The effects of weight loss and apolipoprotein E polymorphism on serum lipids, apolipoproteins A-I and B, and lipoprotein(a)

Erik Muls,1 Kitty Kempen,3 Greet Vansant,1 Christa Cobbaert2 and Wim Saris3

1Department of Endocrinology, Metabolism and Nutrition, and 2Department of Clinical Chemistry, University Hospital Gasthuisberg, B-3000 Leuven, Belgium and 3Department of Human Biology, University of Limburg, PO Box 616, NL-6200 MD Maastricht, The Netherlands

Summary
Serum levels of lipoprotein(a) [Lp(a)], an independent risk factor for coronary heart disease, are strongly influenced by genetic factors. To evaluate the effect of weight loss on serum Lp(a), 54 normolipidemic obese women were examined before and after a two-month weight reduction programme. A mean weight loss of 10.1 kg was associated with decreases of serum triglycerides, total cholesterol (TC), LDL-C, HDL-C, apo B and apo A-I (*P* = 0.0001 for all variables), but not Lp(a). However, initial Lp(a) values were related to Lp(a) responses during dietary intervention (*P* = 0.0001). In subjects with pre-treatment Lp(a) values above 30 mg/dl, a mean 26.3% decrease was observed with weight loss, while no change was found in subjects with low Lp(a) values at baseline (*P* = 0.007 for group comparison). Apo E polymorphism contributes to the variation in lipid levels in the general population and in the obese. The relative contributions of the apo E locus to the total variance of TC, LDL-C and Lp(a) at baseline in these obese women were estimated to be 3.3, 1.7 and 3.2%, respectively, which are less than previously reported in healthy adults. Pre-treatment TC was increased in subjects with the apo E 3/4 phenotype compared to those with the common apo E 3/3 phenotype (*P* = 0.03). Apo E polymorphism did not affect Lp(a) levels at baseline nor the changes in lipoprotein variables, including Lp(a), during weight loss.

In conclusion, short-term substantial weight loss in normolipidemic obese women resulted in a lowering of Lp(a) only in individuals with pre-treatment Lp(a) levels above 30 mg/dl. This effect was independent of apo E polymorphism.

Keywords: apolipoprotein E polymorphism, body fatness, cholesterol, lipoprotein(a), obesity, weight reduction

Introduction
Lipoprotein(a) [Lp(a)], a genetic variant of LDL, is a strong and independent risk factor for cardiovascular disease in Caucasian populations.1,2 Consistent with its mainly genetically determined serum levels, Lp(a) appears to be largely unrelated to endocrine–metabolic or anthropometric variables.3 Most attempts to reduce serum Lp(a) concentrations by pharmacological intervention have been unsuccessful. Among the currently used hypolipidemic drugs, only nicotinic acid has a pronounced Lp(a)-lowering effect.4,5 Early reports6,7 have suggested that Lp(a) levels are also resistant to dietary changes. Recently, however, various types of dietary manipulation have been shown to influence Lp(a) levels: trans fatty acid-enriched diets increase serum Lp(a) in normolipidemic subjects8 and in mildly hypercholesterolemic men,9 while palm oil lowers Lp(a) in normocholesterolemic volunteers.10 Fish oil supplements decrease Lp(a) levels in some11–13 but not all subjects,14,15

Reports on the effect of weight loss on Lp(a) are scarce and contradictory. Significant Lp(a) reductions were previously noticed after weight loss in females but not in males,16 and in both men and women,17 while no changes were observed in other studies.7,13,18

Apolipoprotein (apo) E polymorphism is now considered to be an important genetic factor that influences the metabolism of apo B-containing lipoproteins. It substantially alters the relationship of obesity and abdominal fat accumulation to plasma lipoproteins in women.19 A significant interaction exists between apo E polymorphism and weight gain as they combine to affect plasma triglyceride (TG) and beta-lipoprotein, but not total cholesterol (TC).20

In addition, apo E polymorphism has been reported to influence plasma Lp(a) levels.21

In the present study, we have investigated the effect of short-term substantial weight loss on serum Lp(a) in obese normolipidemic women. In addition, we have examined the
influence of apo E polymorphism on baseline and weight reduction-induced changes in lipoprotein variables that include Lp(a).

