Evidence for a complex risk profile in obese postmenopausal Turkish women with hypertriglyceridaemia and elevated apolipoprotein B

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ABSTRACT

The aim of the present study was to examine possible pathophysiological relationships among a wide array of proatherogenic risk factors in postmenopausal women. Fasting lipids, apoB (apolipoprotein B), BMI (body mass index) and waist circumference were measured in 178 women (59.4 ± 7.2 years) from the Turkish Adult Risk Factor Study. Fasting levels of complement C3, insulin, SHBG (sex hormone-binding globulin), cortisol, oestradiol, testosterone and DHEA-S (dehydroepiandrosterone sulphate) were also determined. This is the first study to examine the relationships of all these variables with apoB. In the first of two different approaches, three groups of obese women were compared. Group 1 comprised women who were normolipidaemic with normal apoB; group 2, women who were hypertriglyceridaemic, but with normal apoB; and group 3, women who were hypertriglyceridaemic with elevated apoB. Complement C3, fasting insulin and glucose were significantly higher and HDL-C (high-density lipoprotein-cholesterol) and SHBG levels were significantly lower in group 3 than in group 1. In the former group, the testosterone/SHBG ratio tended to be higher, indicating more free testosterone, than in group 1. The mean risk score in group 3 and the odds ratio for coronary disease by logistic regression analysis were significantly higher, 2.56 (confidence intervals, 1.12–5.85; \( P = 0.026 \)), compared with the two other groups combined. In examining the whole group, apoB levels correlated significantly with a wider array of pro-atherogenic risk factors than did LDL-C (low-density lipoprotein-cholesterol), particularly being linked to complement C3 and glucose, as well as the risk score. Complement C3 demonstrated the widest associations and was significantly linked with BMI, waist circumference, insulin, glucose, fibrinogen, triacylglycerols (triglycerides) and apoB and was inversely correlated with HDL-C and SHBG. SHBG was also correlated inversely with a wide spectrum of risk variables. In summary, in Turkish women, apoB was linked with a complex array of proatherogenic risk factors, and hypertriglyceridaemia with elevated apoB was associated with a higher risk of coronary disease.

Key words: apolipoprotein B (apoB), coronary disease risk, obesity, risk profile, sex hormone.

Abbreviations: apoB, apolipoprotein B; BMI, body mass index; CHD, coronary heart disease; CRP, C-reactive protein; DBP, diastolic blood pressure; DHEA-S, dihydroepiandrosterone sulphate; E2, oestradiol; HDL, high-density lipoprotein; HDL-C, HDL-cholesterol; LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin.

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INTRODUCTION

It is a fundamental tenet of cardiovascular epidemiology that ischaemic heart disease is more common in men than in women. The differences in frequency are particularly marked before the menopause, but persist even thereafter. No full account for these differences has yet been produced, but two principal hypotheses could be considered. The first is that the differences in risk relate directly to hormonal effects on the vascular wall, whereas the second is that the known risk factors for coronary disease are modulated by the sex hormones, favourably in the case of females, and unfavourably in the case of males. These two hypotheses are not mutually exclusive, but are important to differentiate because prevention strategies are much more practical in the second than in the first.

Two obvious differences between the sexes are their adipose tissue mass and distribution and their plasma lipoproteins. On average, women have more adipose tissue than men and it is principally peripheral, rather than central [1]. Moreover, after puberty, on average, men have higher plasma triacylglycerols (triglycerides), total cholesterol and LDL-C (low-density lipoprotein-cholesterol) and apoB (apolipoprotein B), and lower HDL-C (high-density lipoprotein-cholesterol) and apoA1 (apolipoprotein A1) than women, differences that narrow after menopause in women [2–4]. Recently, there has been much interest in the relationship between adipose tissue function and plasma lipoprotein levels. It is now apparent that the effectiveness of fatty acid trapping by adipocytes can vary substantially. That is, there can be substantial differences in the proportion of fatty acids, which have been liberated by lipoprotein lipase from the triacylglycerol-rich lipoproteins, that are actually taken up by adipocytes and converted into triacylglycerols.

