Relation of Plasma Triglyceride and ApoB Levels to Insulin-Mediated Suppression of Nonesterified Fatty Acids
Possible Explanation for Sex Differences in Lipoprotein Pattern

P.M. McKeigue, A. Laws, Y.D. Chen, M.G. Marmot, and G.M. Reaven

To test whether a sex difference in insulin-mediated suppression of nonesterified fatty acids (NEFAs) could account for sex differences in plasma triglyceride levels, we studied 632 normoglycemic men and women of European and South Asian descent aged 40 to 69 years. Mean fasting NEFA levels were 19% higher in women than in men. Between fasting and 2 hours after a 75-g oral glucose load, NEFA levels fell by 69% in women and 55% in men, so that mean NEFA levels at 2 hours after loading were 19% lower in women than in men. Plasma triglyceride and apolipoprotein B levels were correlated with 2-hour NEFA levels in each sex and ethnic group, and these associations were independent of glucose, insulin, and central obesity. These results are consistent with experimental studies of the effects of insulin and NEFAs on hepatic production of triglycerides and apolipoprotein B. Suppression of NEFA levels in response to insulin is greater in women than in men, and this may account for some of the sex differences in lipoprotein pattern and coronary heart disease risk. (Arteriosclerosis and Thrombosis 1993;13:1187-1192)

KEY WORDS • triglyceride • apo B • insulin • fatty acids, nonesterified • obesity • sex factors • glucose

The higher coronary heart disease risk in men compared with women may be related to sex differences in lipoprotein pattern. In comparison with women, men have higher levels of very-low-density lipoprotein (VLDL) triglyceride and apolipoprotein (apo) B, lower levels of high-density lipoprotein cholesterol (HDL-C), and a higher proportion of small, dense particles in the low-density lipoprotein (LDL) fraction. The metabolic basis of these differences is poorly understood, although in both men and women HDL-C levels and LDL composition are closely dependent on plasma triglyceride levels. Sex differences in the regulation of plasma triglyceride could thus account for some of the other sex differences in lipoprotein pattern.

In a large population survey we demonstrated that elevated triglyceride levels and other metabolic disturbances associated with insulin resistance were more prevalent in people of South Asian (Indian, Pakistani, and Bangladeshi) descent than in Europeans, and that in both of these groups plasma triglyceride levels were higher in men than in women. We also observed that the fall in plasma triglycerides in response to a glucose load was greater in women than in men: in Europeans the average change between fasting and 2 hours after loading was -6% in men and -16% in women, whereas in South Asians the average changes were -1% and -8%, respectively. Most triglycerides in plasma are carried in VLDL particles, which are produced in the liver and contain apoB. Elevation of VLDL triglyceride levels is closely related to an impairment in the ability of insulin to suppress nonesterified fatty acids (NEFAs), which are the principal substrate for hepatic triglyceride synthesis. We suggested that sex differences in the regulation of NEFA levels by insulin might thus account for the sex difference in the plasma triglyceride response to a glucose load. To test this possibility, we examined in a subsample of participants the relationships of plasma triglyceride and apoB concentrations to fasting and postload levels of NEFAs and insulin.

Methods
The methods of the main survey have been described elsewhere. A sample of 2936 men and 537 women aged 40 to 69 years was drawn from industrial work forces and family practitioners’ lists in London, UK. Participants were examined after an overnight fast. From those who were not already diagnosed as diabetic, venous blood samples were taken in the fasting state and 2 hours after a 75-g glucose load. NEFA levels were measured on stored specimens from a subsample of 699
South Asian and European subjects examined during the last year of the study, chosen to yield approximately equal numbers of men and women matched for age and date of examination. The EDTA plasma used for NEFA determinations was separated and frozen on dry ice immediately after taking the blood samples, stored at $-80^\circ\text{C}$, and transported to the Stanford laboratory on dry ice. These precautions are adequate to prevent hydrolysis of triglycerides to NEFAs even when no esterase inhibitor is used. ApoB was measured by immunoturbidimetry. Other laboratory methods have been described previously. The 67 subjects with impaired glucose tolerance or newly detected diabetes by World Health Organization criteria were excluded from the data reported here.

The distributions of body mass index, insulin, glucose, HDL-C, triglyceride, and NEFA measurements were positively skewed (skewness greater than 0.9). Before statistical analysis, these variables were transformed to functions of the form $\log(x+a)$ before analysis, choosing a constant ($a$) for each variable ($x$) so that the skewness of the transform was close to zero.

In Tables 1 through 4 and the Figure these variables have been returned to the original units. Tests of significance for product-moment correlation coefficients are based on the $t$ statistic. Least-squares regression analysis was used to examine the relationships of triglyceride and apoB to NEFA levels while adjusting for the effects of insulin and obesity. In these analyses age is expressed in 10-year units and all other variables have been standardized to unit standard deviation within each group: this means that the regression coefficient represents the predicted change in the dependent variable associated with an increase of one standard deviation in the independent variable. This allows the strengths of the associations to be compared directly. Tests of significance for the regression coefficients are based on the $t$ statistic.

