Influence of Insulin Resistance, Secretion, and Clearance on Serum Cholesterol, Triglycerides, Lipoprotein Cholesterol, and Blood Pressure in Healthy Men

I.F. Godsland, D. Crook, C. Walton, V. Wynn, and M.F. Oliver

Relations between serum lipids, lipoproteins, blood pressure, and insulin metabolism were investigated in 158 healthy men aged 19–77 years and with body mass indexes (BMIs) of 19–41 kg · m⁻². Mathematical modeling analysis of glucose, insulin, and C-peptide concentrations during an intravenous glucose tolerance test was used to measure parameters of insulin metabolism. In univariate analysis, both fasting and postglucose insulin concentrations showed significant positive associations with fasting serum triglyceride levels ($r = 0.33$ and 0.38, respectively) and systolic ($r = 0.22$ and 0.26) and diastolic ($r = 0.21$ and 0.24) blood pressure and negative associations with high density lipoprotein subfraction 2 cholesterol (HDL₂; $r = -0.21$ and $-0.25$). In multivariate analysis, the associations between insulin and HDL₂ cholesterol concentrations were found to depend on triglyceride levels. Insulin resistance and basal pancreatic insulin secretion showed significant positive associations with serum triglycerides, which were independent of the effects of age, BMI, and fat distribution. Hepatic insulin throughput was independently associated with HDL₂ cholesterol. Associations of insulin-related variables with blood pressure were generally dependent on age and BMI. These results underline the importance of insulin sensitivity and insulin concentrations as determinants of triglyceride metabolism. They also indicate a close relation between hepatic insulin handling and HDL₂ concentration that is independent of triglyceride metabolism.


KEY WORDS • insulin resistance • serum lipids • lipoproteins • mathematical models • insulin metabolism

The observation that hyperinsulinemia is an independent predictor of coronary heart disease¹⁻³ has lent support to the possibility that elevated insulin concentrations may have a direct role in atherogenesis.⁴ Furthermore, elevated insulin concentrations are associated with raised blood pressure⁵ and serum triglyceride concentrations⁶ and with reduced serum high density lipoprotein (HDL) cholesterol concentrations,⁷ factors that in themselves may increase coronary heart disease risk.

Resistance of glucose elimination to insulin action (insulin resistance) is a major determinant of the plasma insulin concentration⁸ and may have a stronger relation with cardiovascular disease risk factors than does plasma insulin concentration.⁹⁻¹¹ Determinants of insulin concentrations, such as insulin resistance, may therefore be more directly involved in the relations between insulin and cardiovascular disease risk than are the insulin concentrations themselves.

Computer modeling of plasma glucose, insulin, and C-peptide concentrations during an intravenous glucose tolerance test (IVGTT) allows quantification of the determinants of plasma insulin concentrations, which may be otherwise difficult to measure. We have used this technique in a large group of healthy men to investigate the associations of serum lipid and lipoprotein concentrations and blood pressure with insulin-related variables. A wide range of variation in age and adiposity in this group provided the necessary variation in insulin metabolism against which these associations could be examined.

Methods

Subjects

We studied 158 apparently healthy white men aged 19–77 years and with body mass indexes (BMIs) from 18.9 to 41.1 kg · m⁻² (83–192% of ideal body weight¹²). Of the 158 subjects, seven had systolic and diastolic blood pressures ≥150 and ≥90 mm Hg, respectively, although none were known to be hypertensive. No participants were taking drugs that were likely to affect carbohydrate or lipid metabolism. The group formed part of an executive health screening program. Approval from the Wynn Institute ethics committee was obtained for the study, and participants gave written, informed consent. Relations between insulin metabo-
lism and fat distribution in individuals selected from this group have been reported elsewhere.13

**Procedures**

Subjects were instructed to consume >200 g carbohydrate/day in their diet for 3 days in preparation for an IVGTT and to have fasted overnight (>12 hours). Height and weight were measured on arrival at the institute day ward between 9 and 10 AM, and a general medical history was taken by a clinician. Subscapular and triceps skinfold thicknesses were measured as the mean of three readings at each site. Centrality of body fat distribution was expressed as the ratio of subscapular to triceps skinfold thickness.14

After the subjects rested for 15 minutes in a semirecumbent position, systolic and diastolic blood pressures were measured by a cuff method with a mercury sphygmomanometer. First- and fifth-phase Korotkoff sounds were recorded. An indwelling cannula was inserted into an antecubital vein in each arm, without prolonged venous stasis. Blood for measurement of serum lipoproteins was taken, and serum was separated by low-speed centrifugation. Two successive blood samples (10 minutes apart) were drawn into tubes containing lithium-heparin for measurement of fasting plasma glucose, insulin, and C-peptide levels. An intravenous glucose injection (0.5 g glucose/kg body wt as a 50% [wt/vol] solution of dextrose given over 3 minutes) was then given via the cannula in the opposite arm to the sampling arm. Additional samples were taken at 3, 5, 7, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 minutes.

