Obesity is recognized as an independent risk factor for cardiovascular disease. However, recent advances in obesity research clearly demonstrate that the obesity state is heterogeneous in its morphological presentation (ie, in the site of deposition of the adipose tissue excess) and in its consequences on health. Indeed, recent prospective studies show that accumulation of an excess of adipose tissue in the upper part of the body, and more particularly in the abdominal region, increases the risk of cardiovascular disease and related death, whereas a preferential accumulation of adipose tissue in the lower part of the body has fewer detrimental effects on health. The increased cardiovascular risk associated with abdominal obesity seems to be partly mediated by an environmental factor, such as dietary and physical activity. The respective contributions of genetic variation and of gene-environment interactions to the variance in plasma lipoprotein levels have been investigated in recent studies. Polymorphism in genes relevant to lipoprotein metabolism, such as apolipoprotein (apo) E and apoB-100 genes, has been demonstrated to account for a significant portion of the variance in plasma lipoprotein levels. Moreover, the DNA sequence variation at relevant genetic loci may be involved in determination of the metabolic consequences of abdominal/visceral obesity. (Arterioscler Thromb. 1994;14:527-533.)
Subjects
Participants in the present study were recruited from a larger sample of Caucasian male subjects who had previously participated in studies designed to investigate the associations between obesity, regional adipose tissue distribution as measured by computed tomography (CT), plasma lipoprotein levels, and insulin-glucose homeostasis in our laboratory. Subjects had to be sedentary, nonsmokers, and free from metabolic disorders requiring pharmacological treatment (cardiovascular disease, diabetes, hypercholesterolemia, and hypertension) to be included. These studies were approved by the Medical Ethics Committee of Laval University. From this larger sample, 56 men agreed to a further visit to the laboratory for blood sampling and gave their written informed consent for DNA analysis.

DNA Analysis
DNA was prepared from venous peripheral blood by a standard protocol of digestion with proteinase K and extraction with phenol and chloroform. Purified DNA (4 μg) was digested with EcoRI for 16 hours at 37°C by using 25 units of the enzyme under the buffer conditions recommended by the manufacturer (Pharmacia). The digested fragments were separated by electrophoresis on horizontal 1.2% agarose gels. The DNA fragments were then transferred to nitrocellulose filters (Schleicher and Schuell BA85). DNA fragments were visualized by hybridization with a pB8 probe (American Type Culture Collection), a cDNA probe complementary to a genomic DNA region located near the 3' end of the apoB gene. The probe was labeled with [α-32P]dCTP to a specific activity >1.2 × 10^6 cpm/μg by using random priming. Prehybridization and hybridization were performed as described by Wahl et al. Filters were exposed to x-ray films (Kodak XAR-5) with screens for 72 to 120 hours at −70°C. The size of DNA fragments was then determined by using the size standards. The apoB-100 gene polymorphism detected with EcoRI is due to a base substitution that alters the restriction site: the common allele is cutting, the pB8 probe revealing an 11-kb DNA fragment, and the minor allele is noncutting, the probe revealing a 13-kb DNA fragment.

Measurement of Total Body Fatness
Body density was measured by the hydrostatic weighing technique, and the mean of six measurements was used to estimate percent body fat from density by using the equation of Siri. Fat mass was obtained by multiplying percent body fat by body weight. Pulmonary residual volume was measured before immersion in the hydrostatic tank by using the helium dilution method of Meneely and Kaltreider. Waist and hip circumferences were measured following the procedures recommended by the Airlie Conference.

CT Studies
CT was performed on a Siemens Somatom DRH scanner by using the procedures of Sjöström et al. as described. Briefly, the subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal (between the fourth and fifth lumbar vertebrae) and at the midthigh (middistance between the knee joint and the iliac crest) levels using a radiograph of the skeleton as a reference to establish the position of the scan to the nearest millimeter. Total adipose tissue areas were calculated by delineating the areas with a graph pen and then computing the adipose tissue surface using an attenuation range of −190 to −30 Hounsfield units. Abdominal visceral adipose tissue area was measured by drawing a line within the muscle wall surrounding the abdominal cavity (Fig 1).

