Insulin Sensitivity, Insulinemia, and Coronary Artery Disease

The Insulin Resistance Atherosclerosis Study

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OBJECTIVE — The aim of this study was to evaluate whether low insulin sensitivity (S_i) measured using a modified frequently sampled intravenous glucose tolerance test with minimal model analysis is associated with coronary artery disease (CAD) independent of other cardiovascular risk factors.

RESEARCH DESIGN AND METHODS — We studied 1,482 women and men, age 40–69 years old, African American (28%), Hispanic (34%), or non-Hispanic white (38%), with normal (45%), impaired (23%), or diabetic (32%) glucose tolerance. CAD defined as confirmed past myocardial infarction, coronary artery bypass graft, coronary angioplasty, or presence of a major Q-wave was found in 91 participants.

RESULTS — The odds ratio (OR) for CAD was greatest among individuals in the two lowest quintiles of S_i (2.4, 95% CI 1.0–5.6 and 4.7, 2.1–10.7) compared with the highest S_i quintile. After adjusting for demographic and cardiovascular risk factors, a decrement from the 75th to 25th percentile in S_i was associated with a 56% increase in CAD (P = 0.028). Similar increments in fasting or 2-h insulin levels were associated with, respectively, only 15 (NS) and 3% (NS) increases in CAD. The association between S_i and CAD was partially mediated by insulin, HDL cholesterol and triglyceride levels, hypertension, diabetes, and obesity, but not LDL cholesterol or cigarette smoking.

CONCLUSIONS — Low S_i is associated with CAD independently of and stronger than plasma insulin levels. Part of the association is accounted for by dyslipidemia, hypertension, diabetes, and obesity.

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Abbreviations: CAD, coronary artery disease; ECG, electrocardiogram; FSGT, frequently sampled intravenous glucose tolerance test; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; IRAS, Insulin Resistance Atherosclerosis Study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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nente health maintenance organizations. The centers in San Antonio, Texas and San Luis Valley, Colorado recruited non-Hispanic whites and Hispanics from ongoing population-based studies (21,22). Race and ethnicity were assessed by self-report using the U.S. census definitions; African-Americans comprised 29%, Hispanics 34%, and non-Hispanic whites 37% of the study participants. Exclusion criteria included insulin treatment in the past 5 years, fasting glucose ≥16.7 mmol/l [300 mg/dl], unstable angina, decompensated congestive heart failure, or serious illness within the past month. All study protocols were approved by institutional review boards, and informed consent was obtained from all participants.

Measurement of glucose tolerance, insulin, and insulin sensitivity
An oral glucose tolerance test with glucose tolerance classification according to the WHO criteria (23) and a frequently sampled intravenous glucose tolerance test (FSIGT) were performed on two separate days 2–28 days apart. Participants were asked to refrain from heavy exercise and alcohol consumption for 24 h and fast for 12 h before each visit and abstain from smoking the morning of examination. Plasma glucose was measured with the glucose oxidase method on an automated analyzer (Yellow Springs Equipment). Plasma insulin levels were measured using the dextran-charcoal radioimmunoassay method (24).

Insulin sensitivity was assessed by the FSIGT with minimal model analysis (25). Glucose (0.3 g/kg in 50% solution) was injected through an intravenous catheter at 0 min, and regular human insulin (0.03 U/kg) was injected at 20 min. Blood was collected at −5, 2, 4, 8, 19, 22, 30, 40, 50, 70, 100, and 180 min for insulin and glucose determination. S/ was calculated by mathematical modeling (MINMOD, version 3.0, 1994). The injection of insulin was necessary to ensure adequate plasma insulin levels for accurate computation of insulin sensitivity in a diabetic person (26). This version of the FSIGT was validated by comparison with the hyperinsulinemic-euglycemic clamp (27).

