Relation of Fasting Plasma Insulin Concentration to High Density Lipoprotein Cholesterol and Triglyceride Concentrations in Men

Ami Laws, Abby C. King, William L. Haskell, and Gerald M. Reaven

Low plasma high density lipoprotein (HDL) cholesterol concentration is a risk factor for coronary heart disease (CHD) and is frequently associated with high triglyceride concentration. Both of these abnormalities have been related to insulin resistance as estimated by plasma insulin concentrations and to measures of obesity, regional adiposity, and physical fitness. To determine which of these variables (fasting plasma insulin, obesity as measured by body mass index [BMI], or regional adiposity as measured by waist to hip ratio [WHR]) best identifies men with low HDL cholesterol and high triglyceride concentrations, we divided 83 men, aged 50–65 years, who were free of CHD or diabetes, into tertiles based on BMI, WHR, or fasting plasma insulin concentration. Only for plasma insulin tertiles were there statistically significant differences in HDL cholesterol (tertile 1, mean±SEM, 134±0.08 mmol/l; 2, 1.16±0.05 mmol/l; 3, 1.10±0.06 mmol/l; p<0.03) and triglyceride (tertile 1, 1.05±0.08 mmol/l; 2, 1.48±0.12 mmol/l; 3, 1.82±0.17 mmol/l; p<0.005) concentrations. In forward stepwise regressions with HDL cholesterol and triglyceride as dependent variables, fasting insulin concentration but not BMI, WHR, or maximal oxygen uptake (VO₂max), a measure of physical fitness, predicted HDL cholesterol (R²=0.07, p<0.02) and triglyceride (R²=0.20, p<0.001) concentrations. The data suggest that plasma insulin concentration is an important predictor of HDL cholesterol and triglyceride concentrations independent of BMI, WHR, or VO₂max. (Arteriosclerosis and Thrombosis 1991;11:1636–1642)

Low plasma high density lipoprotein (HDL) cholesterol concentration has been shown in prospective studies to be a risk factor for coronary heart disease (CHD) in men and women, independent of total cholesterol and other risk factors.1-7 Although the role of hypertriglyceridemia as an independent cardiovascular risk factor remains controversial, a majority of studies examining the relation of plasma triglyceride concentration to CHD have found a positive relation in univariate analysis.8 In addition, a high plasma triglyceride concentration may have increased importance as a CHD risk factor in certain subgroups, namely those with normal or low total cholesterol,9 or those with impaired glucose tolerance or diabetes.10 Plasma HDL cholesterol and triglyceride concentrations are strongly negatively correlated,8 and data from Framingham suggest that those with low HDL cholesterol and high triglycerides are at particularly increased risk for CHD.11 The basis for the association of high plasma triglyceride with low HDL cholesterol concentrations has not been well defined. A previous study has shown that both a low HDL cholesterol and a high triglyceride concentration are associated with hyperinsulinemia,12 and it has been suggested that all three of these abnormalities are secondary to a defect in the ability of insulin to stimulate glucose uptake.13 On the other hand, there is evidence that plasma triglyceride and HDL cholesterol concentrations are modulated by a number of other factors: variations in general degree of obesity, regional fat distribution, and level of physical activity.14,15 Because insulin-stimulated glucose uptake and plasma insulin concentrations are also associated with these latter three variables,16-18 it could be argued that the relation between plasma insulin concentration...
and plasma triglyceride and HDL cholesterol concentrations is explained by obesity, regional fat distribution, and/or physical activity. This study was undertaken to attempt to determine whether fasting plasma insulin or measures of obesity, fat distribution, or physical activity best predict individuals with low HDL cholesterol and high triglyceride concentrations. Our hypothesis was that plasma insulin concentration would be associated with HDL cholesterol and triglyceride concentrations independently of these other factors.