There is, necessarily, an inverse relationship between the proportion of fatty acids taken up by adipocytes and the proportion that are released into the systemic circulation from which they can be taken up by the liver and skeletal muscle. Moreover, the rate at which apoB particles are secreted by the liver is related directly to fatty acid flux to the liver, and that relationship explains why hypertriglyceridaemia and elevated apoB is the consequence of ineffective fatty acid trapping by adipose tissue. Peripheral obesity in females tends to be associated with effective fatty acid trapping by adipose tissue, relatively non-atherogenic plasma lipoprotein profiles and a low incidence of coronary disease. By contrast, central obesity in males is associated with ineffective fatty acid trapping by adipose tissue, an atherogenic lipoprotein profile and a high incidence of coronary disease (for reviews, see [4–6]).

The incidence of coronary disease is increasing in all developing countries in parallel with the adoption of energy excessive, fatty-acid-rich and carbohydrate-poor diets that characterize most affluent Western societies. In Turkey, the major differences in coronary incidence between males and females, which are easily apparent everywhere else, are markedly narrowed [7,8]. This loss of resistance to vascular disease in women is particularly intriguing given that the levels of LDL-C remain relatively low in both genders [8]. On the other hand, hypertriglyceridaemia with elevated apoB (i.e. hypertriglyceridaemic hyperapo B) [9,10] and low HDL-C [8,11] is common.

The aims of the present study, therefore, were to examine the relationships among plasma lipids and apoB and the sex hormones, cortisol, insulin and certain cytokines in selected groups of overweight Turkish women. Our hypothesis was that hypertriglyceridaemia with elevated apoB would be associated with a more androgenic hormonal pattern. We also tested the hypothesis that apoB would be associated with a wider array of risk factors than with LDL-C.

METHODS

Women were selected from the cohort of the Turkish Adult Risk Factor Study. The Turkish Adult Risk Factor Study is a prospective survey of the prevalence of risk factors and cardiac disease in adults, which has been carried out periodically since 1990 in seven geographical regions of Turkey [8]. The last follow-up was conducted in 2002/2003. All subjects gave informed consent, and the study was approved by the Committee of the Turkish Society of Cardiology, Istanbul. The survey is stratified so as to be representative for sex and age and urban versus rural distribution. Data were obtained via questionnaires, physical examinations of the cardiovascular system, ECGs and fasting blood samples.

Among the original cohort of the last nationwide screening [12], 576 women between 47 and 74 years were surveyed. For inclusion in the present study, women had to be menopausal and to have a serum determination of apoB and CRP (C-reactive protein) as well as a fasting value of triacylglycerols (triglycerides) and insulin. Those with waist circumference < 78 cm were excluded as were those with normal (< 140 mg/dl) concentrations of triacylglycerols in conjunction with high apoB values. Diabetes or coronary disease were not criteria for exclusion.

Women who met these criteria (n = 178) were divided into three obese groups as follows: (i) normotriglyceridaemia with normal apoB (n = 71; waist ≥ 78 cm, triacylglycerols < 140 mg/dl and apoB < 120 mg/dl); (ii) hypertriglyceridaemia with normal apoB (n = 47; triacylglycerols ≥ 140 mg/dl and apoB < 120 mg/dl); and (iii) hypertriglyceridaemia with elevated apoB (n = 60; triacylglycerols ≥ 140 mg/dl and apoB ≥ 120 mg/dl). An apoB cut-off of ≥ 120 mg/dl has been used in several studies [13,14] to distinguish normal from elevated apoB and has been shown to correspond to a substantially...
increased risk of vascular disease. No participant was on hormone-replacement therapy when sampled, and clinical signs of hyperandrogenism suggestive of polycystic ovary syndrome were not present in any of the subjects.

Blood pressure was measured in the right arm in the sitting position after at least 5 min at rest. Each reading was determined to the nearest even number and the final value was the average of two measurements taken 3 min apart. With the subject standing, waist circumference was measured midway between the lowest rib and the iliac crest.