Methods

Subjects
Fifty-four Dutch women, aged 19 to 53 years (mean ± s.d. 36.4 ± 8.7 years), with a BMI of 25.4 to 41.9 kg/m² were studied before and after an eight-week weight reduction programme. The subjects ingested a 1967 kJ (470 kcal) formula diet (Modifast; Sandoz Nutrition, Basle, Switzerland) containing 52 g protein, 7 g fat and 50 g carbohydrate for four weeks, followed by a 3347 kJ (800 kcal) mixed diet for an additional four-week period. Except for being obese, all subjects were judged to be in good health according to their medical history and physical examination. The participants gave informed consent to the study protocol, which was reviewed and approved by the Ethical Committee of the University of Limburg.

Analytical techniques
Body fat distribution was assessed before and after weight loss by measuring the waist girth to the nearest 0.5 cm at the minimum circumference and the hip circumference to the nearest 0.5 cm at the widest point of the hip area. All measurements were taken twice and the mean values were used to determine the waist-to-hip ratio (WHR). Body composition was evaluated by densitometry and deuterium isotope dilution.

Before and after the weight reduction programme, blood for lipoprotein analyses was drawn from each subject after an overnight 12 h fast. Serum levels of TG and TC were assayed enzymatically using GPO-PAP (glycerol phosphate oxidase-phenol 4-amino phenazine) and CHOD-PAP (cholesterol oxidase-phenol 4-amino phenazine) kits from Boehringer Mannheim, German. HDL-C was determined after precipitation with phosphotungstic acid-Mg²⁺, and LDL-C after polyvinyl sulphate/magnesium chloride precipitation. Apo A-I and B were assayed by immunonephelometry with kits from Boehringer Mannheim, Germany. Lp(a) was measured by the 'TintElize' Lp(a) enzyme immunoassay (Biopool AB, Umea, Sweden). Apo E phenotyping was performed by isoelectric focusing and immunoblotting techniques.

Statistics
Results are shown as means ± standard deviations. The body composition data on fat-free mass and fat mass are given as the means of measurements made by densitometry and deuterium isotope dilution.

Statistical evaluations were performed with SPSS statistical software (SPSS/PC+, V2.0). The skewed distribution of Lp(a) data was normalized by logarithmic conversion. For comparison of data, the two-sided Student’s t test for paired or non-paired data was used. To study relationships, Pearson correlation coefficients were calculated. A stepwise multiple regression analysis was used to quantify the effect of study variables on serum lipoproteins. Except for the regression analysis in Figure 1, changes (Δ) were calculated as the value before weight loss minus the value after weight reduction. Apo E allele frequencies were estimated using the gene-counting method. The relative contribution of genetic variance associated with the apo E locus to the total phenotypic variance of TC, LDL-C, apo B and Lp(a) was estimated according to the method of Sing and Davignon.

Results
Anthropometric and metabolic parameters in 54 obese women before and after the eight-week weight reduction programme are given in Table 1. Energy restriction significantly reduced body weight, BMI and WHR. Loss of fat-free mass accounted for 20.8% of the weight reduction. Serum TG, TC, LDL-C, apo B, HDL-C and apo A-I were all significantly decreased. Overall, no significant change in serum Lp(a) concentrations was observed. Median Lp(a) values before and after dietary intervention were 4.80 and 4.45 mg/dl, respectively. However, Lp(a) responses were dependent on pre-treatment Lp(a) values, as a highly significant negative relationship was observed between initial Lp(a) and the difference after dietary intervention (r = -0.44; P = 0.0001), as shown in Figure 1. In nine subjects with initial Lp(a) values above 30 mg/dl, a 26.3% reduction (−10.9 ± 8.9 mg/dl) in Lp(a) was observed with weight loss, while no change (−0.3 ± 3.5 mg/dl) was found in 45 subjects with baseline levels below 30 mg/dl (P = 0.007 for group comparison by Mann–Whitney test for log-transformed data). Lp(a) responses during weight loss in subjects with elevated Lp(a) at baseline were not related to changes in TG, LDL-C or apo B.