Methods

Subjects

Subjects were recruited for participation in a physical activity trial through a random-digit dial telephone survey of the community of Sunnyvale, Calif., and by city-wide promotion. Eligibility criteria for participation included age between 50 and 65 years; absence of CHD, peripheral vascular disease, or stroke (determined by medical history, physical examination, and resting and exercise electrocardigrams); absence of musculoskeletal problems that would prevent participation in moderate levels of physical activity; not currently engaged in a regular exercise program; not currently taking medication for the treatment of hypertension or hyperlipidemia; cholesterol level below 300 mg/dl; triglyceride level below 500 mg/dl; and blood pressure below 160/95. One hundred sixty men were recruited for participation in the exercise study and were randomly assigned to one of four treatment groups. Subjects in two of these groups (n=83) underwent oral glucose tolerance tests (see below) and are included in this analysis. No subject had fasting plasma glucose greater than 140 mg/dl. Two subjects were excluded who had 2-hour values in the diabetic range per World Health Organization criteria. More than 90% of the subjects were Caucasian and of middle socioeconomic status; most held professional jobs. In summary, subjects included in this report were relatively healthy, generally sedentary older men without clinical or laboratory evidence of CHD or diabetes.

Measurements

Subjects reported to the Stanford Outpatient Clinical Research facility in the morning after an overnight fast. Examinations took place on two mornings at least 72 hours apart. At the first visit all subjects underwent a medical history and physical examination. Body weight (in kilograms) and height (in centimeters) were determined with the participant in a hospital gown and shoes. Body mass index (BMI), weight in kilograms divided by height in meters squared, was calculated. Waist girth was measured in centimeters at the level of the umbilicus and hip girth at the widest circumference of the buttocks, with the patient standing. Waist to hip ratio (WHR) was determined each minute by a semiautomated metabolic analysis system, as described previously. Maximal oxygen uptake (VO_{2max}), a measure of physical fitness, was defined as the highest value determined during the last 2 minutes of exercise.

On the second visit, 72 or more hours later, subjects had fasting (>12 hours) blood drawn for measurement of lipoproteins, glucose, and insulin. Subjects were then given a 75-g oral glucose load, and blood was drawn 2 hours later for measurement of glucose and insulin. Venous blood samples were drawn into Vacutainer tubes containing 1.5 mg/ml Na_{2}EDTA while the subject was seated. Plasma was prepared from blood within 2 hours, and blood and plasma were stored at 4°C. Plasma total cholesterol and triglyceride concentrations were determined by enzymatic procedures with an Abbott ABA 200 instrument (Abbott Laboratories, North Chicago, Ill.). Plasma HDL and HDL subfractions 2 and 3 (HDL_{2} and HDL_{3} cholesterol) were determined by a dextran sulfate-magnesium precipitation procedure. Glucose was determined by the glucose oxidase method and insulin by double-antibody radioimmunoassay.

Smoking and alcohol use information were obtained with a survey adapted from the National Health Interview Survey.

The study was approved by the Stanford Human Subjects Committee, and each subject gave written informed consent at the time of entry into the study.

Data Analysis

Spearman correlation coefficients were used to determine simple correlations among the clinical and metabolic variables. Analysis of variance, performed by the general linear models procedure of the Statistical Analysis System (Carey, N.C.), was used to determine differences among groups divided into tertiles of BMI, WHR, and fasting plasma insulin. A probability value less than 0.05 was considered significant. A series of forward stepwise multiple regression analyses were calculated to determine relations among fasting insulin, HDL cholesterol, VO_{2max}, and measures of body weight and body composition. In the forward stepwise regression analysis, only variables that significantly (p<0.05) contributed to the R^2 were considered independent determinants of each dependent variable.
Results

Clinical and metabolic characteristics of the 81 men are listed in Table 1. There was a broad range of BMI, WHR, and %BF in the study population. Mean systolic and diastolic blood pressures were relatively low for this age group,\(^2\&\) and the mean VO\(_{2}\text{max}\) was consistent with that of a relatively sedentary population. Total and low density lipoprotein cholesterol and triglyceride concentrations were slightly above the mean for white American males of this age by use of the Lipid Research Clinics value as a reference, whereas HDL cholesterol level was approximately at the mean.\(^2\&\) Glucose levels were clearly in the non-diabetic range.