**Laboratory Determinations**

Plasma glucose level was measured by a glucose oxidase procedure.15 Plasma insulin and C-peptide concentrations were measured (on samples stored at −20°C) by using the radioimmunoassay procedure of Albano et al16 and the radioimmunoassay kit supplied by Guildhay Ltd., Surrey, UK, respectively. Serum total cholesterol and triglyceride concentrations were measured by fully enzymatic procedures.17,18 Concentrations of HDL and HDL subtraction 3 (HDL3) cholesterol were measured after sequential precipitation with heparin/manganese ions and dextran sulfate, respectively.19 HDL subtraction 2 (HDL2) cholesterol was calculated as the difference between HDL and HDL3 cholesterol. The Friedewald equation (Friedewald et al20) was used to calculate low density lipoprotein cholesterol. Quality control was monitored by use of commercially available lyophilized sera and by participation in national standardization schemes. Overall within- and between-batch coefficients of variation were 1–2% (serum total cholesterol and serum triglycerides), 2–4% (HDL cholesterol), 5–7% (HDL2 cholesterol), 6–9% (HDL3 cholesterol), 1–2% (plasma glucose), 4–6% (plasma insulin), and 7–9% (plasma C-peptide).

**Modeling Analyses**

Modeling analyses were carried out with programs written in FORTRAN 77 and run on a PDP 11/33 minicomputer. Glucose elimination was analyzed by using the minimal model of glucose disappearance of Bergman and coworkers.22 The equations of this model provide measures of the sensitivity of glucose elimination to insulin (S, inversely proportional to insulin resistance) and glucose-dependent glucose elimination (Sg). Estimates of insulin sensitivity and glucose-dependent glucose disposal from this model have been validated against established experimental procedures.23–25

The plasma insulin concentration profile during the IVGTT reflects the distinct first and second phases of pancreatic insulin secretion, and these concentration changes were described according to the minimal model of posthepatic insulin delivery of Toffolo et al.26 This model provides measures of the sensitivity of net first-phase posthepatic insulin delivery to glucose (ϕ1, first-phase insulin responsiveness), the sensitivity of second-phase posthepatic insulin delivery to glucose (ϕ2, second-phase insulin responsiveness), and the insulin elimination constant (n = ln 2/insulin half-life). This model applies only to posthepatic insulin delivery because about 50% of the insulin secreted by the pancreas is taken up by the liver before entering the general circulation.27

True pancreatic insulin secretion was analyzed by the model of Vølund et al28 by using the model identification system of Watanabe et al.29 The model uses insulin and C-peptide concentrations during the IVGTT. The model equations provide measures of the fractional hepatic throughput of insulin (f, inversely related to hepatic insulin uptake), the insulin-elimination constant (κs), net basal insulin secretion, and net incremental first- and second-phase pancreatic insulin secretion during the IVGTT. Measures of insulin secretion derived from this model have been validated in animal studies.29,30

For a modeling analysis to be acceptable, parameter estimates were required to have positive fractional standard deviations <100%. Only model identifications that resulted in parameters that lay within ±2 SDs from the mean were accepted. A further exclusion criterion, applied to analyses using the pancreatic insulin secretion model, was a ratio >1 between insulin and C-peptide concentrations during the first 10 minutes of the IVGTT together with a value >2 for the fractional hepatic insulin throughput index. This requirement related to a minority of tests in which there was overlap between insulin and C-peptide concentrations during the first 10 minutes of the IVGTT,30 which apparently led to physiologically unrealistic solutions for the pancreatic insulin secretion model. Numbers of cases remaining after application of exclusion criteria are given in Table 1.

**Data Analyses**

Total areas under the plasma glucose, insulin, and C-peptide profiles were calculated by the trapezoidal rule. In the present study, we have used the incremental area (i.e., total area −180×mean fasting concentration), as this provides a measure of IVGTT response unfounded by changes in the fasting level. First- and second-phase incremental insulin and C-peptide areas were calculated by partitioning the profile at the 10-minute time point.

