Evaluation of Two Insulin Assays in Insulin Resistance Syndrome (Syndrome X)

Steven M. Haffner, Leena Mykkänen, Rodolfo A. Valdez, Michael P. Stern

Abstract Recent data suggest that proinsulin is associated with cardiovascular risk factors in nondiabetic and diabetic subjects. Since most conventional insulin assays cross-react with proinsulin, it has been suggested that the associations of insulin concentrations with dyslipidemia and hypertension could actually reflect associations with proinsulin. We examined these associations by using both a conventional immunoreactive insulin assay and a specific Linco insulin assay that does not cross-react with proinsulin in 623 nondiabetic and in 180 non-insulin-dependent diabetic subjects who participated in the San Antonio Heart Study, a population-based study of diabetes and cardiovascular disease. Both the immunoreactive insulin assay and the specific Linco insulin assay were equally correlated with cardiovascular risk factors in nondiabetic subjects. Insulin concentrations were moderately correlated with high triglyceride and low high-density lipoprotein cholesterol levels and were weakly correlated with increased blood pressure. In diabetic subjects there were only weak associations between proinsulin and cardiovascular risk factors using either assay. We conclude that the association of insulin concentrations with cardiovascular risk factors is not a function of using insulin assays that cross-react with proinsulin and that for epidemiological studies of cardiovascular risk factors, conventional immunoreactive insulin assays are as good as the newer specific insulin assays. (Arterioscler Thromb. 1994;14:1430-1437.)

Key Words • insulin • proinsulin • lipoproteins • lipids • blood pressure

Many epidemiological and clinical studies have highlighted the association between insulin concentration and various metabolic and physiological disorders, including hypertension, dyslipidemia, and glucose intolerance. 

In this report, we compare a conventional immunoreactive insulin assay to a specific insulin assay that does not react with proinsulin in relation to cardiovascular risk factors in 623 nondiabetic and 180 diabetic subjects from a population-based study.

Methods

The San Antonio Heart Study is a population-based study of diabetes and cardiovascular disease in Mexican Americans and non-Hispanic whites. From 1979 through 1982 (phase I) and
from 1984 through 1988 (phase II) we randomly selected households from low-income (barrio), middle-income (transitional), and high-income (suburban) census tracts in San Antonio. All men and nonpregnant women 25 through 64 years of age who resided in the randomly sampled households were eligible to participate. Only Mexican Americans were sampled in the barrio, but approximately equal numbers of each ethnic group were studied in the other types of neighborhoods. Mexican Americans were defined as individuals whose ancestry and cultural traditions derived from a Mexican national origin. Detailed descriptions of the two study phases (I and II) are available. This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. All subjects gave informed consent.

In October 1987, we began an 8-year follow-up of the phase I cohort to determine the incidence of NIDDM and cardiovascular disease. This survey was completed in November 1990. Beginning in October 1990, we began a similar 7-year follow-up of the phase II cohort. The results presented in this report are based on the first three of six census tracts in the barrio, but approximately equal numbers of each ethnic group were studied in the other types of neighborhoods. Mexican Americans were defined as individuals whose ancestry and cultural traditions derived from a Mexican national origin. Detailed descriptions of the two study phases (I and II) are available. This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. All subjects gave informed consent.

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Systolic blood pressure (first phase) and diastolic blood pressure (fifth phase) were measured to the nearest even digit with a random-zero sphygmomanometer (Hawksley-Gelman). Three readings were recorded for each individual, and the average of the second and third readings was defined as the subject's blood pressure. Hypertension was defined according to the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC) as a systolic blood pressure > 140 mm Hg and/or a diastolic blood pressure >90 mm Hg and/or current use of antihypertensive medication.

Anthropometric measurements (height, weight, and waist and hip circumferences) were made after participants had removed their shoes and upper garments and donned an examining gown. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured at the level of the umbilicus and hip circumference at the level of the greater trochanters. The waist-to-hip ratio (WHR) was used as a measure of upper-body adiposity.

On the basis of previous prospective epidemiological studies, we considered four metabolic conditions to be most closely related to IRS in nondiabetic subjects: impaired glucose tolerance, hypertension, and low HDL-C and high triglyceride concentrations. Triglyceride concentrations were dichotomized as >200 mg/dL or <200 mg/dL and HDL-C was dichotomized as >35 mg/dL or <35 mg/dL as recommended by the Second Report of the National Cholesterol Education Program. We used WHO criteria for the diagnosis of impaired glucose tolerance and the mild hypertension definition of the JNC as discussed above. In subjects with NIDDM we considered three metabolic disorders (high triglyceride, low HDL-C, and hypertension) as being related to IRS.

Statistical analysis included ANOVA, correlation coefficients, χ² tests, and forward stepwise multiple linear regression. The percent of variance explained (multiple R²) was calculated for multiple linear regression models. A value of P<.05 was used as the criterion for entry and removal at each step. Interactions between ethnicity and other variables (eg, gender, obesity, number of metabolic disorders) were examined by using multiple linear regression. Because no significant interactions between ethnic groups and other variables were found (P>.10), both ethnic groups were pooled for greater statistical power and ease of presentation.