Plasma Lipoprotein Measurements
Blood samples were obtained in the morning after a 12-hour fast from an antecubital vein and placed in Vacutainer tubes containing EDTA. Cholesterol and triglyceride levels in plasma and lipoprotein fractions were measured enzymatically on an RA-1000 analyzer (Technicon Instruments Corp) following the procedures of the Centers for Disease Control and Prevention, Atlanta, Ga. Very-low-density lipoprotein (VLDL, d < 1.006 g/mL) was isolated by ultracentrifugation, and the HDL fraction was obtained after precipitation of LDL (d > 1.006 g/mL) with heparin and MnCl2. ApoB concentration was measured in plasma and the infranatant (d > 1.006) (LDL apoB) by using the rocket immunoelectrophoretic method of Laurell as described. Briefly, poly-
clonal monospecific rabbit antisera to apoB was obtained from Behringwerke AG. The specificity of the antisera was tested by radial double immunodiffusion. Immunoelectrophoresis in 1% agarose was performed in a water-cooled electrophoresis cell (Bio-Rad) for 16 hours using barbital buffer, pH 8.6. The concentration of apoB was calculated by comparison of peak heights (peak areas give similar results) of samples and standards. The serum standards were prepared in our laboratory, calibrated against reference sera from the Centers for Disease Control and Prevention, lyophilized, and stored at -85°C until use. Peak heights between 15 mm and 35 mm gave linear and reproducible results with a cumulative coefficient of variation of less than 3%.

**Statistical Analyses**

Analysis of covariance was used to adjust the morphological traits as well as the lipid, lipoprotein, and apolipoprotein variables either for age alone or for both age and percent body fat. Differences between genotype groups were then tested for statistical significance by using Student’s t test. Partial correlation coefficients were used to quantify the age-adjusted interrelationships between body fatness, abdominal obesity measurements, and the plasma variables within each EcoRI genotype group. All statistical analyses were performed with the SAS statistical package (SAS Institute).

**Results**

Subjects were classified into two groups on the basis of their EcoRI genotype: subjects homozygous for the major 11-kb allele, the 11/11 group (n=40), and subjects carrying the minor 13-kb allele, the 13/11 group (n=16). None of the subjects was homozygous for the 13-kb allele. The relative allele frequencies in this sample were 0.857 and 0.143 for the 11-kb and 13-kb alleles, respectively. Mean age was 32.1±8.3 years in the 13/11 group and 33.4±6.8 years in the 11/11 group. The age-adjusted morphological characteristics of the subjects are presented in Table 1. The group of subjects carrying the 13-kb allele had significantly lower percent body fat and lower levels of total abdominal adipose tissue compared with the group homozygous for the 11-kb allele (P=.04). They also showed a tendency for lower mean waist-to-hip ratio values (P=.08) and for abdominal visceral adipose tissue area (P=.07). Age-adjusted plasma lipid, lipoprotein, and apoB levels are shown in Table 2 for each EcoRI genotype group. Although leaner, the 13/11 group did not show a more favorable lipoprotein-lipid profile than the group homozygous for the 11-kb allele. After statistical adjustment for both age and the difference in percent body fat, the 13/11 group showed significantly higher serum cholesterol levels (P=.02) and a tendency for higher apoB levels than the 11/11 group (P=.06).

The age-adjusted correlation coefficients between total adiposity, regional adipose tissue distribution, and plasma lipid, lipoprotein, and apoB levels within each EcoRI genotype group are shown in Table 3. The association patterns were markedly different between the two EcoRI genotype groups. Indeed, only in the 13/11 group was the abdominal visceral adipose tissue area, which is generally a critical correlate of the adverse metabolic effects of abdominal obesity, significantly associated with the plasma variables. Furthermore, indices of total and regional adiposity were more consistently associated with apoB concentration in plasma and the LDL fraction in the 13/11 than in the 11/11 group. The relationships between the abdominal visceral adipose tissue area and the cholesterol and apoB concentrations in plasma and the LDL fraction are illustrated in Fig 2. The steeper slopes for these associations in the 13/11 group suggest that increases in abdominal visceral adipose tissue accumulation are associated with greater increases in the concentration of plasma cholesterol and apoB levels in subjects carrying the 13-kb allele than in the group homozygous for the 11-kb allele.
Table 4. Pearson Correlation Coefficients Between Total Adiposity and Regional Adipose Tissue Distribution and Plasma Lipid, Lipoprotein, and ApoB Levels by EcoRI Genotype