The apo E phenotype distribution and allele frequencies among the 54 obese women are presented in Table 2. The gene frequencies in the Dutch women enrolled in this weight reduction programme were similar to those in two

| Table 1 Means ± standard deviations of anthropometric and metabolic variables in 54 obese women before and after the eight-week weight reduction programme |
|----------------------------------|-----------------|-----------------|
| Attrib. | Before | After | P     |
|----------------------------------|-----------------|-----------------|
| Height (cm)                      | 166 ± 5         |                 |
| Weight (kg)                      | 87.5 ± 12.0     | 77.4 ± 10.7     | 0.0001 |
| Body mass index (kg/m²)          | 31.8 ± 4.0      | 28.0 ± 3.5      | 0.0001 |
| Waist circumference (cm)         | 93 ± 10         | 83 ± 9          | 0.0001 |
| Hip circumference (cm)           | 113 ± 9         | 103 ± 9         | 0.0001 |
| WHR                              | 0.83 ± 0.05     | 0.81 ± 0.04     | 0.001 |
| Fat mass (kg)                    | 36.9 ± 8.8      | 29.0 ± 7.5      | 0.0001 |
| Fat-free mass (kg)               | 50.9 ± 5.1      | 48.8 ± 4.9      | 0.0001 |
| Lipoproteins (mg/dl)             |                 |                 |
| Triglycerides                    | 118 ± 51        | 93 ± 36         | 0.0001 |
| Total cholesterol                | 203 ± 34        | 174 ± 32        | 0.0001 |
| LDL cholesterol                  | 142 ± 38        | 116 ± 33        | 0.0001 |
| Apolipoprotein B                 | 79 ± 19         | 66 ± 16         | 0.0001 |
| HDL cholesterol                  | 45 ± 10         | 42 ± 8          | 0.0001 |
| Apolipoprotein A-I               | 119 ± 19        | 104 ± 16        | 0.0001 |
| Lipoprotein(a)                   | 11.6 ± 14.9     | 10.0 ± 12.3     | 0.07  |
| ln Lp(a)                         | 3.96 ± 1.35     | 3.92 ± 1.22     | 0.50  |

a WHR = waist-to-hip circumference ratio. P values are from paired t test.
Table 2 Apolipoprotein E phenotype and allele frequencies

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number observed</th>
<th>Relative frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2/E2</td>
<td>1</td>
<td>1.85</td>
</tr>
<tr>
<td>E2/E3</td>
<td>4</td>
<td>7.41</td>
</tr>
<tr>
<td>E2/E4</td>
<td>1</td>
<td>1.85</td>
</tr>
<tr>
<td>E3/E3</td>
<td>34</td>
<td>62.96</td>
</tr>
<tr>
<td>E3/E4</td>
<td>14</td>
<td>25.93</td>
</tr>
<tr>
<td>E4/E4</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>100</td>
</tr>
</tbody>
</table>

Gene frequencies
E2: 0.065
E3: 0.706
E4: 0.139

previously-reported random population samples from the Netherlands.21,26 The relative contributions of apo E polymorphism to the total variance of TC, LDL-C, apo B and Lp(a) at baseline were calculated to be 3.3, 1.7, 1.7 and 3.2%, respectively.

Pre-treatment anthropometric parameters were not statistically different between subjects with the apo E 3/3 (n = 34) and apo E 3/4 (n = 14) phenotypes. Among the lipoprotein variables, TC at baseline was significantly higher in the apo E 3/4 subset compared to those homozygous for apo E 3 (221 ± 30 vs. 199 ± 31 mg/dl; P = 0.03). Similar trends for LDL-C, apo B and A-I did not reach statistical significance. The apo E*4 locus did not affect Lp(a) values. Within the apo E 3/3 group, WHR at baseline, but not BMI, correlated positively with TG (r = 0.33; P = 0.03) and negatively with HDL-C (r = -0.29; P = 0.05). In addition, fat mass correlated negatively with LDL-C and apo B (for both, r = -0.37; P = 0.02) and apo B showed a positive relationship with fat-free mass (r = 0.40; P = 0.01) in apo E 3/3 subjects. These associations between pre-treatment anthropometric variables and lipoproteins at baseline were not observed within the apo E 3/4 group. Changes in anthropometric and metabolic parameters in response to the low calorie diet and weight loss were identical in the apo E 3/3 and apo E 3/4 subgroups.