Serum cholesterol, triacylglycerols and glucose were determined by enzymic dry chemistry methods, and HDL-C by direct automated measurement using Roche Diagnostics kits (Mannheim, Germany) as described previously [12]. LDL-C was calculated using the Friedewald equation. Triacylglycerol values > 400 mg/dl were encountered in only three women, namely with concentrations 410–495 mg/dl; the calculated LDL-C value in each was augmented by 1–3 mg/dl respectively. Insulin concentrations were estimated by the chemiluminescent immunometric method. Plasma apoB levels were measured by an immunoturbidometric method (Turbitimer; Behring Diagnostics, Marburg, Germany). Only two sera exhibited mild visual turbidity and the samples were centrifuged again to obtain clarity before apoB determination. Plasma non-esterified fatty acids were not measured because, except in the postpartum period, they have not been shown to distinguish hypertriglyceridaemia with elevated apoB from the other dyslipidaemias. CRP concentrations were determined by particle-enhanced immunonephelometry (Behring Diagnostics). Geometric means for triacylglycerols, insulin and CRP were calculated from log-transformed distributions. Concentrations of complement C3 were measured by nephelometry (Behring Diagnostics). Serum levels of E2 (oestradiol), testosterone, DHEA-S (dihydroepiandrosterone sulphate), cortisol and insulin were determined by chemiluminescent immunometric methods using Roche Diagnostic kits. SHBG (sex hormone-binding globulin) was measured by RIA. The following conversions were used: for DHEA-S units, 100 µg/dl = 2.7 nmol/l; for SHBG units, 34.7 mg/dl = 1 nmol/l; for testosterone, 3.467 ng/ml = 1 nmol/l.

In a random sample of 5% of the participants, measurements of plasma lipids, lipoproteins and apoB were repeated in a reference laboratory (Biochemistry Laboratory, American Hospital, Istanbul, Turkey) and adjustments made accordingly.

**Diagnosis of CHD (coronary heart disease) and assessment of global risk in individual women**

CHD was diagnosed based on the presence of angina pectoris or a history of myocardial infarction without or with accompanying Minnesota code electrocardiographic abnormalities [15]. The global CHD risk in each woman was evaluated by the scoring scheme of the Turkish Adult Risk Factor Study, which was based on the Framingham risk score and validated prospectively in the Turkish Adult Risk Factor Study cohort [16]. This method includes nine risk variables: age, SBP (systolic blood pressure), smoking status, presence of diabetes, levels of LDL-C, HDL-C and triacylglycerols, waist circumference and physical activity grade. Individual point counts of ⩾ 21 and ⩾ 27 points correspond to intermediate- and high-risk categories. Our aim was to assess the relationship of various risk factors and hormone levels with CHD risk as well as the mean risk in the three groups that were studied.

**Data analysis**

Statistical analyses of the data were carried out using SPSS-10 for Windows. Given their skewed distribution, CRP, fasting insulin, triacylglycerol, E2 and DHEA-S values were log-transformed for calculations. Univariate correlations were determined with Spearman’s test, except for those with a normal distribution. Differences among groups were tested by ANOVA with post-hoc Dunnett *t* test. Estimates of odds ratio and 95% confidence intervals were obtained by use of regression analysis. A value of *P* < 0.05 was considered statistically significant.

**RESULTS**

**Risk factor status by groups**

Table 1 shows the baseline risk factor status for the three groups. By design, triacylglycerols in groups 2 and 3 are higher than in group 1. Similarly, apoB is highest in group 3 and this accounts for the fact that total cholesterol and LDL-C are also highest in this group as well. HDL-C was similar in groups 2 and 3, but both were lower than in group 1, differences that are likely to be secondary to the differences in triacylglycerol levels between group 1 and groups 2 and 3. All these differences, therefore, were the direct consequence of case selection. That does not mean, however, they are not determinants of risk.

The following differences were not the direct consequence of case selection and, therefore, appear to be characteristics that are associated with those that determined case selection, namely the lipid status as given by plasma triacylglycerol and apoB. Fasting serum insulin and glucose differed significantly between group 1 and group 3. In the study group, 33 women (19%) were diabetic (11% in group 1, 28% in group 2 and 22% in group 3). The metabolic syndrome was present in 39% of women in group 1 (*P* < 0.001), in contrast with 89% and 88% in groups 2 and 3 respectively. Interestingly, there
were no clear differences in obesity or the pattern of obesity among the groups, although there was a trend for higher waist circumference and waist-to-hip ratio in groups 2 and 3. On the other hand, complement C3 rose progressively, being significantly higher in group 3 compared with group 1, and CRP was highest, although not significantly, in group 3. Furthermore, both the total cholesterol/HDL-C ratio, a good predictor of CHD events in the overall cohort of the Turkish Risk Factor study [8], and the individual global risk score in group 3 were significantly higher than in each of the other two groups. This was in agreement with the observed distribution of CHD (15.5 % in group 1, 23.4 % in group 2 and 33.3 % in group 3; $P = 0.023$ between groups 1 and 3, but not significant among the other groups).

