Specific insulin assays, insulin sensitivity and blood pressure

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Summary

Serum insulin concentrations have been used as markers of insulin resistance in population studies examining the relationship between insulin resistance and blood pressure, but the relationship is variable among studies. We hypothesized that differences in cross-reactivity of insulin assays with proinsulin and its split/des-amino products might account for the variation. We therefore examined fasting and post-glucose load serum insulin concentrations (determined by both specific and conventional assays), insulin sensitivity (measured by the euglycaemic clamp technique), and blood pressure, in a group of 56 diabetic (NIDDM) and non-diabetic subjects. Insulin concentrations as measured by the two methods were highly correlated \((r = 0.97, p < 0.0001)\), and the relationships among serum insulin concentrations, insulin sensitivity and blood pressure were independent of assay method; for example, in non-diabetic subjects the univariate correlation between \(\log_{10}\text{AUC insulin}\) and insulin sensitivity index was similar with both methods \([r = -0.81\text{ vs. } r = -0.82, p < 0.0001 (\text{specific vs. conventional assay})]\). Discrepancies between studies in the relationship between serum insulin concentrations and blood pressure are unlikely to be due to cross-reactivity of conventional insulin assays with proinsulin-like molecules.

Introduction

Resistance to insulin-mediated glucose uptake is a feature of non-insulin-dependent diabetes mellitus (NIDDM), obesity, essential hypertension and coronary heart disease.\(^1,2\) In non-diabetic obese and hypertensive individuals, normal glucose tolerance is maintained, at least in the short term, by increased pancreatic \(\beta\)-cell secretion of insulin. Serum insulin concentrations in such individuals are raised in proportion to the degree of insulin resistance, and the resulting hyperinsulinaemia has been implicated in the pathogenesis of cardiovascular disease.\(^3,4,5\) Blood pressure appears to be more closely related to insulin sensitivity than to serum insulin concentrations,\(^6\) but measurement of insulin sensitivity is relatively labour-intensive, and circulating insulin concentrations (fasting and post-glucose load) have been used as surrogate measurements in many of the large-scale studies which have implicated insulin resistance in the pathogenesis of essential hypertension.\(^6,7\) However, the relationship between insulin concentrations and blood pressure is variable among studies and ethnic groups, particularly after adjustment for confounding variables such as body mass index (BMI), and its existence and significance remain controversial.\(^8,9\)

Commercially-available radioimmunoassays for insulin cross-react with intact proinsulin and its partially-processed split and des-amino products. However, sensitive and specific assays have now been developed for insulin and its precursor hormones.\(^10,11,12\) While partially-processed proinsulin products have decreased biological activity in terms of glucose disposal when compared with insulin, they have longer half-lives, and are not converted to insulin in the circulation.\(^13\) It has been reported that serum concentrations of proinsulin-like molecules...
are more strongly related than serum insulin concentrations to cardiovascular risk factors in both non-diabetic and NIDDM populations, and adults who were of low birth weight appear to have abnormal proinsulin processing. It is possible that the cardiovascular risk attributed to insulin resistance and hyperinsulinaemia might in part reflect cross-reactivity of proinsulin-like molecules in conventional insulin assays.

This study was designed to clarify, for the first time, whether the relationships among insulin concentrations, insulin sensitivity (measured using the ‘gold standard’ euglycaemic clamp technique), and blood pressure are affected by the specificity of the assay used to determine serum insulin concentrations and hence whether some of the variability between studies in the relationship between insulin concentrations and blood pressure might be accounted for by use of insulin assays with different degrees of cross-reactivity with proinsulin and its split/des-amino products.

**Methods**

Fifty-six Caucasian subjects (49 male, 7 female; Table 1) gave informed consent to participate in the study, which was approved by the Ethics Committee of the West Glasgow Hospitals University NHS Trust. Non-diabetic hypertensive subjects were recruited from patients attending the Glasgow Blood Pressure Clinic, while patients with NIDDM were attending the Diabetes Clinic. Non-diabetic normotensive control subjects were recruited by advertisement. Those aged <30 or >75 years were excluded, as were those with a clinical history of myocardial infarction, angina pectoris, intermittent claudication, or stroke, and those who gave a history of alcohol intake >20 units weekly. All had a serum creatinine within the laboratory reference range. For the purposes of the study, body mass index of <30 kg/m² was classified as ‘non-obese’; otherwise patients were deemed ‘obese’. Hypertension was defined as a mean supine diastolic BP of ≥95 mmHg or systolic BP ≥160 mmHg on three readings after 10 min supine rest (Dinamap Critikon) on at least two separate occasions. Diabetes was confirmed by OGTT according to WHO criteria (75 g). Hypertensive subjects were either newly diagnosed or withdrawn from all antihypertensive medication at least 4 weeks prior to the study; none had been taking thiazide diuretics in the previous 6 months. Patients treated with insulin or oral hypoglycaemic agents were excluded.