Spearman correlation coefficients between plasma HDL cholesterol and triglyceride concentrations and other variables are shown in Table 2. These data show that statistically significant (\(p<0.05\)) negative correlations were seen between HDL cholesterol and BMI (\(r=-0.23, p<0.05\)), WHR (\(r=-0.24, p<0.05\)), fasting glucose (\(r=-0.25, p<0.05\)), and fasting insulin (\(r=-0.28, p<0.01\)). Significant direct correlations were present between plasma triglyceride concentration and many of the same variables: BMI (\(r=0.25, p<0.05\)), fasting glucose (\(r=0.48, p<0.001\)), 2-hour glucose (\(r=0.34, p<0.01\)), fasting insulin (\(r=0.40, p<0.001\)), and 2-hour insulin (\(r=0.24, p<0.05\)). HDL cholesterol was negatively correlated with triglyceride concentration (\(r=-0.51, p<0.001\)). Of note, the correlation of HDL cholesterol with fasting insulin was accounted for by the HDL\(_2\) subfraction (\(r=-0.29, p<0.01\)); there was no correlation between HDL\(_3\) cholesterol and fasting insulin (\(r=-0.07, p=\text{NS}\)). Age (not shown) did not correlate significantly with any of the variables, most likely due to the narrow age range of the participants. It is important to note that BMI, %BF, and WHR were all highly correlated with each other, that is, BMI was correlated with WHR (\(r=0.57, p<0.01\)) and with %BF (\(r=0.75, p<0.01\)). Additionally, the measures of obesity and fat distribution were highly negatively correlated (\(p<0.01\)) with VO\(_{2}\text{max}\) (BMI, \(r=-0.70\); WHR, \(r=-0.65\); and %BF, \(r=-0.42\)).

To determine if measures of obesity, regional fat distribution, or plasma insulin concentration best identified subjects with low HDL cholesterol and high triglyceride levels, subjects were divided into groups on the basis of these variables. Table 3 displays results where subjects were divided into tertiles on the basis of BMI. As expected, the higher the BMI, the greater the WHR and %BF. In addition, the higher the BMI, the lower the VO\(_{2}\text{max}\). The only other variable that significantly increased with the degree of general obesity, as estimated by BMI, was fasting insulin concentration. However, the expected gradient of decreasing HDL cholesterol and increasing triglyceride concentrations
with increasing BMI was seen, although it was not statistically significant.

Table 4 shows similar data for subjects who were separated into tertiles based on WHR. As before, the greater the degree of abdominal obesity (as estimated by WHR), the higher the BMI and %BF and the lower the \( V_{\text{O}_2}\text{max} \). Again, the only other variable that significantly increased with increasing abdominal obesity was fasting insulin concentration. As with BMI, HDL cholesterol concentration decreased and triglyceride concentration increased with increasing WHR, but this was not statistically significant.

In contrast to the results in Tables 3 and 4 are the data shown in Table 5. It is apparent that when patients were divided into tertiles based on fasting plasma insulin concentration, significant relations were seen between insulin level and both plasma triglyceride and HDL cholesterol concentrations. The difference in HDL cholesterol by insulin tertile was due to differences in the HDL 2 subfraction (tertile 1, 0.36 mmol/l; tertile 2, 0.23 mmol/l; and tertile 3, 0.18 mmol/l; \( p<0.02 \)), while there were no differences between tertiles in HDL 1 cholesterol. As would be expected, fasting glucose and 2-hour insulin values were also higher in those with the highest fasting insulin concentration. BMI, WHR, and \( V_{\text{O}_2}\text{max} \) also varied as a function of fasting plasma insulin concentration. Of note, there were no significant differences among the groups in alcohol consumption per week or in percent smokers (data not shown), which might explain the differences in HDL cholesterol and triglycerides.

