T	T/T	Carriers versus Noncarriers
Number of subjects	60	44	7	
LDL size, nm	24.9 ± 0.5	25.2 ± 0.5	25.2 ± 0.5	<0.05
ApoB, g/l	1.18 ± 0.19	1.11 ± 0.21	1.17 ± 0.38	0.14
ApoC-III, mg/l	152 ± 35	146 ± 32	151 ± 32	0.43
LPL activity, nmol/ml/min	35.2 ± 18.2	33.4 ± 15.6	35.8 ± 14.7	0.71
HL activity, nmol/ml/min	221.3 ± 79.2	211.6 ± 89.8	124.8 ± 63.5	0.29
Insulin, pmol/l	137 ± 101	122 ± 62	103 ± 55	0.26
Glucose, mmol/l	5.55 ± 0.57	5.48 ± 0.50	5.19 ± 0.31	0.27

Apo, apolipoprotein; HL, hepatic lipase; LPL, lipoprotein lipase. Values are presented as mean ± standard deviation.

Fasting plasma lipid concentrations of subjects separated according to the different apoC-III polymorphisms are shown in **Table 1**. We found that men with the S2 allele had higher fasting TG concentrations compared with S1/S1 subjects (+23.5%, $P < 0.05$). Men with the S2 allele were also characterized by lower LDL cholesterol and HDL_3 cholesterol levels, as well as higher VLDL cholesterol, LDL, and HDL TG concentrations compared with S1/S1 subjects (data not shown). **Table 2** shows other metabolic characteristics among abdominally obese men separated according to the different apoC-III gene polymorphisms. Although apoC-III levels were slightly higher in the S2 allele carriers

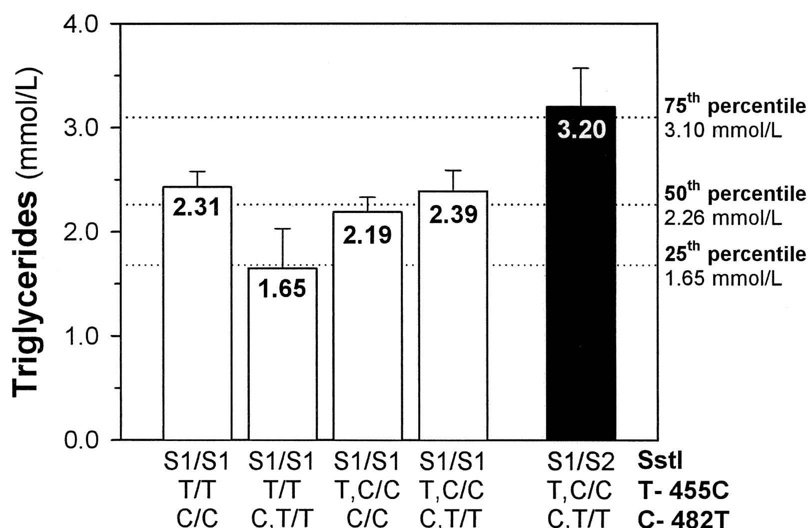

Fig. 3. Fasting plasma triglyceride concentrations in men classified on the basis of the different combinations of apoC-III gene polymorphisms. T,C/C represents men heterozygous or homozygous for the T-455C variant. C,T/T represents men heterozygous or homozygous for the C-482T variant. Values are means ± SEM.

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(~9.0%), the difference was not statistically significant (P = 0.18). Furthermore, whereas no difference was found in postheparin plasma LPL activity between both groups of men, S2 allele carriers were characterized by lower HL activity compared with S1/S1 subjects ($P < 0.05$). Viscerally obese men carrying the S2 allele also showed a significantly lower mean LDL peak particle diameter ($P < 0.05$) compared with those lacking the apoC-III *Sst*I polymorphic site.

On the other hand, the two variants within the IRE of the apoC-III gene promoter did not appear to be related to either lipoprotein-lipid or metabolic profile variables, with the exception of a tendency for larger LDL particles in carriers of the T-455C or C-482T polymorphism. Furthermore, **Fig. 3** indicates that, irrespective of the presence of the T-455C or C-482T minor alleles, only men carrying the *Sst*I polymorphism were showing fasting plasma TG above the 75th percentile.

Multivariate regression analyses were also conducted in order to sort out the independent contributions of the different apoC-III gene polymorphisms and other physical or metabolic variables to the variance in fasting plasma TG concentrations (**Table 3**). When all apoC-III gene polymorphism, body fatness, and AT distribution variables were included in the statistical model, the *Sst*I polymorphism was the best predictor (6.5%, $P < 0.01$) of fasting plasma TG levels. In this model, waist circumference (4.4%, $P < 0.05$) also contributed to the variance of fasting plasma TG concentrations. On the other hand, when metabolic variables were included in the statistical model, fasting apoC-III concentration (41.9%, $P < 0.0001$) was, by far, the strongest predictor of fasting plasma TG. Waist circumference (5.6%, $P < 0.005$) and apoB (3.0%, $P < 0.05$) also significantly predicted the variance of fasting plasma TG levels. In this model, the *Sst*I polymorphism was no longer a significant predictor of the variance of plasma TG (2.2%, P = 0.06).