**Results**

Mean levels of body mass index, waist-to-hip ratio, serum insulin, and plasma lipids in the 632 men and women with normal glucose tolerance are shown in Table 1. These levels are similar to those previously reported on the main sample of 3477 participants in which subjects with glucose intolerance were included. Fasting NEFA levels were higher and 2-hour NEFA levels lower in women than in men, so that the percent fall in NEFA in response to a glucose load was greater in women than in men. There were no ethnic differences in mean fasting or 2-hour NEFA levels, even though mean 2-hour insulin levels were twice as high in South Asians as in Europeans.

Correlations of fasting and 2-hour NEFA levels with insulin, glucose, triglyceride, apoB, and measures of obesity are shown in Table 2. Fasting NEFA levels were correlated with 2-hour glucose but not with fasting glucose. Triglyceride levels were correlated with both fasting and 2-hour NEFA levels, with the strongest correlations between 2-hour NEFA and 2-hour triglyceride levels. ApoB levels were correlated with fasting NEFA levels in South Asian women and with 2-hour NEFA levels in all four sex/ethnic groups.

In regression models with age, fasting NEFA, and 2-hour NEFA as independent variables, fasting triglycerides were significantly associated with 2-hour NEFA but not with fasting NEFA in each sex/ethnic group except European women (Table 3). When fasting and 2-hour insulin were included in these analyses, the association of triglycerides with 2-hour NEFA was significant in all four sex/ethnic groups (Table 3). The relationships of fasting triglycerides to 2-hour NEFA remained significant when fasting glucose, 2-hour glucose, and waist-to-hip ratio were included in the regression models. With 2-hour triglycerides instead of fasting triglycerides as the dependent variable, the relationships with insulin and 2-hour NEFA were slightly stronger.

To examine further the associations of triglycerides with 2-hour NEFA and 2-hour insulin, we defined tertiles of 2-hour insulin and tertiles of 2-hour NEFA for each sex/ethnic group so that stratifying by both variables divided the group into nine cells. In each sex/ethnic group, mean 2-hour triglyceride levels were approximately twice as high in those who were in the highest tertiles for both 2-hour NEFA and 2-hour insulin as in those who were in the lowest tertile for both of these variables (Figure). The relationships of fasting triglycerides to fasting insulin and 2-hour NEFA were similar, although the relationships to 2-hour NEFA were weaker in women than in men.

When the regression analyses were repeated with fasting apoB instead of triglycerides as the dependent variable, the relationships between apoB and 2-hour NEFA were similar to those described for triglycerides and 2-hour NEFA (Table 4). In contrast to the relationships of triglycerides to insulin, which remained significant even when NEFA was included in the models, apoB levels were not related to insulin (Table 4).

In Europeans there were no differences in mean fasting NEFA or 2-hour NEFA between the 53 premenopausal women and the 69 postmenopausal women not on estrogen therapy. In South Asians mean fasting NEFA was significantly higher in the 56 postmenopausal women than in the 72 premenopausal women ($P=.001$), but mean 2-hour NEFA did not differ significantly between postmenopausal women ($P=.97$) and premenopausal women ($P=.01$). In regression models with plasma triglycerides as the dependent variable, the relationship of triglycerides with 2-hour NEFA remained significant even when menopausal status, fasting insulin, 2-hour insulin, and fasting NEFA were simultaneously included as independent variables.

**Discussion**

This study demonstrates that suppression of NEFA levels by a glucose challenge is greater in women than in men, even though postload insulin levels were similar in women and men. This sex difference in NEFA response is of similar magnitude in Europeans and South Asians even though fasting and postload insulin levels differ markedly between these two ethnic groups. We have also demonstrated that plasma triglyceride and apoB levels are related to postload NEFA levels within each sex and ethnic group. The underlying associations with triglycerides and apoB may be stronger than those observed in this study, since a single measurement of NEFAs at 2 hours is only a crude measure of the NEFA response to a glucose load. In a clamp study, insulin-
mediated NEFA suppression accounted for 66% of the variation in plasma triglycerides in hypertglyceremic men and control subjects. The highest triglyceride levels in this study occurred in those individuals who had both high insulin and high postload NEFA levels. In contrast, apoB levels were correlated with NEFAs only, consistent with findings that plasma VLDL apoB turnover is correlated with plasma NEFA flux but not with plasma insulin.

Although impaired clearance by lipoprotein lipase could account for the association of elevated triglyceride levels with impaired insulin-mediated NEFA suppression, such a mechanism would not account for the correlation of postload NEFAs with apoB, which is not cleared by lipoprotein lipase. Experimental studies have demonstrated that elevated NEFA levels increase triglyceride synthesis and apoB secretion by hepatocytes in vitro. Triglyceride secretion increases in response to loading with either glucose or NEFAs, whereas apoB secretion increases only in response to loading with NEFAs.