Variables were transformed as necessary (logarithmic or square root) to normalize their distributions. Statistical analyses were carried out by using BMDP statistical software (BMDP, Los Angeles, Calif.). Univariate correlations of insulin concentrations and model-derived insulin concentration determinants with lipids, lipoproteins, and blood pressure were derived. In view of the number of comparisons made, univariate correlation
TABLE 1. Age, BMI, Blood Pressures, Concentration Measures, and Parameters of Insulin Metabolism

<table>
<thead>
<tr>
<th>Demographic data (n=158)</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.1±10.8</td>
<td></td>
</tr>
<tr>
<td>BMI (kg · m⁻²)</td>
<td>25.3±3.1</td>
<td></td>
</tr>
<tr>
<td>Subscapular/triceps skinfold ratio</td>
<td>1.65±0.57</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>123.2±17.1</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>78.0±10.1</td>
<td></td>
</tr>
</tbody>
</table>

Fasting concentration measures (n=158)

| Triglycerides (mmol · l⁻¹) | 0.98±0.39, +0.65 |
| Total cholesterol (mmol · l⁻¹) | 5.39±0.90 |
| LDL cholesterol (mmol · l⁻¹) | 3.54±0.80 |
| HDL cholesterol (mmol · l⁻¹) | 1.31±0.26, +0.32 |
| HDL₂ cholesterol (mmol · l⁻¹) | 0.33±0.15, +0.27 |
| Glucose (mmol · l⁻¹) | 0.96±0.15 |
| Insulin (pmol · ml⁻¹) | 0.037±0.022, +0.051 |
| C-peptide (pmol · ml⁻¹) | 0.157±0.059, +0.093 |

IVGTT concentration measures (n=158)

| Incremental glucose area (mmol · l⁻¹ · min) | 493±170 |
| Phase 1 incremental insulin area (pmol · ml⁻¹ · min) | 2.94±1.38, +2.59 |
| Phase 2 incremental insulin area (pmol · ml⁻¹ · min) | 15.0±7.2, +13.8 |
| Phase 1 incremental C-peptide area (pmol · ml⁻¹ · min) | 1.72±0.97, +1.36 |
| Phase 2 incremental C-peptide area (pmol · ml⁻¹ · min) | 28.7±11.2, +13.9 |

Minimal model of glucose disappearance (n=149)

| Insulin sensitivity index, S₁ (min⁻¹ · μU · ml⁻¹) | 2.93±1.75, +2.54 |
| Glucose-dependent glucose disposal constant, S₂ (min⁻¹) | 1.52±0.58, +0.93 |

Posthepatic insulin delivery model (n=140)

| Net phase 1 plasma insulin responsiveness, φ₁ (μU · ml⁻¹ · min⁻¹ · mg⁻¹ · dl⁻¹) | 3.72±1.65, +2.98 |
| Phase 2 plasma insulin responsiveness, φ₂ (μU · ml⁻¹ · min⁻² · mg⁻¹ · dl⁻¹) | 10.3±5.4, +11.2 |
| Insulin elimination constant, n (min⁻¹) | 0.141±0.055, +0.069 |

Pancreatic insulin secretion model (n=141)

| Basal pancreatic insulin secretion (pmol · ml⁻¹) | 1.38±0.88, +1.18 |
| Phase 1 incremental pancreatic insulin secretion (pmol · ml⁻¹) | 0.83±0.45 |
| Phase 2 incremental pancreatic insulin secretion (pmol · ml⁻¹) | 3.31±1.18, +1.44 |
| Insulin elimination constant, k (min⁻¹) | 0.093±0.041, +0.072 |
| Hepatic insulin throughput index, f | 0.73±0.27, +0.42 |

BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; IVGTT, intravenous glucose tolerance test. Values are mean ± SD, ±SD (asymmetric SDs derived by back-transformation).

- Logarithmically transformed data.
- Square-root-transformed data.

significance values between 0.01 and 0.05 were considered as borderline. Multiple linear regression was used to explore whether the associations found depended on age, BMI, fat distribution, or triglyceride concentrations. An upper limit of p < 0.05 was accepted as significant in multivariate analyses.

Results

The mean age of men in the study group was 50.1±10.8 years and the mean BMI 25.3±3.1 kg · m⁻². Mean values and standard deviations for all variables considered in the study are given in Table 1.

There were no significant or borderline-significant associations between insulin-related variables and HDL₃ cholesterol. Neither were there any associations between lipids, lipoproteins, and blood pressure and mean fasting glucose, IVGTT incremental glucose area, glucose-dependent glucose disposal (S₁), first-phase posthepatic insulin responsiveness to glucose (φ₁), and the pancreatic insulin-secretion model insulin-elimination constant (k). Univariate correlation coefficients between insulin and insulin-related variables showing significant or borderline-significant associations with lipids, lipoproteins, and blood pressure are shown in Table 2. Basal insulin and IVGTT insulin concentrations correlated negatively with HDL₂ cholesterol and positively with fasting serum triglyceride levels and systolic blood pressure. In addition, IVGTT insulin concentrations correlated positively with total serum cholesterol level and diastolic blood pressure. Basal C-peptide concentrations correlated positively with triglyceride concentrations.

Insulin sensitivity correlated negatively with fasting serum triglyceride concentrations. Basal and second-
phase pancreatic insulin secretion correlated positively with triglyceride concentrations. Hepatic insulin throughput correlated negatively with HDL subfraction 2 cholesterol concentrations (Figure 1).