As the presence of the apoC-III *Sst*I polymorphism was associated with increased fasting TG levels, we were interested in its effect on the relationships between fasting TG, body fat distribution, and other metabolic variables (**Table 4**). In S1/S1 subjects, elevated TG levels were associated with a preferential accumulation of fat in the abdominal region, as suggested by the positive associations with waist circumference (r = 0.26, $P < 0.05$) and visceral AT accumulation (r = 0.27, $P < 0.05$). However, these associations were not statistically significant in men with the S2 allele. Contrary to insulin, which was positively correlated with TG in S1/S1 subjects only, high TG levels were significantly associated with lower HDL cholesterol and higher apoC-III concentrations, and an increased total/HDL cholesterol ratio as well as smaller, denser LDL particles irrespective of the presence or absence of the S2 allele. When subjects were divided into noncarriers versus carriers of the T-455C or C-482T polymorphisms, similar associations were found between fasting plasma TG and metabolic variables than with *Sst*I. We must emphasize that, although differences in the significance of associations were noted, further analyses revealed that the correlation coefficients were not statistically significant between noncarriers versus carriers of the different apoC-III gene variants, with the exception of the relationship of TG versus HDL cholesterol in S1/S1 versus S1/S2 controls.

TABLE 3. Multivariate regression analyses showing the independent contributions of physical and metabolic characteristics to fasting plasma triglyceride concentrations

Dependent Variable	Independent Variable	Partial ($R^2 \times 100$)	P	Total ($R^2 \times 100$)
Model 1				
Plasma TG[a]	ApoC-III *Sst*1 genotype	6.5	<0.01	10.9
	Waist circumference	4.4	<0.05	
Model 2				
Plasma TG	ApoC-III	41.9	<0.0001	50.5
	Waist circumference	5.6	<0.005	
	ApoB	3.0	<0.05	
	ApoC-III *Sst*1 genotype	2.2	0.0595	
	Insulin	1.6	0.0985	

[a] Log_{10} transformed values.

AT, adipose tissue; TG, triglyceride. Model 1 included age, apoC-III *Sst*I, T-455C, and C-482T genotypes, body fat mass, waist circumference, and abdominal visceral and subcutaneous AT. Model 2 included all variables of Model 1 as well as fasting insulin and apoB, apoCII, apoC-III, and apoE levels.

TABLE 4. Associations between adiposity and metabolic variables in carriers versus noncarriers of different apoC-III gene polymorphisms

Variables	Plasma TG	
*Sst*I	Noncarriers (n = 98)	Carriers (n = 24)
Body mass index	0.26[a]	0.03
Fat mass	0.15	0.32
Waist circumference	0.26[a]	0.16
Visceral AT	0.27[a]	0.06
Insulin	0.35[b]	0.10
HDL cholesterol	−0.34[b]	−0.68[a]
Total/HDL cholesterol	0.53[b]	0.66[a]
LDL particle size	−0.53[b]	−0.42[c]
ApoC-III	0.70[b]	0.71[b]
T-455C	Noncarriers (n = 41)	Carriers (n = 62)
Body mass index	0.08	0.19
Fat mass	−0.04	0.20
Waist circumference	0.12	0.20
Visceral AT	0.17	0.10
Insulin	0.30[c]	0.28[a]
HDL cholesterol	−0.30[c]	−0.43[b]
Total/HDL cholesterol	0.58[b]	0.50[b]
LDL particle size	−0.53[b]	−0.31[a]
ApoC-III	0.74[b]	0.66[b]
C-482T	Noncarriers (n = 60)	Carriers (n = 51)
Body mass index	0.20	0.02
Fat mass	0.09	0.09
Waist circumference	0.20	0.04
Visceral AT	0.21	0.03
Insulin	0.30[a]	0.16
HDL cholesterol	−0.27[a]	−0.52[b]
Total/HDL cholesterol	0.44[b]	0.56[b]
LDL particle size	−0.46[b]	−0.69[b]
ApoC-III	0.60[b]	0.74[b]

[a] $P < 0.05$.
[b] $P < 0.005$.
[c] $P < 0.07$.