<table>
<thead>
<tr>
<th>EcoRI Genotype</th>
<th>Fat Mass</th>
<th>WHR</th>
<th>Abdominal AT Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>13/11</td>
<td>.35</td>
<td>-.04</td>
</tr>
<tr>
<td></td>
<td>11/11</td>
<td>.21</td>
<td>.27</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>13/11</td>
<td>.36</td>
<td>.46</td>
</tr>
<tr>
<td></td>
<td>11/11</td>
<td>.28</td>
<td>.14</td>
</tr>
<tr>
<td>LDL chol</td>
<td>13/11</td>
<td>.44</td>
<td>.65*</td>
</tr>
<tr>
<td></td>
<td>11/11</td>
<td>.27</td>
<td>.10</td>
</tr>
<tr>
<td>HDL chol</td>
<td>13/11</td>
<td>-.39</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>11/11</td>
<td>-.15</td>
<td>-.12</td>
</tr>
<tr>
<td>Total apoB</td>
<td>13/11</td>
<td>.55*</td>
<td>.43</td>
</tr>
<tr>
<td></td>
<td>11/11</td>
<td>.28</td>
<td>.26</td>
</tr>
<tr>
<td>LDL apoB</td>
<td>13/11</td>
<td>.59*</td>
<td>.55*</td>
</tr>
<tr>
<td></td>
<td>11/11</td>
<td>.28</td>
<td>.26</td>
</tr>
</tbody>
</table>

ApoB indicates apolipoprotein B; WHR, waist-to-hip ratio; AT, adipose tissue; LDL, low-density lipoprotein; chol, cholesterol; and HDL, high-density lipoprotein. Correlation coefficients are age adjusted.

*P<.05.
†P<.01.

homozygous for the 11-kb allele. However, the interaction between EcoRI genotype and abdominal visceral adipose tissue area reached statistical significance only for total plasma apoB (P<.05; data not shown). It is also apparent from Fig 2 that for a given abdominal visceral adipose tissue area, subjects carrying the 13-kb allele generally have higher plasma cholesterol and apoB levels than subjects homozygous for the 11-kb allele, particularly when a certain level of visceral adipose tissue is reached, ie, approximately 90 to 100 cm². To further examine this possibility, we compared the two EcoRI genotype groups by only including in the analyses those subjects with abdominal visceral adipose tissue areas greater than 90 cm² (n=28 in the 11/11 group and n=8 in the 13/11 group) (Fig 3). The results showed that despite a trend for a lower total adiposity (percent body fat, 24.8±5.5 and 28.5±4.1 in the 13/11 and 11/11 groups, respectively; P=.06), subjects carrying the 13-kb allele had significantly higher total and LDL cholesterol levels and...
groups, subjects carrying the minor 13-kb allele showed significantly higher plasma cholesterol concentrations than subjects homozygous for the common 11-kb allele. Furthermore, when only men with elevated amounts of abdominal visceral adipose tissue were included in the analyses, significantly higher total cholesterol and apoB levels as well as higher LDL cholesterol and apoB levels were observed in the 13/11 group compared with the 11/11 group, although the 13/11 group tended to be leaner. Previous studies have shown that the amount of abdominal visceral adipose tissue measured by CT is a critical correlate of the metabolic complications found in obesity and more particularly in abdominal obesity. Our observations therefore suggest that small differences in plasma cholesterol and apoB levels exist between apoB-100 EcoRI genotypes and that these differences may be exacerbated by relevant environmental "triggers" such as a high accumulation of abdominal visceral adipose tissue. In the light of the recent work of Monsalve and colleagues, who report that heterozygosity for the EcoRI restriction fragment length polymorphism (RFLP) is associated with larger within-individual variability in serum cholesterol levels over time, it may be hypothesized from our observations that the increase in abdominal visceral adipose tissue accumulation that is known to occur with aging may be one of the environmental factors contributing to the larger within-individual variability in serum cholesterol over time in subjects carrying the minor EcoRI allele at the apoB gene locus.