Thus in a group selected for a specific dyslipidaemia, group 3 (hypertriglyceridaemia with elevated apoB), a series of other abnormalities, all of which increase the risk of vascular disease, were also present, creating a constellation of risk factors within a single cohort of subjects. It is of interest that logistic regression analysis in a model comprising group 3 and the remaining groups as independent variables demonstrated that the odds ratio for CHD, the dependent variable, in group 3 was 2.05 (confidence intervals, 1.03–0.41; $P = 0.041$) compared with the two other groups, and 2.73 ($P = 0.019$) compared with the obese group when the three groups were included in the model separately as independent variables.

### Hormonal status by group

The results of serum hormones are shown in Table 2. No significant differences existed among the three groups of women with regard to E2, cortisol, DHEA-S and total testosterone concentrations. SHBG levels in group 3 were significantly lower ($P = 0.031$) than in group 1, whereas the testosterone/SHBG ratio was (not significantly) higher by 19 % in group 3 compared with group 1.

### Overall relationships

The overall relationships were more informative than the intergroup comparisons. Table 3 shows the significant Spearman correlation coefficients within the overall cohort. LDL-C correlated significantly and positively with apoB and triacylglycerols and inversely with SHBG. Considerably more significant associations with triacylglycerol were observed. These included positive relationships with apoB, glucose, LDL-C, complement C3 and CRP and inverse significant relationships with SHBG and HDL-C. ApoB was positively correlated with LDL-C, triacylglycerols, glucose and complement C3 and inversely to SHBG. Although HDL- and LDL-C values showed high correlations with the total cholesterol/HDL-C ratio, being direct constituents of the latter, triacylglycerols and apoB displayed still higher correlations. Among the non-lipid parameters, the most interesting associations were with complement C3, which correlated positively with BMI, waist circumference, insulin, glucose, triacylglycerols, apoB, CRP and fibrinogen, and inversely with HDL-C. SHBG correlated inversely with complement C3, BMI, glucose,
The findings of the study were mixed. On one hand, there was a positive and significant correlation between apoB and free testosterone, as reflected by the testosterone/SHBG ratio, in the total group of subjects. On the other hand, although there was a trend to a higher free testosterone level in the group with hypertriglyceridaemia and elevated apoB, the group in which fatty acid trapping by adipose tissue should be least effective, the differences were not statistically significant. The data, therefore, do not provide clear cut support for the hypothesis we tested. By the same token, given the relatively small size of the groups studied and the overall relationship that emerged between plasma apoB and free testosterone, further study would appear to be warranted.

It is worth underlining that marked hypertriglyceridaemia (>400 mg/dl), which precludes accurate estimation of LDL-C by the Friedewald formula, hardly existed in this group of women. In 2% of women in whom these triacylglycerol limits were slightly exceeded, reasonable adjustments for underestimating the LDL-C concentrations were made.

The relationship of the sex hormones with obesity and dyslipidaemia is complex and remains incompletely understood [17]. Plasma levels of SHBG are inversely associated with BMI, and an obesity-induced fall in SHBG results in an increased turnover of androgens. In addition, after adjustment for adiposity, SHBG is positively correlated with HDL-C in pre- [18] and postmenopausal [19] women and inversely associated with insulin [19]. As is the case with SHBG, total testosterone diminishes with increasing (central) obesity, but the total testosterone/SHBG ratio rises.

Moreover, an androgenic sex hormone profile characterized by low levels of SHBG, high levels of DHEA-S

**DISCUSSION**

The incidence of CHD is multiplying in men in many of the countries of the developing world, but, as in the West, the risk remains much less in women. This is not the case in Turkey, where vascular disease is multiplying in both men and women. The aim of the present study was to begin to examine the phenomenon in which gender-associated risk appears to have disappeared or, at least, largely diminished. To do so, we studied obese postmenopausal Turkish females. Our specific aim was to study the relationship between the plasma lipids that reflect the adequacy of fatty acid trapping, triacylglycerols and apoB, and the plasma levels of the androgenic hormones.