A multivariate procedure was used to quantify the relative contribution of apo E polymorphism and anthropometric parameters to the variance in serum lipoproteins. The impact of apo E polymorphism was evaluated using three patient groups: E 2/2 and 2/3, E 3/3 or E 3/4, age, BMI, WHR, fat mass and fat-free mass as independent continuous variables in 54 obese women before weight loss.

Table 3 Multiple stepwise regression analysis with lipoprotein parameters as dependent variables and apo E phenotype (E 2/2 and 2/3, E 3/3 or E 3/4), age, BMI, WHR, fat mass and fat-free mass as independent continuous variables in 54 obese women before weight loss

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Step</th>
<th>Independent variable</th>
<th>Multiple r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryglicerides</td>
<td>1</td>
<td>Age</td>
<td>0.11</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Fat-free mass</td>
<td>0.21</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1</td>
<td>WHR</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>1</td>
<td>Age</td>
<td>0.10</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Total cholesterol
LDL cholesterol
Apolipoprotein B
Apolipoprotein A-I
In Lipoprotein(a)

No variables reach the 0.05 limit

Table 4 Multiple stepwise regression analysis with changes in lipoprotein parameters during weight loss as dependent variables and apo E phenotype and changes in BMI, WHR, fat mass and fat-free mass as independent variables in 54 obese women

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Step</th>
<th>Independent variable</th>
<th>Multiple r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryglicerides</td>
<td>1</td>
<td>Δ fat mass</td>
<td>0.13</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Δ fat-free mass</td>
<td>0.30</td>
<td>0.0003</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1</td>
<td>Δ fat-free mass</td>
<td>0.09</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Total cholesterol
LDL cholesterol
Apolipoprotein B
Apolipoprotein A-I
Lipoprotein(a)
In Lipoprotein(a)

No variables reach the 0.05 limit

Changes (Δ) were calculated as the value before weight loss minus the value after weight reduction.

Discussion

Serum Lp(a) concentrations in this group of normolipidemic obese women were unrelated to anthropometric variables. This observation extends previous reports on non-obese healthy adults3 and diabetics.27

The decreases in TC, LDL-C and TG induced by a 10 kg weight loss in this study were substantially greater than those predicted from a recent meta-analysis of 70 studies
designed to examine the effects on blood lipids of weight reduction by dieting. This was not unexpected since blood lipid reductions have been shown to be more pronounced during active weight loss on a very low calorie diet than on a normal food intake. However, changes in lifestyle as well as intra-individual, between-sample variations in lipid levels reflecting both biological and methodological variability may also have contributed to the changes in lipid variables observed in this study.

Despite the considerable decreases in TC, LDL-C and TG, no overall significant change in serum Lp(a) levels was observed. These data confirm previous reports showing no change of Lp(a) concentrations after weight loss in obese subjects, but are in disagreement with another study documenting mean Lp(a) decreases of 19% in men and of 30% in premenopausal women after moderate weight loss. The reasons for these discrepancies are not clear, especially since the average decrease in BMI was greater in the studies reporting no overall Lp(a)-lowering effect of weight reduction.

Our data, together with those from Sönntichsen et al. and Corsetti et al. suggest, however, that weight loss may have a Lp(a)-lowering effect in the subgroup of obese subjects with elevated initial Lp(a) values (i.e. above 25 to 30 mg/dl). This observation appears to be of clinical relevance since Lp(a) concentrations above this level are associated with a 1.5- to 2-fold increased risk for coronary heart disease. Interestingly, Lp(a) responses to other types of dietary or pharmacological manipulation seem to be dependent on baseline Lp(a) values, since both increases of Lp(a) on diets high in trans-mono-unsaturated fatty acids and decreases by palm oil, fish oil and combined nemooycin-niacin treatment are related to intrinsic Lp(a) levels.