The study design was such that patients and controls were studied concurrently: for each non-obese hypertensive subject recruited (NIDDM or non-diabetic), two age- and sex-matched controls were identified: one non-obese normotensive and the other obese hypertensive. All attended an initial screening visit, when baseline characteristics were recorded, followed by two further study days. On the first of these, a standard 75 g oral glucose tolerance test (OGTT) was performed, with venous blood samples being withdrawn from an indwelling 21G venous cannula at 0, 30, 60, 90, and 120 min for glucose and insulin measurements (all time-points), and fasting cholesterol and triglycerides (baseline sample only). On the second day, a modified (3 h) hyperinsulinaemic euglycaemic clamp was performed (5.2 mmol/l): a primed infusion of soluble human insulin (1.5 mU/kg/min; Actrapid, NovoNordisk) along with a variable rate infusion of 20% dextrose (Baxter Healthcare) was administered via a left antecubital vein for 3 h. The right hand was placed in a heated-air hand box at 55°C (University of Nottingham, Department of Physiology and Pharmacology), and the dextrose infusion rate was adjusted on the basis of glucose concentrations measured in arterialized venous blood samples at the bedside. In the patients with NIDDM, serum glucose concentrations were gradually normalized with an infusion of soluble insulin (2 U/h) prior to commencing the procedure.

Insulin sensitivity index was defined as:

\[ S_I = \frac{\Delta R_g}{\Delta I \times G} \]

where: \( \Delta R_g \), increment in glucose uptake (basal to steady state); \( \Delta I \), increment in insulin concentration (basal to steady state); \( G \), steady-state glucose concentration. It was calculated at steady state from the glucose infusion rate and ambient insulin and glucose concentrations during the final 40 min.

**Laboratory methods**

Glucose concentrations were measured using the glucose oxidase method (Beckman 2 glucose analyser, Beckman Instruments; inter-assay coefficient of variation 1.5%). Serum insulin concentrations were determined by a commercially available assay highly specific for insulin (Lifescreeen, Insulin EASIA; inter-assay CVs 6.7% at 4.2 mU/l, 3.5% at 9.8 mU/l, and 3.3% at 81 mU/l; intra-assay CV was lower than this) and also by a conventional assay with a 62% molar cross-reaction with intact proinsulin (Pharmacia insulin RIA 100; inter-assay CVs 5.8% at 11.6 mU/l, 6.4% at 32.7 mU/l, and 6.5% at 65.2 mU/l).

**Statistical analysis**

All data were checked for normality using the Shapiro-Wilks test (Minitab statistical package), and logarithmic transformation (log_{10}) was performed...
### Table 1  Characteristics of non-diabetic and NIDDM subjects (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Non-obese normotensive</th>
<th>Non-obese hypertensive</th>
<th>Obese hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-diabetic subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>38 ± 8.0</td>
<td>44 ± 8.8</td>
<td>49 ± 10.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.8 ± 2.93</td>
<td>26.5 ± 2.35</td>
<td>32.9 ± 2.79***</td>
</tr>
<tr>
<td>M/F</td>
<td>1/10</td>
<td>8/2</td>
<td>9/0</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>92 ± 10.2</td>
<td>119 ± 7.0***</td>
<td>121 ± 11.6***</td>
</tr>
<tr>
<td>Glucose intolerant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.4 ± 0.44</td>
<td>5.3 ± 0.55</td>
<td>5.9 ± 0.56</td>
</tr>
<tr>
<td>Fasting specific insulin (µU/ml) (geometric mean)</td>
<td>5.47</td>
<td>7.89</td>
<td>16.9*</td>
</tr>
<tr>
<td>Fasting conventional insulin (µU/ml) (geometric mean)</td>
<td>7.14</td>
<td>9.97</td>
<td>21.2*</td>
</tr>
<tr>
<td>Duration hypertension (months: median (range))</td>
<td>–</td>
<td>3 (2–300)</td>
<td>3 (2–36)</td>
</tr>
<tr>
<td><strong>NIDDM subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 ± 7.5</td>
<td>67 ± 6.2**</td>
<td>57 ± 11.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 2.91</td>
<td>25.8 ± 2.74</td>
<td>34.4 ± 4.11***</td>
</tr>
<tr>
<td>M/F</td>
<td>9/2</td>
<td>6/1</td>
<td>7/1</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>96 ± 6.3</td>
<td>118 ± 8.0***</td>
<td>123 ± 5.6***</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>9.1 ± 2.49</td>
<td>8.0 ± 1.42</td>
<td>8.2 ± 2.09</td>
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<tr>
<td>Fasting specific insulin (µU/ml) (geometric mean)</td>
<td>7.23</td>
<td>7.81</td>
<td>14.4*</td>
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<tr>
<td>Fasting conventional insulin (µU/ml) (geometric mean)</td>
<td>10.0</td>
<td>11.0</td>
<td>20.7*</td>
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<tr>
<td>Duration hypertension (months: median (range))</td>
<td>–</td>
<td>2 (2–36)</td>
<td>2 (2–41)</td>
</tr>
<tr>
<td>Duration diabetes</td>
<td>19 (4–98)</td>
<td>10 (2–60)</td>
<td>16 (2–84)</td>
</tr>
</tbody>
</table>