Definition of CAD
CAD was defined conservatively as past myocardial infarction, coronary artery bypass graft, or percutaneous transluminal coronary angioplasty only if confirmed by review of medical records or a major Q-wave on IRAS examination electrocardiogram (ECC). The IRAS Events Committee (M.R., S.H., and J.S.) reviewed using standard criteria (28) all events reported to occur before the IRAS examination. Myocardial infarction was confirmed in 39 (72%) of 54, coronary artery bypass graft in 19 of 21, and percutaneous transluminal coronary angioplasty in 7 of 9 of case subjects. Standard, resting 12-lead ECG was performed using the MAC/PC electrocardiograph (Marquette Electronics, Milwaukee, WI). ECG tracings were read centrally using NOVACODE ECG software and the Minnesota Code (29) and revealed a major Q-wave (Minnesota code 1.1–1.2, except for 1.28) in 59 of the participants. Of the 1,482 IRAS participants who completed FSIGT, 91 (47 non-diabetic and 44 diabetic participants) had at least one of these events and were classified as case subjects.

Other measurements
Resting systolic and diastolic blood pressure were measured three times, and the second and third measurements were averaged. Hypertension was defined as systolic blood pressure ≥140 or diastolic blood pressure ≥90 mmHg or if they were currently taking antihypertensive medication. BMI was used as an estimate of overall adiposity. The waist-to-hip ratio was used as an estimate of body fat distribution. Cigarette smoking was categorized into “none,” “past,” or “current” using a standard questionnaire. Plasma HDL and LDL cholesterol were measured in fresh fasting plasma using the β-quantification according to the Lipid Research Clinics. Triglycerides were measured by enzymatic method in a glycerol blanked assay (Hitachi Autoanalyzer).

Statistical analysis
All analyses were performed in SAS version 6.08 statistical package (SAS Institute, Cary, NC) using Student’s t test and χ² test for univariate comparisons and logistic regression to estimate the relationship between S/ and CAD, controlling for potential confounders and effect modifiers.

The S/ was estimated to be 0 for 231 of the 298 participants in the lowest S/ quintile. In all logistic regression models shown, an indicator variable was included for individuals with S/ = 0, as previously described (17), but it was statistically not significant (P > 0.05).

RESULTS — This report includes 91% (1,482 of 1,624) of the study participants who completed the FSIGT. Univariate comparison of the characteristics of the 91 case subjects and 1,391 control subjects studied (Table 1) confirmed known associations between CAD and type 2 diabetes, male sex, older age, central obesity (higher waist-to-hip ratio), dyslipidemia (low HDL cholesterol and high triglycerides), hypertension, and cigarette smoking. Case subjects had significantly lower S/ levels than control subjects. Fasting insulin levels were only on the borderline of being higher among case subjects than control subjects. There was no difference in the levels of 2-h insulin between case subjects and control subjects.

To explore the linearity of the relationship between S/ and the CAD, the ORs of CAD were estimated by quintiles of the distribution, adjusting for age, sex, ethnicity, and clinic (Fig. 1). Adjusted CAD ORs for quintiles of fasting and 2-h insulin levels were included for comparison. The quintile of highest S/ or lowest fasting insulin or 2-h insulin levels served as the reference. The ORs for CAD were greatest among individuals in the second lowest S/ quintile (OR = 4.7, 95% CI 2.1–10.7), followed by those with the lowest S/ (2.4, 1.0–5.6). The ORs were nearly identical when the analysis was stratified by diabetic status. For instance, the ORs for CAD in nondiabetic participants were greatest among individuals in second lowest S/ quintile (OR = 4.7), followed by those with the lowest S/ (OR = 2.5).

After adjustment for demographic factors CAD (Table 2, model 1a), an interquartile decrement from the 75th to 25th percentile in S/ (2.21 to 0.41 × 10⁻⁴ min · μU⁻¹ · ml⁻¹) was associated with a 91% increase in CAD (P = 0.001). Similar interquartile differences in fasting insulin (from 60 to 132 pmol/l) (model 1b) or 2-h insulin levels (from 216 to 816 pmol/l) (model 1c) were associated with, respectively, only 34% (P = 0.034) and 16% (NS) increases in CAD. A simultaneous estimation of the effects of S/, fasting, and 2-h insulin (model 1d) indicated that only S/ was significantly and independently associated with CAD (OR = 1.84, P < 0.006).

Because nearly a one-half of the case subjects had type 2 diabetes, which is
known to increase the risk of CAD, we carried the analyses also stratified by diabetic status. The results were virtually identical for diabetic and nondiabetic participants, and the interaction between the effects of $S_i$ and diabetes was nonsignificant ($P > 0.9$) in all of the models. In further analyses, we combined diabetic and nondiabetic participants.