Table 2. Correlations Between Fasting Insulin and Demographic, Anthropometric, and Metabolic Variables

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic Subjects (n=623)</th>
<th>Diabetic Subjects (n=180)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunoreactive Insulin</td>
<td>Specific Insulin</td>
</tr>
<tr>
<td></td>
<td>Fasting 2 h</td>
<td>Fasting 2 h</td>
</tr>
<tr>
<td>Age</td>
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<td>-.02</td>
</tr>
<tr>
<td>BMI</td>
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<td>.31*</td>
</tr>
<tr>
<td>WHR</td>
<td>.11*</td>
<td>.07</td>
</tr>
<tr>
<td>Fasting glucose</td>
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<td>.21*</td>
</tr>
<tr>
<td>2-h glucose</td>
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<td>.57*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>.16*</td>
<td>.20*</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-.16*</td>
<td>-.21*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-.05</td>
<td>-.03</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-.03</td>
<td>-.01</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>.05</td>
<td>.12*</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>.14*</td>
<td>.13*</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; WHR, waist-to-hip ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and BP, blood pressure.

*P<.05; †P<.01; ‡P<.001.
Results

Table 1 shows the clinical, anthropometric, and metabolic characteristics of the population by gender. A higher percentage of women than men were Mexican American. Men had higher WHR and diastolic blood pressure than women. Women had higher BMI and 2-hour glucose, insulin concentrations, and HDL-C levels. There were no significant differences by gender in the prevalence of NIDDM or in levels of triglyceride, low-density lipoprotein cholesterol, systolic blood pressure, or fasting insulin and glucose.

Table 2 shows the Spearman correlations of immunoreactive and specific insulin to anthropometric and metabolic variables in nondiabetic and diabetic subjects. In general, the correlations of both insulin measures were of similar magnitude with metabolic variables. For example, in nondiabetic subjects the correlation of triglyceride with fasting immunoreactive insulin was .16 ($P<.001$), and the correlation with fasting specific insulin was .17 ($P<.001$). Insulin concentrations were significantly correlated with BMI and high glucose and triglyceride and low HDL-C levels. Weaker but still significant correlations were observed between insulin and WHR and blood pressure. In diabetic subjects the correlations of insulin with lipids, lipoproteins, and blood pressure was weaker than in nondiabetic subjects, although this may also reflect the lower number of diabetic subjects.

We also calculated the partial correlation coefficients between insulin concentrations and metabolic variables adjusting for age, BMI, WHR, gender, and ethnicity (data not shown). These correlations were also similar using either immunoreactive or specific insulin concentrations. Insulin (measured by either method) was correlated with high triglyceride and low HDL levels and weakly with blood pressure in nondiabetic subjects. In diabetic subjects, insulin concentrations were not correlated with lipids, lipoproteins, or blood pressure.

Figs 1 through 4 show the associations between insulin concentrations and the four metabolic disorders closely related to IRS: high triglyceride and low HDL-C levels, hypertension, and impaired glucose tolerance (in nondiabetic subjects). In nondiabetic subjects (Figs 1 and 2) both fasting and 2-hour specific and immunoreactive insulin concentrations were related to all four metabolic disorders except for a borderline significant association between fasting immunoreactive insulin and hypertension. In diabetic subjects, fasting specific insulin concentrations were significantly associated with high triglyceride ($P=.001$) and low HDL-C ($P=.001$) (Fig 3). Fasting immunoreactive insulin was significantly associated with low HDL ($P=.021$), but the association...
FIG 3. Bar graphs showing mean insulin in relation to high triglyceride (TG) and low high-density lipoprotein cholesterol (HDL) in subjects with non-insulin-dependent diabetes mellitus.

FIG 4. Bar graph showing mean insulin in relation to hypertension in subjects with non-insulin-dependent diabetes mellitus.

Fig 5. Bar graphs showing mean insulin in relation to number of metabolic disorders in nondiabetic subjects.

status in the overall population, men and women separately and in Mexican Americans and non-Hispanic whites separately (data not shown). There was little evidence to suggest a different relation between insulin concentrations and the number of metabolic disorders. Moreover, the insulin concentrations in men and women and in nondiabetic subjects of both ethnic groups appeared to be similarly related to the number of metabolic disorders. In nondiabetic subjects, the number of metabolic disorders varied from zero to four and included high triglyceride and low HDL-C levels, impaired glucose tolerance, and hypertension (Fig 5). In these analyses both fasting and 2-hour immunoreactive insulin were significantly associated with the number of metabolic disorders in nondiabetic subjects ($P<.001$). The percent of variance explained was somewhat higher when immunoreactive insulin was used instead of specific insulin. In diabetic subjects, three metabolic disorders were considered: hypertension, high triglyceride, and low HDL-C levels. In diabetic subjects, fasting immunoreactive and specific insulin (but not the 2-hour values) were significantly associated with the number of metabolic disorders (Fig 6).