On the basis of these findings we suggest that plasma concentrations of triglycerides and apoB in the population depend on a mechanism in which the combination of carbohydrate loading with impaired insulin-mediated suppression of NEFAs drives the synthesis of VLDL. At least some of the sex difference in plasma triglycerides and apoB levels may thus be attributable to the sex difference in NEFA suppression. The correlations between 2-hour glucose and fasting NEFA levels may result from the effect of elevated plasma NEFAs on hepatic glucose production.
The NEFA response to a glucose challenge is determined by the ability of insulin to inhibit lipolysis and stimulate re-esterification in fat cells. Insulin-stimulated glucose transport has been found to be higher in women's subcutaneous fat cells than in men's subcutaneous fat cells, and it is therefore plausible that there may be corresponding sex differences in re-esterification. Alternatively, the sex difference in NEFA suppression may result from a sex difference in suppression of lipolysis. Intracellular fat cells are more resistant to insulin-mediated suppression of lipolysis than fat cells from other depots, and the proportion of body fat stored intracellularly is higher in men than in women. Central obesity is associated with a slower decline of NEFAs in response to insulin infusion, at least in men. Such a mechanism would be consistent with the finding that sex differences in body fat pattern account statistically for the sex difference in plasma triglycerides.

Sex differences in other lipoproteins may be mediated through effects on VLDL triglycerides. For example, higher plasma VLDL triglyceride levels are associated with lower plasma HDL-C levels, and smaller, denser LDL particles, and increased postprandial accumulation of triglyceride-rich particles of intestinal origin. All of these effects may enhance the atherogenicity of circulating lipoproteins and increase coronary disease risk in men compared with women.

The differences in lipoprotein pattern between men and women (higher triglycerides, lower HDL-C, and smaller, denser LDL particles) are paralleled by the differences in lipoprotein pattern between people with non-insulin-dependent diabetes (NIDDM) and people with normal glucose tolerance. These differences between diabetic and nondiabetic individuals are greater in women than in men; in effect, diabetic women have a lipoprotein profile similar to diabetic men. NIDDM carries a higher relative risk for coronary heart disease in women than in men, so that in people with NIDDM the sex difference in coronary risk is attenuated or abolished. Our results suggest that women are

### Table 2. Product-Moment Coefficients of Correlation of Fasting and 2-Hour NEFAs With Metabolic and Anthropometric Measurements by Sex and Ethnicity

<table>
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<tr>
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<th>Fasting NEFAs</th>
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<tr>
<td>Fasting apoB</td>
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<tr>
<td>Waist/hip ratio</td>
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NEFAs, nonesterified fatty acids; TGs, triglycerides; apo, apolipoprotein.

*P<.05; †P<.01; ‡P<.001.

### Table 3. Regression of Fasting Plasma Triglycerides on Insulin and NEFAs by Sex and Ethnicity

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>European</th>
<th>South Asian</th>
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<tr>
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<td>Men</td>
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<td>2-H insulin</td>
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<tr>
<td>2-H NEFAs</td>
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<td>.19*</td>
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</table>

NEFAs, nonesterified fatty acids. Values are standardized regression coefficients for models containing all variables listed in the far left column.

*P<.05; †P<.01; ‡P<.001.

Mean 2-hour triglyceride (TG) levels by tertile of 2-hour insulin and 2-hour nonesterified fatty acids by sex and ethnicity. FFA, free fatty acids; S, South.
protected from dyslipidemia by the ability to suppress NEFA levels in response to insulin. We may therefore be in a position to explain the loss of this protection in NIDDM, in which both insulin-stimulated glucose uptake and insulin-mediated suppression of NEFAs are impaired. The loss of sex differences in coronary heart disease risk that accompanies NIDDM is consistent with the hypothesis that this protection from dyslipidemia underlies the sex difference in coronary risk in the general population.

Although the data reported here are derived from an epidemiological survey, we have cited experimental evidence for the mechanisms suggested. Confirmation will depend on the results of detailed metabolic studies. The effect of impaired insulin-mediated suppression of NEFAs on VLDL triglyceride synthesis may be a fundamental link in the relationships between insulin resistance, disturbances of lipid metabolism, and coronary heart disease.

Acknowledgments

This work was supported by the Medical Research Council, the British Heart Foundation, and the British Diabetic Association. We thank Jane Ferrie, Tracey Pierpoint, Edward Kearney, Martin Mattock, Andrea Collins, and Ben Varasteh for assistance.

References


Table 4. Regression of Fasting ApoB on Insulin and NEFAs by Sex and Ethnicity

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<td>2-H NEFAs</td>
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<tr>
<td>Age</td>
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<tr>
<td>2-H insulin</td>
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<td>-.02</td>
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<tr>
<td>Fasting NEFAs</td>
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<tr>
<td>2-H NEFAs</td>
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<td>.23†</td>
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<td>.22*</td>
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</table>

Apo. apolipoprotein; NEFAs, nonesterified fatty acids. Values are standardized regression coefficients for models containing all variables listed in the far left column.

*P<.05; †P<.01; ‡P<.001.


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doi: 10.1161/01.ATV.13.8.1187

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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