Adjustment for HDL and LDL cholesterol levels, triglycerides, smoking, and hypertension attenuated the independent association between CAD and $S_i$ (model 2a) and removed any association between CAD and fasting (model 2b) or 2-h insulin levels (model 2c). Models 2d, 2e, and 2f further suggested that $S_i$, rather than fasting or 2-h insulin levels, was the independent determinant of CAD. The decrease in the CAD ORs with adjustment for cardiovascular disease risk factors, from 1.91 for (model 1a) to 1.56 (model 2a), was consistent with the likely scenario that some of these factors could mediate the association between $S_i$ and CAD.

A stepwise addition of these risk factors to model 1a (data not shown) indicated that HDL cholesterol levels or closely correlated triglyceride levels and hypertension, but not LDL cholesterol and cigarette smoking, might mediate the effect of $S_i$.

Further adjustment for diabetes status (model 3) and obesity (model 4) removed most of the remaining association between $S_i$ and CAD. One may, however, argue that this could be a case of over adjustment, because the vast majority of participants with low $S_i$ were diabetic and/or obese. After adjustment for all covariates (model 5), insulin levels did not have any effect on CAD, but the interquartile decrement in $S_i$ was still associated with a 29% increase in the odds of CAD. Although not statistically significant, this finding may suggest that additional factors not included in these analyses may also play a role in the association between low $S_i$ and CAD.

**CONCLUSIONS** — This is the largest epidemiological study to date that has assessed directly insulin sensitivity and related it to fasting and postload insulin levels, traditional cardiovascular risk factors, and CAD. Our findings of an association between low insulin sensitivity and CAD, largely independent of the effects of major cardiovascular risk factors, are consistent with previous studies that used fasting insulin levels as a marker of insulin sensitivity (6–13). In contrast to some of these previous studies (8,9,30), the association between $S_i$ and CAD was highly significant and independent of the effects of lipids, hypertension, and cigarette smoking. These results are also consistent

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**Table 1** — Univariate comparison of the levels of selected cardiovascular risk factors among IRAS participants with and without confirmed CAD

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>CAD cases</th>
<th>Control subjects</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>91</td>
<td>1,391</td>
<td>0.712</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African Americans</td>
<td>22 (24.2)</td>
<td>391 (28.1)</td>
<td></td>
</tr>
<tr>
<td>Hispanics</td>
<td>32 (35.2)</td>
<td>473 (34.0)</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic whites</td>
<td>37 (40.6)</td>
<td>527 (37.9)</td>
<td></td>
</tr>
<tr>
<td>Glucose tolerance</td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>Normal</td>
<td>27 (29.7)</td>
<td>644 (46.3)</td>
<td></td>
</tr>
<tr>
<td>Impaired</td>
<td>20 (22.0)</td>
<td>312 (22.4)</td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>44 (48.3)</td>
<td>433 (31.3)</td>
<td></td>
</tr>
<tr>
<td>Sex (women)</td>
<td>37 (40.7)</td>
<td>775 (55.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.5 ± 6.7</td>
<td>55.3 ± 8.5</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2 ± 5.7</td>
<td>29.3 ± 5.8</td>
<td>0.123</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.98 ± 0.07</td>
<td>0.94 ± 0.08</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$S_i$ (10⁻⁴ min⁻¹ µU⁻¹ · ml⁻¹)</td>
<td>1.07 ± 1.22</td>
<td>1.68 ± 1.92</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>144 ± 93</td>
<td>129 ± 115</td>
<td>0.083</td>
</tr>
<tr>
<td>Mean fasting insulin (pmol/l)*</td>
<td>129 ± 72</td>
<td>115 ± 86</td>
<td>0.201</td>
</tr>
<tr>
<td>2-h insulin (pmol/l)</td>
<td>753 ± 517</td>
<td>710 ± 667</td>
<td>0.385</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.03 ± 0.31</td>
<td>1.16 ± 0.39</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.92 ± 1.20</td>
<td>1.63 ± 1.21</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.72 ± 0.96</td>
<td>3.65 ± 0.91</td>
<td>0.572</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132 ± 18</td>
<td>124 ± 17</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 ± 10</td>
<td>78 ± 9</td>
<td>0.346</td>
</tr>
<tr>
<td>Hypertension</td>
<td>52 (58.4)</td>
<td>527 (37.9)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td>0.049</td>
</tr>
<tr>
<td>None</td>
<td>30 (33.0)</td>
<td>614 (44.2)</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>47 (51.6)</td>
<td>542 (39.0)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>14 (15.4)</td>
<td>234 (16.8)</td>
<td></td>
</tr>
</tbody>
</table>