We also calculated mean insulin concentrations in relation to the number of metabolic disorders adjusted
Discussion

We have shown that insulin, whether measured by a conventional antibody or a specific antibody that does not cross-react with proinsulin, is equally correlated with the components of IRS, especially with increased triglyceride and decreased HDL-C levels in nondiabetic subjects. The results were similar in men and women and in Mexican Americans (a high-risk group for NIDDM) and in non-Hispanic whites (a low-risk group for NIDDM). This observation is important for two reasons. Recent data suggest that proinsulin is increased in subjects with NIDDM and impaired glucose tolerance. Some studies suggest that proinsulin concentrations are related to cardiovascular risk factors in both subjects with NIDDM or impaired glucose tolerance and nondiabetic subjects. Thus it is possible that the associations between insulin concentrations and cardiovascular risk factors reported in epidemiological studies could be due to the use of a conventional immunoreactive insulin antibody that cross-reacts with proinsulin. However, in the current report, insulin concentrations measured with a specific insulin antibody were as strongly correlated with cardiovascular risk factors as were the conventionally measured concentrations. Thus it appears that the previously reported associations of insulin with cardiovascular risk factors are not due to contamination with proinsulin. Interestingly, fasting specific insulin was no more correlated with the number of metabolic disorders in diabetic subjects than was immunoreactive insulin in this population.

On the other hand, the use of specific insulin antibodies could be useful in diabetes research. Subjects with NIDDM have peripheral hyperinsulinemia, insulin resistance, decreased insulin secretion, and overproduction of hepatic glucose. However, the role of impaired insulin secretion versus insulin resistance with resulting hyperinsulinemia in prediabetic subjects is still controversial. Thus, the evaluation of the measurement of specific insulin (which does not react with proinsulin) and the separate use of proinsulin (thought to be a marker of pancreatic failure) are potentially useful in research on the etiology of diabetes. However, our data imply that insulin antibodies with different degrees of cross-reactivity with proinsulin are equally well correlated with cardiovascular risk factors in nondiabetic subjects, which suggests that the choice of an assay for epidemiological studies emphasizing cardiovascular disease may turn on cost and ease of use. Whatever type of assay is preferred, participation in a standardization program will help to reduce the variability of assays over time.

The strongest correlations of insulin concentration with cardiovascular risk factors were with increased triglyceride and decreased HDL-C levels. Even so, these correlations are about 0.2 and thus explain only 4% of the variance in triglyceride or HDL-C. Insulin concentrations were not significantly correlated with low-density lipoprotein cholesterol levels. Insulin resistance may be more strongly correlated with dyslipidemia than is insulinemia in nondiabetic subjects. In two studies, both insulin concentrations and insulin resistance were equally predictive of dyslipidemia. We also found weak but significant associations between insulin concentrations and blood pressure. Previous data on the relation between blood pressure and insulin concentrations have been contradictory; some studies support an association, while others do not. The reasons for these inconsistent associations are unknown, but differences according to race or level of obesity may play a role. The present report suggests, however, that the inconsistency is not likely to be due to different insulin assays with variable degrees of cross-reactivity with proinsulin.

We also showed strong associations between both specific and immunoreactive insulin concentrations and the number of metabolic disorders in nondiabetic subjects. Although the percent of variation explained was
similar when immunoreactive insulin was used. In diabetic subjects, only fasting specific and immunoreactive insulin were significantly related to the number of metabolic disorders.

We observed higher insulin concentrations with a specific insulin assay (which does not recognize proinsulin) than with a "nonspecific" insulin assay that does recognize proinsulin. The measured cross-reactivity of the immunoreactive insulin (DCP) with proinsulin is from 70% to 100%. Intuitively, we would expect "specific" insulin levels to be lower than "conventional" insulin values by precisely the contribution of proinsulin measured by the conventional assay. However, differences in absolute insulin values are commonly observed (eg, in the College of American Pathologists program), even between assays displaying similar proinsulin cross-reactivity. This is likely due to other factors, including methodology and reagent differences. For example, the Linco assay uses the traditional double-antibody methods, whereas the immunoreactive insulin is a coated-tube assay. We make no claims regarding differences in absolute values between assays. The statistical analyses reported in this article are based on relative changes within assays.

In summary, we have shown that immunoreactive insulin (which cross-reacts with proinsulin) and a specific insulin are equally predictive of the features of IRS. We conclude that for epidemiological studies of cardiometabolic subjects, only fasting specific and immunoreactive insulin works as well as the newer specific insulin are equally predictive of the features of IRS.

Valdez is supported by the American Diabetes Association mentor-based postdoctoral fellowship program.

Acknowledgments

This work was supported by the National Heart, Lung, and Blood Institute grants RO1 HL24799 and R37 HL36820. Dr Valdez is supported by the American Diabetes Association mentor-based postdoctoral fellowship program.

References

33. Hazuda HP, Comeaux PJ, Stern MP, Haffner SM, Eifler CW, Rosenthal M. A comparison of three indicators for identifying...


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Arterioscler Thromb Vasc Biol. 1994;14:1430-1437
doi: 10.1161/01.ATV.14.9.1430
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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