Apo E polymorphism contributes to the variation in lipid levels seen in the population. Three common alleles (E*2, E*3 and E*4) code for the three major isoforms (E2, E3, and E4), giving rise to six apo E phenotypes in plasma. Absorption efficacy of dietary cholesterol is increased and serum TC and LDL-C are higher in subjects with the E*4 allele, while cholesterol absorption and both TC and LDL-C levels are lower in people with E*2 when compared to those with E*3. In addition, TG levels are elevated and HDL-C concentrations are decreased in apo E 2/2, 2/3, 2/4 and 3/4 subsets compared to the more common apo E 3/3 group. The relationships between the apo E phenotype and plasma lipid levels previously documented in the population at large have now also been shown to exist in the obese, since the E*4 allele increases both the risk of hypercholesterolemia and hypertriglyceridemia among obese individuals.

Within our group of obese women, TC at baseline was higher in the subjects carrying the E 3/4 phenotype than in those with E 3/3, but, probably due to the small sample size, no significant differences were observed in LDL-C, apo B, TG, HDL-C and apo A-I. Also, the relative contribution of apo E polymorphism to the total variance of TC, LDL-C and Lp(a) was less pronounced than previously observed in non-obese subjects. No association was observed between pre-treatment Lp(a) and apo E polymorphism in these obese Dutch women, although a previous report from the Netherlands has described an apo E genotype effect on Lp(a) similar to that on LDL-C. Apo E polymorphism substantially alters the association between body fatness, body fat distribution and plasma lipoprotein levels in obese women. In women homozygous for apo I/I, Pouliot et al. have observed positive associations of body fat mass, WHR and computerized tomography-derived total and intra-abdominal fat areas with VLDL lipids, LDL-C and LDL-apo B, and negative correlations of these body fatness variables with HDL-C. In our apo E 3/3 subjects, WHR was positively related to TG and negatively to HDL-C, but no association was observed with LDL-C or apo B. In contrast to the observations of Pouliot et al., we found an inverse relationship between fat mass and both LDL-C and apo B, while apo B was positively related to fat-free mass. These conflicting results emphasize the need for further studies that explore the interplay between genes relevant to lipoprotein metabolism and body fatness or its components.

The response to dietary fat and cholesterol may vary between individuals with different apo E phenotypes. However, two recent studies have failed to detect this association. In both investigations, only relatively small numbers of subjects were studied. We have therefore explored whether apo E polymorphism might modulate the response to weight reduction. The data were analysed firstly by comparing changes in anthropometric and lipoprotein variables in subjects with apo E 3/3 or E 3/4 phenotypes, and secondly by multivariate stepwise analyses that included apo E polymorphism together with anthropometric parameters as independent variables. Neither approach was able to demonstrate a modulating effect of apo E polymorphism on changes induced by weight loss. This could partly be related to the relative homogeneity of baseline lipid values in our normal weight subjects. In two earlier reports, which observed an association of the E*4 allele with a greater sensitivity to diet, pre-treatment cholesterol levels were significantly higher in carriers of the E*4 allele than in subjects without E*4. In a third study showing that both baseline total cholesterol and apo E4 phenotype independently predicted the degree of cholesterol reduction following dietary intervention in dyslipidemic men, baseline levels of TC and LDL-C were similar in subjects with and without the E*4 allele.

In conclusion, short-term, substantial weight loss in normal weight obese women was associated with decreases of VLDL, LDL and HDL. A lowering of serum Lp(a) was observed only in individuals with pre-treatment Lp(a) levels above 30 mg/dl. Although pre-treatment TC was increased in subjects with the apo E 3/4 phenotype compared to those with the common apo E 3/3 phenotype, apo E polymorphism did not affect Lp(a) at baseline, nor did it affect the changes in lipoprotein variables, including Lp(a), during weight loss.

Acknowledgements
This study was supported in part by National Fonds voor Wetenschappelijk Onderzoek, Belgium, and by the Netherlands Organisation for Scientific Research (Grant No. 900–562–090).
References