Data are n (%) or mean ± SD. *Average fasting insulin on the oral glucose tolerance test day and on the FSIGT day of IRAS examination 2–28 days apart.
with the previously reported (17–19) association between low $S_i$ and carotid artery wall thickness, which is an index of atherosclerosis. A comparison of the intima-media thickness of the internal carotid arteries in the IRAS CAD case subjects and control subjects (Fig. 2), confirmed that the insulin-resistant CAD case subjects had the most extensive carotid atherosclerosis. Thus, low insulin sensitivity is associated with both subclinical carotid atherosclerosis and clinical CAD.

The association between $S_i$ and carotid wall thickness (17) or CAD (current report) was independent of and much stronger than the associations with fasting or 2-h insulin levels. The exact contribution of the proposed atherogenic effect of insulin (13) to the association between insulin resistance and CAD is difficult to quantify in this cross-sectional analysis but appears to be relatively small (Table 2, model 1a versus 1d). This is consistent with the variable and generally weak associations between insulin levels and CAD reported previously (30). On the other hand, our data confirm that hypertension (31), dyslipidemia (32), and diabetes (acting through hyperglycemia or other risk factors [33,34]) mediate a significant part of the association between low $S_i$ and CAD.

This study is the first to measure insulin sensitivity directly in a large population of people with normal, impaired, or diabetic glucose tolerance. Whereas it is easier than that of fasting insulin levels, the effectiveness of insulin on glucose kinetics (e.g., cirrhosis). The major

**Figure 2**—Mean internal carotid artery intima-media wall thickness among CAD case ($n = 91$) and control ($n = 1,391$) subjects by quintiles of insulin sensitivity ($S_i$, adjusting for age, sex, ethnicity, and clinic); IRAS 1992–1995.
advantage of the IRAS protocol was the ability to measure insulin sensitivity in individuals with diabetes who are at a two- to fourfold increased risk of CAD (12, 35). They have typically been excluded from previous studies (6–11), yet diabetes affects, in the U.S., 6–14% of people aged 30–64 years and 18–32% of those over 64 years (36).

Despite the advantages of the minimal model analysis in assessing insulin sensitivity, the method resulted in a “zero $S_i$” estimate in $\sim$16% of IRAS participants (in 2% of those with normal, 13% with impaired, and 36% of those with diabetic glucose tolerance). “Zero insulin sensitivity” is a difficult concept to accept; however, we have demonstrated that IRAS participants with $S_i = 0$ had more features of the metabolic syndrome than other insulin-resistant IRAS participants with $S_i > 0$ (37). The phenomenon has been recently explained (38) as an artifact of a single compartment glucose distribution assumption underlying the minimal model estimation of $S_i$, which does not include insulin action on hepatic glucose metabolism. A more exact two-compartment modeling is not suitable for field studies due to complexity and use of a radiolabeled tracer. However, allowing $S_i$ to assume apparently negative values could partly correct the deviation and improve the correlation with euglycemic clamp derived measure of insulin sensitivity (39). When we recalculated $S_i$ allowing negative values, the rank of $S_i$ values was virtually unchanged. The ORs for CAD by quintile of such calculated $S_i$ (data not shown) looked nearly identical to those shown in Fig. 1, which were calculated using traditional $S_i$ values. This could be expected because $S_i$ estimates from the two-compartment model correlate perfectly with the one-compartment model $S_i$ estimates (38). Therefore, whereas the minimal model systemically underestimated insulin sensitivity, compared with the euglycemic clamp or a two-compartment model, it provided a dependable, cost-efficient, and minimally invasive way to measure insulin sensitivity in a large free-living population.