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Finally, we examined the insulin and glucose responses to a 75 g OGTT in abdominally obese men separated on the basis of the three apoC-III gene polymorphisms (**Fig. 4**). We did not observe any significant difference between carriers versus noncarriers of the *Sst*1, T-455C, or C-482T variants in glucose or insulin concentrations measured before or during the glucose challenge.

DISCUSSION

Genetic variation in the apoC-III gene has been associated with fasting hypertriglyceridemia and impaired responses to oral fat and glucose tolerance tests (4, 6, 11, 20). Abdominal obesity, especially in the presence of an increased visceral AT accumulation, has also been associated with disturbances in the fasting plasma lipoprotein-lipid profile that include high TG and apoB levels and low HDL cholesterol concentrations, as well as an increased number of small, dense LDL particles (25–27). However, no study has yet examined the impact of the apoC-III gene variants in subjects prone to higher TG concentrations and insulin resistance, such as viscerally obese individuals (25, 26).

Frequency of the different variants of the apoC-III gene observed in the present study was comparable to the prevalence already reported in the greater Québec City area (43) and other Caucasian populations (13, 20), which suggests that it is unlikely that there is an increased prevalence of carriers of either the apoC-III *Sst*I, T-455C, or C-482T genotypic variations among subjects with high visceral AT accumulation compared with nonobese normolipidemic subjects.

Of all apoC-III gene polymorphisms examined in the present study, only the *Sst*I variant appeared to be associated with plasma TG concentrations. Indeed, we found that the carriers of the S2 allele were characterized by significantly higher TG concentrations compared with S1/S1 subjects. This observation supports the association between hypertriglyceridemia and the apoC-III *Sst*I polymorphism (4, 11). However, we also found that, irrespective of the presence or absence of the *Sst*I polymorphism, viscerally obese subjects were characterized by elevated fasting TG concentrations. Indeed, both groups of subjects had mean TG levels above 2.0 mmol/l, an upper limit to define elevated TG concentrations as recommended by the Canadian Working Group on Hypercholesterolemia and Other Dyslipidemias (44). Thus, it seems unlikely that the hypertriglyceridemic state that characterizes visceral obesity (25, 26) results from a greater proportion of S1/S2 individuals in the viscerally obese population.

The *Sst*I site could be in linkage disequilibrium with other mutations that affect apoC-III levels. It has been shown that five other polymorphic sites in the promoter of the apoC-III gene have a strong allelic association with the *Sst*I polymorphism (19). However, results from two recently published studies suggest that such linkage disequilibrium between the *Sst*I polymorphism and these other apoC-III gene promoter variants (14, 22) does not explain the association between high TG levels and the S2 allele. Indeed, Surguchov et al. (22) showed that, although the minor alleles of six polymorphic sites (positions −935, −641, −630, −625, C-482T, and T-455C) were in strong linkage disequilibrium with each other and with the S2 polymorphic nucleotide, they were unable to detect an association of any specific S2 haplotype with elevated levels of plasma TGs over and above those conferred by the presence of the S2 polymorphic nucleotide alone. This is consistent with our results showing no independent contribution of the T-455C and C-482T polymorphisms to fasting plasma TG levels. Furthermore, in men carrying the minor T-455C or C-482T alleles, only those bearing the S2 allele had TG levels above the 75th percentile (3.1 mmol/l). On the other hand, a strong allelic association has been reported between the *Sst*I site and two variants within the apoC-III gene itself (45). However, no study has yet demonstrated a link between these polymorphic sites and any functional alteration of apoC-III that could explain the proposed as-