The findings of the study were mixed. On one hand, there was a positive and significant correlation between triacylglycerols, apoB and LDL-C and positively with E2. The testosterone/SHBG ratio, an index of circulating free testosterone, correlated positively with DHEA-S and fibrinogen. DHEA-S was also positively correlated with testosterone. Not only conventional risk factors were significantly correlated (r = 0.3 to 0.5) with the individual global risk score, but also complement C3, fibrinogen and insulin. SHBG, along with the testosterone/SHBG ratio, were the only hormonal estimates which significantly correlated with the risk score.

Taken together, there appears to be a complex series of significant associations between triacylglycerol and apoB and a wide variety of proatherogenic risk factors, and between SHBG and a wide variety of proatherogenic risk factors.
and free testosterone has recently been reported in young women with the metabolic syndrome [20]. In addition, low serum levels of SHBG have been associated with CHD, independently of apoB and conventional risk factors in 55 postmenopausal women with CHD compared with 32 women without [21]. Finally, Van Pottelbergh et al. [22] observed a positive correlation between free testosterone and apoB in a cohort of 715 healthy middle-aged men.

That android obesity is associated with hypertriglyceridaemia with elevated apoB, whereas gynoid obesity is not, has been repeatedly observed [23]. It is of interest, therefore, that the three groups of obese postmenopausal women in the present study could not be confidently distinguished on the basis of adipose tissue distribution. Moreover, significant relationships between waist circumference and either triacylglycerols or apoB were not observed in the overall group. This suggests that assessment of risk by triacylglycerols and waist circumference only is not adequate for individuals and certainly not for all individuals in all cultures. If so, measuring waist circumference does not substitute for measuring apoB.

Other interesting relationships were uncovered in the present study. In the whole group, triacylglycerol was positively correlated with insulin and complement C3 and inversely correlated with SHBG. Other than age, triacylglycerols and apoB, LDL-C was only significantly inversely correlated with SHBG. By contrast, apoB was significantly correlated with triacylglycerol, LDL-C, complement C3 and fasting glucose and inversely correlated with SHBG. Moreover, insulin and glucose were significantly higher in the hypertriglyceridaemic group with elevated apoB (group 3) than in the obese normolipidaemic group (group 1). CRP is a well-recognized risk factor for vascular disease [24–26] and was highest in the hypertriglyceridaemic group with elevated apoB (group 3), but the difference was not statistically significant. However, in a much larger cohort, apoB and LDL-C were significantly linked to an array of proatherogenic dyslipoproteinaemia in Type II diabetes [9]. This link should not be surprising given that increased fatty acid flux to the liver is the progenitor of hypertriglyceridaemia with elevated apoB, and there is considerable evidence to indicate that increased fatty acid flux to the liver and pancreas can lead to insulin resistance and hyperglycaemia.

The data in the present study do not establish the pathophysiological basis for the clustering of risk factors in obese Turkish women with hypertriglyceridaemia with elevated apoB. Clearly, it is not simply obesity that is responsible, because BMI was the same in all three groups. Rather, it appears that, just as hypertriglyceridaemia is heterogeneous with respect to very-LDL secretion and LDL particle number, obesity is heterogeneous in pathophysiology and clinical consequence as well. Our working hypothesis is that differences in fatty acid trapping by adipose tissue may be responsible, but considerably more work is required before this hypothesis can be fully tested.

In summary, hypertriglyceridaemia with elevated apoB is an important proatherogenic dyslipoproteinaemia in obese Turkish women. We believe this is of particular clinical significance given the high prevalence of CHD in Turkish women that has been documented previously and confirmed in the present study. Of interest, there were significantly lower levels of SHBG and a trend to higher free testosterone levels in this group, suggesting a link between hormonal dysfunction and adipose tissue dysfunction. Since statin therapy appears to be more effective in patients with hypertriglyceridaemia with elevated apoB [38] than in those with uncomplicated hypercholesterolaemia and apoB-guided statin therapy is more effective than LDL-C-guided statin therapy [39–42], we believe the present findings are important for clinical practice.
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