The present study has several limitations. First, the relation between $S_i$, insulin levels, and CAD were assessed cross-sectionally, and the proposed role of low insulin sensitivity as one of the causes of CAD needs to be confirmed in longitudinal studies. The IRAS cohort is being followed prospectively with major cardiovascular disease end points ascertained through annual participant interviews and committee review of medical records of reported fatal and nonfatal events. A 10-year follow-up of the study cohort will be completed in 2005.

Second, the IRAS cohort is not strictly population based. The study participants were drawn from two existing population-based epidemiological studies and from two health maintenance organization populations; however, individuals with IGT and diabetes were over-sampled by design. On the other hand, demanding protocol and specific exclusion criteria removed from the study population individuals with the most severe diabetes or CAD. Less than expected carotid artery atherosclerosis among the most insulin-resistant IRAS participants reported previously (17) and lower than expected CAD prevalence found in the current study in that group could be due to a “survivor bias.” This could occur if individuals with the most severe CAD have died, elected not to participate, or were excluded. This potential selection bias would tend to underestimate the true association between $S_i$ and CAD.

Third, the study population included Hispanic and non-Hispanic whites as well as African Americans, but relatively few end points in each of these subgroups limited our ability to detect any ethnic differences in the relation between low $S_i$ and CAD. There were no clear interactions between $S_i$ and ethnicity ($P > 0.4$, data not shown), and the present analyses were adjusted for, but not stratified by, ethnicity.

Fourth, there could have been some misclassification of the CAD status using the study criteria. Only 91 participants with the most severe clinical or ECG manifestations of CAD were classified as “case subjects,” whereas obviously many more had some degree of CAD but were classified as “control subjects.” More precise procedures to document CAD, such as coronary angiography or electron beam tomography for coronary calcification, were too invasive or expensive for this large study. Our definition of CAD most likely underestimated the true associations between CAD and risk factors, including $S_i$. Recently, a study of just 13 case subjects with arteriographically documented CAD and 10 control subjects (3) found a significant difference in their insulin sensitivity, consistent with that reported here.

Fifth, the minimal model measurement of insulin sensitivity is technically difficult in clinical practice. In search for a simpler solution, we substituted $S_i$ with the homeostasis model assessment (HOMA) measurement of insulin sensitivity that can be derived from the FSIGT (39). In none of the models, except for the simplest model 1a, was HOMA associated with CAD. Although easier to obtain than $S_i$, the HOMA estimate of insulin sensitivity appears to be insufficiently precise for studies of IRAS size.

Finally, $S_i$ and insulin levels display significant variability, partially related to precision of measurements and partially due to acute day-to-day and diurnal changes (40). The interclass correlation for $S_i$ measured twice within 1 week in 58 IRAS participants was 0.67 compared with 0.76 for fasting insulin. Thus, it is unlikely that we measured $S_i$ with more precision than fasting insulin levels and that this could account for the stronger association of CAD with $S_i$ than with fasting insulin. We confirmed that by using the average of two fasting insulin measurements (on the oral glucose tolerance test day and on the FSIGT day) instead of a single measurement in alternative models 1, 2, and 5. Although some of the ORs for fasting insulin increased slightly, the ORs for $S_i$ and the associated P values virtually did not change. We did not estimate the reproducibility of 2-h insulin levels in IRAS, but they may vary by >30% in normal subjects studied 48 h apart (41), which is comparable with the reproducibility of $S_i$ and fasting insulin. Therefore, differential measurement precision of $S_i$ and insulin levels is unlikely to explain the apparent independence and greater strength of the association between $S_i$ and CAD compared with that between insulin levels and CAD.

In middle-aged women and men representative of the three major U.S. ethnic groups and including individuals with normal, impaired, and diabetic glucose tolerance, we found that CAD was cross-sectionally associated with low insulin sensitivity. This association was independent of and stronger than that between CAD and fasting or postload insulin levels. Dyslipidemia, hypertension, diabetes, obesity, and fat centrality explained part of the association between low insulin sensitivity and CAD.
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References

9. Yarnell JWG, Sweetnam PM, Marks V, Teale JD, Bolton CH: Insulin in ischaemic heart disease: are associations explained by triglyceride concentrations. Br Heart J 171:293–296, 1994