Variations in plasma LDL cholesterol and apoB levels may result from differences in synthetic rates of apoB, in LDL removal rates, and in the balance between these two phenomena. The EcoRI polymorphism of the apoB gene alters the amino acid sequence in the mature protein (residue 4154 is altered from glutamic acid to lysine), but the functional significance of this change on the metabolism of apoB-containing lipoproteins is still unclear. Indeed, only a few studies have investigated the possible mechanisms whereby the EcoRI polymorphism of the apoB gene is likely to affect plasma cholesterol and apoB levels. Studying LDL kinetics in relation to apoB polymorphism in 5 different populations, Houlston et al observe that in 4 of these 5 populations the LDL fractional catabolic rate is lower in subjects carrying the minor EcoRI allele, the difference reaching statistical significance in 1 population. These data suggest that variation in the apoB gene may influence LDL metabolism and that the EcoRI polymorphism may more particularly influence the LDL catabolic rate. However, Gallagher and Myant do not support the hypothesis of an influence of EcoRI polymorphism on the LDL catabolic rate. Indeed, whereas the major pathway for removal of LDL from the plasma is through binding of apoB-100 on LDL particles to the LDL receptor, Gallagher and Myant find no difference in binding affinities to human skin fibroblasts between LDL particles from individuals homozygous for the major EcoRI allele and LDL particles from individuals homozygous for the minor EcoRI allele. It is possible that the absence of a relevant environmental trigger (eg, abdominal/visceral obesity, lipid-rich diet) in such in vitro experiments may have prevented the observation of small differences in LDL kinetics. However, another possibility is that apoB gene EcoRI polymorphism may affect plasma cholesterol and apoB levels by altering

**Discussion**

The frequency of the minor allele for the apoB-100 EcoRI polymorphism is increased in patients with coronary or peripheral arterial disease. In most studies in which an association is observed between the apoB EcoRI polymorphism and coronary artery disease, no relation is found with plasma lipid levels, which suggests that the polymorphism may be an independent risk factor for coronary artery disease. One study, however, has demonstrated an association between the minor EcoRI allele and several VLDL components, thus suggesting that the EcoRI polymorphism or another polymorphism within the apoB gene in linkage disequilibrium with the EcoRI polymorphism may affect coronary artery disease risk by contributing to the interindividual variability in serum lipoprotein levels. In the present study, apoB EcoRI polymorphism was associated with variations in cholesterol and apoB levels measured in plasma and the LDL fraction. Indeed, after statistical control for the differences in age and percent body fat between the two EcoRI genotype groups, subjects carrying the minor 13-kb allele showed
the affinity of apoB for the LDL receptor but by affecting apoB synthesis or, alternatively, by modifying apoB catabolism at postbinding steps. Additional studies are needed to understand the mechanisms whereby the DNA sequence variation in the apoB-100 gene creating the EcoRI polymorphic site or another polymorphism in linkage disequilibrium with the EcoRI polymorphism affects the metabolism of apoB-containing lipoproteins.

Although the present study was not aimed at investigating the associations between apoB polymorphism and obesity, we noted that the group of subjects carrying the minor 13-kb allele had significantly lower adiposity than subjects homozygous for the 11-kb allele. These results suggest that the minor EcoRI allele or another mutation within or near the apoB gene in linkage disequilibrium with the EcoRI polymorphism may be associated more frequently with leanness and normal body weight. Interestingly, an association between polymorphism of the apoB gene (the RFLPs detected with Pvu II and Xba 1) and obesity has been reported. The mechanisms by which variation at the apoB gene locus may influence the degree of adiposity are undefined. Nevertheless, a role for apoB in determination of adiposity is theoretically plausible, since this protein plays a central role in lipid metabolism. Additional research is needed to establish the true association between apoB gene polymorphism and variation in body fatness and to elucidate the mechanisms responsible for this association.

Conclusions

In the present study, the low-frequency 13-kb allele of EcoRI polymorphism was associated with increased plasma cholesterol and apoB levels, especially in subjects with high levels of abdominal visceral adipose tissue. In addition, the association patterns between abdominal visceral adipose tissue area and plasma cholesterol and apoB levels differed depending on the EcoRI genotype. These results suggest that the EcoRI polymorphism or another polymorphism within the apoB gene in linkage disequilibrium with the EcoRI polymorphism may interact with environmental factors, such as the level of abdominal visceral adipose tissue, to determine interindividual variability in plasma cholesterol and apoB levels. They further suggest that obesity, and more particularly abdominal/visceral obesity, may exacerbate minor disturbances in lipid metabolism that could result from DNA variation in genes relevant to lipid metabolism. In other words, genetic variation at the apoB gene locus may be involved in determination of the metabolic consequences of obesity and, more particularly, abdominal/visceral obesity. These observations may help to explain the metabolic heterogeneity that is reported in obesity and abdominal/visceral obesity.

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ApoB-100 gene EcoRI polymorphism. Relations to plasma lipoprotein changes associated with abdominal visceral obesity.

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