To determine the relative contributions of fasting insulin, obesity, and physical fitness to HDL cholesterol concentrations, we entered fasting insulin, BMI, and \( V_{\text{O}_2}\text{max} \) into a forward stepwise multiple regression program, with HDL cholesterol as the dependent variable. The program selected fasting insulin and no other variables (\( R^2=0.07, p<0.02 \)). We ran this program again, with WHR replacing BMI, and obtained the same results. We repeated the analysis with fasting plasma triglyceride as the dependent

### Table 3. Clinical and Metabolic Variables by Tertile of Body Mass Index

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertile 1 (kg/m²)</th>
<th>Tertile 2 (25.6–28.5)</th>
<th>Tertile 3 (≥28.5)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4</td>
<td>27.1</td>
<td>32.0</td>
<td>0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.948</td>
<td>0.977</td>
<td>1.008</td>
<td>0.001</td>
</tr>
<tr>
<td>%BF</td>
<td>19</td>
<td>24</td>
<td>31</td>
<td>0.001</td>
</tr>
<tr>
<td>( V_{\text{O}_2}\text{max} ) (ml/kg/min)</td>
<td>34</td>
<td>30</td>
<td>26</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.59</td>
<td>6.05</td>
<td>5.56</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.29</td>
<td>1.22</td>
<td>1.11</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.22</td>
<td>1.5</td>
<td>1.65</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.2</td>
<td>5.4</td>
<td>5.6</td>
<td>NS</td>
</tr>
<tr>
<td>2-Hour glucose (mmol/l)</td>
<td>5.2</td>
<td>5.6</td>
<td>5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>93</td>
<td>122</td>
<td>144</td>
<td>0.001</td>
</tr>
<tr>
<td>2-Hour insulin (pmol/l)</td>
<td>481</td>
<td>610</td>
<td>703</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI, body mass index; WHR, waist to hip ratio; %BF, percent body fat; \( V_{\text{O}_2}\text{max} \), maximal oxygen uptake; HDL, high density lipoprotein.

### Table 4. Clinical and Metabolic Variables by Tertile of Waist to Hip Ratio

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertile 1 (WHR)</th>
<th>Tertile 2 (0.963–0.998)</th>
<th>Tertile 3 (≥0.998)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2</td>
<td>26.7</td>
<td>30.7</td>
<td>0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.924</td>
<td>0.984</td>
<td>1.026</td>
<td>0.001</td>
</tr>
<tr>
<td>%BF</td>
<td>21</td>
<td>24</td>
<td>30</td>
<td>0.001</td>
</tr>
<tr>
<td>( V_{\text{O}_2}\text{max} ) (ml/kg/min)</td>
<td>32</td>
<td>31</td>
<td>28</td>
<td>0.002</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.59</td>
<td>6.08</td>
<td>5.53</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.29</td>
<td>1.19</td>
<td>1.11</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.25</td>
<td>1.5</td>
<td>1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.3</td>
<td>5.4</td>
<td>5.4</td>
<td>NS</td>
</tr>
<tr>
<td>2-Hour glucose (mmol/l)</td>
<td>5.2</td>
<td>5.7</td>
<td>5.7</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>100</td>
<td>108</td>
<td>144</td>
<td>0.004</td>
</tr>
<tr>
<td>2-Hour insulin (pmol/l)</td>
<td>509</td>
<td>552</td>
<td>725</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI, body mass index; WHR, waist to hip ratio; %BF, percent body fat; \( V_{\text{O}_2}\text{max} \), maximal oxygen uptake; HDL, high density lipoprotein.
variable with the same sets of regressions. In each case the program entered fasting insulin and no further variables ($R^2=0.20$, $p<0.001$).

Because fasting plasma glucose was also shown to be correlated with plasma HDL cholesterol concentration (see Table 2), we determined the relative contributions of fasting glucose, obesity, and physical fitness to HDL cholesterol concentration. Thus, we entered fasting glucose, BMI or WHR, and $V_{O_2}\text{max}$ into forward stepwise multiple regression programs, with HDL cholesterol as the dependent variable. In both cases fasting plasma glucose was the only variable that approached statistical significance, and this was borderline ($R^2=0.05$, $p<0.06$).