Standardized linear regression coefficients from analyses including the predictors of age, BMI, and subscapular-to-triceps skinfold thickness ratio for those variables showing significant associations in univariate analysis are shown in Table 3. Relations of triglyceride levels with fasting insulin and C-peptide concentrations, second-phase insulin incremental area, insulin sensitivity index ($S_I$), and basal pancreatic insulin secretion ($S_B$) were independent of age, BMI, and fat distribution. The relation between HDL subfraction 2 cholesterol and hepatic insulin throughput index ($f$) was also independent. Relations between HDL subfraction 2 cholesterol and other insulin-related variables were generally dependent on age (results not shown). Relations between blood pressure and insulin-related variables were generally dependent on age and BMI (results not shown), although the association of systolic blood pressure with fasting plasma insulin concentration and hepatic throughput index was still apparent in multivariate analysis.

HDL subfraction 2 cholesterol showed a strong inverse relation ($r=-0.58$, $p<0.001$) with triglyceride concentrations. When triglyceride concentration was added to the list of insulin-related variables predicting HDL subfraction 2 cholesterol concentration, only one significant association, HDL subfraction 2 cholesterol and fractional hepatic insulin throughput index ($f$), remained significant ($r=-0.17$, $p=0.02$).

**Discussion**

Reduced sensitivity of glucose elimination to insulin (insulin resistance) has recently received attention as a...
potential central factor in a number of disturbances associated with coronary heart disease. These include elevated triglyceride concentrations and blood pressure and reduced HDL2 cholesterol concentrations. However, studies of the relations between insulin resistance and these risk factors have generally involved obese, diabetic, or hypertensive individuals. Furthermore, relations between risk factors and determinants of variation in insulin concentrations other than insulin resistance do not appear to have been explored.

In the present study of a large group of healthy individuals with a wide range of age and BMI, we found strong relations between fasting serum triglyceride concentrations and insulin and C-peptide concentrations, insulin sensitivity, and pancreatic insulin secretion. These relations were generally independent of age, BMI, and fat distribution, indicating that the variation in age and adiposity in the group we studied simply provided a source of variation in insulin sensitivity and secretion against which independent links between insulin and lipoprotein metabolism could be explored. Previous studies have suggested that the relation between triglyceride concentration and insulin sensitivity was stronger than the relation between triglyceride and insulin concentrations. Although this was not necessarily the case in normal healthy individuals, the strongest relations we found were between triglyceride and basal and glucose-stimulated insulin concentrations. These findings would support a causal chain whereby variation in plasma insulin concentrations resulting from variation in insulin sensitivity and secretion is responsible for the insulin-related variation in triglyceride concentrations.

Hepatic synthesis of triglycerides can result from elevated insulin concentrations, although there is evidence from studies of isolated hepatocytes that favors an inhibitory effect of insulin on triglyceride release. A further factor that could contribute to the association between increased triglyceride concentrations and insulin resistance could be resistance of adipose tissue lipolysis to inhibition by insulin. This and the resistance of glucose elimination to stimulation by insulin may be linked conditions. Thus, poorly suppressed lipolysis resulting in increased triglyceride synthesis could be associated with elevated insulin concentrations resulting from insulin resistance.

Insulin can stimulate cholesterol synthesis, and this process could underlie the positive association we found between total cholesterol and IVGTT insulin and C-peptide concentrations. HDL2 cholesterol concentrations also showed associations with insulin and C-peptide concentrations, but the only significant relation with insulin concentration determinants was with the hepatic insulin throughput index. These associations between HDL2 cholesterol and insulin-related variables could have been secondary to the widely recognized inverse association between HDL2 and triglyceride concentrations. In multivariate analyses after inclusion of triglycerides in the regression equations, the associations between HDL2 cholesterol and insulin and C-peptide concentrations were no longer significant. Nevertheless, the association with hepatic insulin throughput index was still apparent, and this association was independent of age, BMI, and fat distribution. These observations suggest the novel possibility that hepatic insulin metabolism and HDL2 metabolism could be closely associated. Possible links between the two could be an effect of hepatic insulin metabolism on hepatic lipase activity or inhibition of insulin binding and degradation by free fatty acids.

The positive association we found between insulin concentration and blood pressure is well established and could be due to a direct effect of insulin on renal sodium reabsorption or increased activity of the sympathetic nervous system. No single determinant of insulin concentration was significantly associated with blood pressure, and only the association with fasting insulin concentration was independent of age and BMI. Interestingly, the borderline association between systolic blood pressure and hepatic insulin throughput index was still present when age, BMI, and fat distribution were included in the regression equation.

The present study supports the central importance of variation in insulin sensitivity in insulin-related variation in triglyceride concentrations and suggests the previously unreported possibility of a close association between hepatic insulin and HDL2 metabolism.
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