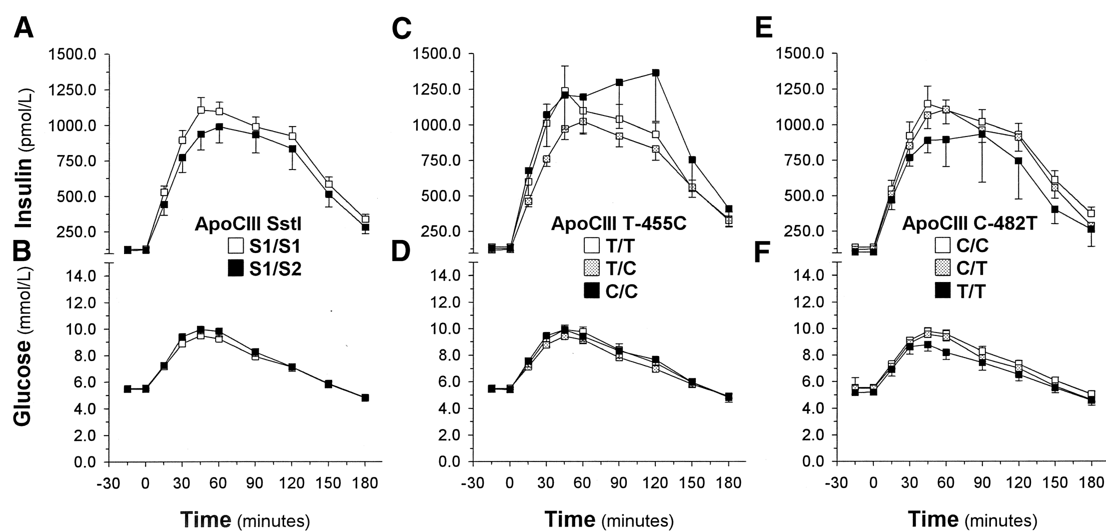

Fig. 4. Plasma insulin (A, C, E) and glucose (B, D, F) levels during a 75 g oral glucose tolerance test in abdominally obese men separated on the basis of the *Sst*I, T-455C, and C-482T apoC-III gene polymorphisms. Values are means ± SEM.

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sociation between hypertriglyceridemia and *Sst*I. We cannot exclude the possibility of linkage disequilibrium with others variants from genes located in the apoC-III chromosomal region. For instance, variants in the newly discovered apoA-V gene (46) near the apoC-III gene need to be further investigated and may be revealed as having a significant role in the association between higher TG and the apoC-III *Sst*I polymorphism. In addition, as already proposed, a potential effect of the *Sst*I polymorphism on mRNA stability cannot be excluded (47).

Elevated apoC-III concentrations have been associated with high plasma TG levels through the inhibition of LPL activity and reduction of TG-rich lipoprotein clearance by the liver (2, 3). This is certainly supported by results from our multivariate analyses that showed that the plasma apoC-III level was the strongest predictor of plasma TG concentration in our group of men. Furthermore, to our knowledge, the relationship between the apoC-III gene *Sst*I polymorphism and apoC-III circulating levels has never been documented in viscerally obese individuals. In our study, a trend for higher plasma apoC-III levels was found in S1/S2 men, although the difference with S1/S1 subjects did not reach statistical significance (156 ± 29 vs. 144 ± 36 mg/l, respectively, $P = 0.18$), probably due to lack of statistical power. Furthermore, as we only measured total plasma apoC-III concentrations, further studies should also be conducted in order to identify a possible contribution of the *Sst*I polymorphism to the distribution of apoC-III in different lipoprotein subclasses e.g., VLDL, LDL, and HDL, which could possibly explain the association between hypertriglyceridemia and the *Sst*I polymorphism. The importance of this kind of information has been recently underlined as Sacks and colleagues (48) showed that the apoC-III concentration in VLDL+LDL, but not in HDL, was an independent predictor of recurrent coronary events. On the other hand, the hypertriglyceridemic state of S1/S2 men compared with S1/S1 subjects may be explained, at least in part, by the lower HL activity in the S2 allele carriers. The significant negative correlations between HL activity and the TG content of LDL ($r = -0.41$, $P < 0.0001$) suggest such a possibility. In support of this, S1/S2 men were characterized by higher LDL-TG and HDL-TG levels, while showing lower HL activity compared with S1/S1 subjects. Interestingly, the apoC-III *Sst*I genotype was a weak yet significant predictor of postheparin plasma HL activity (4.9%, $P = 0.0474$).

The insulin response to an OGTT has been reported to be affected by the apoC-III gene polymorphism (6, 20). In the present study, no difference was found in the glucose or insulin response to the glucose challenge among men carrying the minor allele of either the *Sst*I, T-455C, or C-482T variants. However, it is well known that visceral obesity is associated with disturbances in glucose-insulin homeostasis (49). Whereas Salas et al. (6) and Waterworth et al. (20) studied apparently nonobese individuals, we investigated only viscerally obese men. This discrepancy in subject characteristics between the two studies may account for the difference in the response to the OGTT between carriers versus noncarriers of the *Sst*I, T-455C, or C-482T polymorphism. Possibly, the impact of visceral obesity on glucose-insulin metabolism could be greater than the contribution of the apoC-III gene polymorphism to plasma glucose-insulin homeostasis.

In summary, our results suggest that the apoC-III *Sst*I polymorphism is associated with hypertriglyceridemia in viscerally obese subjects who are already characterized by moderately elevated plasma TG concentrations. As the relationships between fasting TG levels and body fat distribution indices appear to be altered by the presence of the rare S2 allele, and considering that the associations between TG and other metabolic risk profile variables are observed both in S1/S1 and S1/S2 men, we conclude that the etiology of higher TG levels in apoC-III *Sst*I polymorphism carriers appears to be independent of adiposity and abdominal fat accumulation, but that the impact of increased TG concentrations on HDL cholesterol levels and LDL particle size is not affected by the presence of the apoC-III *Sst*I polymorphism, at least in viscerally obese men.

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