### Discussion

We determined metabolic and behavioral covariates of plasma HDL cholesterol and triglyceride concentrations in a group of 50–65-year-old sedentary men free of CHD or diabetes and found statistically significant correlations between fasting plasma insulin and both HDL cholesterol and triglyceride concentrations that were not explained by obesity, abdominal obesity, or physical fitness level. When subjects were divided into tertiles on the basis of BMI, WHR, or insulin, fasting insulin best delineated those with low HDL cholesterol and high triglyceride concentrations. Division of subjects into tertiles of BMI or WHR did show the expected gradients of decreasing HDL cholesterol and increasing triglyceride concentrations, but they were not statistically significant. The lesser strength of these associations may be a true effect, or it may be due to the small sample size or the variability of these measurements. However, in forward stepwise multivariate regressions, only plasma insulin was significantly related to plasma HDL cholesterol and triglyceride levels. No additional amount of the variance in either was explained by the addition of BMI, WHR, or $V_{O_2}\text{max}$ to the model. This suggests that insulin may have a more central role than BMI or WHR in the determination of HDL cholesterol and triglyceride concentrations; however, assessing the relative strengths of these associations is limited by intrapersonal variability and laboratory repeatability of the measurements.

The cross-sectional nature of this study precludes causal inferences; however, the results are consistent with an important role for plasma insulin in the regulation of HDL cholesterol and triglyceride concentration. It is most likely that plasma insulin concentration is only serving as a marker for insulin resistance. Plasma insulin levels, both fasting and in response to an oral glucose challenge, have been shown in normals to correlate negatively with insulin-stimulated glucose uptake, a measure of insulin resistance; that is, the higher the insulin levels, the greater the resistance. Therefore, the associations of plasma insulin with plasma HDL cholesterol and triglyceride concentrations are most likely due to variations in the degree of insulin resistance. Of note, plasma insulin concentration was more strongly related to triglyceride than to HDL cholesterol concentration. It has been shown that hepatic very low density lipoprotein triglyceride secretion rate and plasma triglyceride concentration are significantly correlated with insulin resistance and hyperinsulinemia and that very low density lipoprotein catabolism plays a major role in the rate at which HDL particles are formed in vivo. This suggests that the relationship between insulin and triglycerides may be the more direct one.

On a broader level, the results of this and other studies showing an independent relation between fasting plasma insulin and HDL cholesterol and triglyceride concentrations add some perspective to the assessment of CHD risk factors in epidemiological studies. Three of the metabolic variables examined in this study, that is, plasma insulin, HDL cholesterol, and triglyceride concentration, have been identified as risk factors for CHD. As is apparent from this and other studies, however, significant correlations exist among these variables. Despite this, most published studies of CHD risk factors do not take these interactions into consideration.
For example, prospective epidemiological studies have shown that plasma insulin levels are an independent risk factor for CHD in nondiabetic subjects.43-45 However, in these studies HDL cholesterol concentrations were not measured. It seems likely that the CHD risk attributed to increased plasma insulin is related, at least in part, to associated decreases in HDL cholesterol concentration.

Similarly, a number of prospective studies have shown low HDL cholesterol to be an independent risk factor for CHD,1-7 but in these studies plasma insulin was not measured. Because plasma insulin has been shown to correlate negatively with HDL cholesterol and positively with triglyceride concentrations in our study as in others,32-36 it would be of interest to know whether plasma insulin levels are elevated in most subjects with low HDL cholesterol, particularly those with high triglycerides.

One hypothesis linking insulin, triglyceride, and HDL cholesterol as risk factors for CHD is that these abnormalities exist as a cluster, with the underlying defect being insulin resistance. This clustering has been demonstrated to occur in at least one population with a high prevalence of CHD despite levels of total cholesterol that are not elevated. Specifically, Asian Indians in London have been shown to have higher plasma insulin and triglyceride concentrations and lower HDL cholesterol concentrations than do British men and women,40 and this former population has been shown to have a CHD risk that is about 50% higher than that of the British population.41

Finally, it is notable that two recent reviews of the relation of HDL cholesterol to atherosclerosis make no mention of diabetes or hyperinsulinemia in the lists of factors contributing to low serum HDL cholesterol.42,43 Given the demonstrated relations of the states of insulin resistance, namely non-insulin-dependent diabetes mellitus, abnormal glucose tolerance, and hyperinsulinemia to low HDL cholesterol and elevated triglyceride concentrations, it would seem fruitful for future studies investigating the contributions of these risk factors to CHD to examine their possible clustering rather than their independence.

References


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KEY WORDS • plasma insulin • high density lipoprotein cholesterol • triglycerides • coronary heart disease risk
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