*2p<0.05; **2p<0.01; ***2p<0.001, non-obese hypertensive vs. non-obese normotensive.

*2p<0.05, **2p<0.01, ***2p≤0.001, obese hypertensive vs. non-obese hypertensive.

where necessary. Area-under-the-curve (AUC) insulin was calculated as a summary measure for each individual. A Bland-Altman plot of insulin concentrations as measured by the two assays was performed. Log_{10} serum insulin and glucose concentrations were back-transformed to give the geometric mean for presentation of oral glucose tolerance test data. One-way ANOVA was used in the analyses of steady state serum insulin concentrations and insulin sensitivity index by subgroup. Unpaired t-tests were used for comparisons between subgroups: in order to adjust for multiple comparisons, statistical significance was assessed using 97.5% CIs (2p < 0.05).

In univariate analyses, simple correlations were plotted of the relationships among serum insulin concentrations, insulin sensitivity, and blood pressure for the specific and conventional assays. To examine the best predictors of insulin sensitivity index, multiple regression analysis was performed using the following predictor variables: age, sex, fasting and post-load glucose concentrations, systolic and diastolic blood pressure, BMI, waist-to-hip ratio, smoking status, and fasting cholesterol and triglyceride concentrations. This analysis was performed separately for both specific and conventional assays.

### Results

All 56 subjects completed the protocol without complication. Six of the non-diabetic subjects screened (one non-obese normotensive, one non-obese hypertensive, and four obese hypertensive) were found on the basis of their post-load glucose concentrations to have impaired glucose tolerance (IGT). The former two subjects were excluded from the study. Satisfactory matching was achieved (Table 1) between comparable subgroups for age, sex, and BMI, although within the NIDDM subjects the non-obese normotensive and obese hypertensive subgroups were younger than the non-obese hypertensive subgroup.

### Simple assay comparison

Insulin concentrations as measured by the two methods were highly correlated (r = 0.97,
A Bland-Altman plot revealed that AUC insulin concentrations by the conventional method were greater than or equal to those by the specific method in 52 of the 56 subjects (Figure 1b). For the non-diabetic subjects, the percentage (mean ± SD) of immunoreactive insulin which was accounted for by specific insulin was 89 ± 11.6, 90 ± 8.8, and 92 ± 5.5% in the non-obese normotensive, non-obese hypertensive, and obese hypertensive subgroups, respectively. For those with NIDDM, the equivalent percentages were 84 ± 15.8, 87 ± 15.3, and 88 ± 7.4%. The largest differences detected were in the obese NIDDM subjects and in obese non-diabetic subjects who were glucose intolerant (Figure 1b). In the four subjects in whom specific insulin concentrations were apparently greater than conventional insulin concentrations, the magnitude of the difference was small, and reflected a difference at a single time-point in the later part of the OGTT.

Insulin and glucose concentrations

Insulin

Fasting and AUC insulin results were similar for both specific and conventional assays (Figure 2). In the non-diabetic subjects, AUC insulin was greater in obese hypertensive compared with non-obese hypertensive subjects (2p < 0.05, Figure 2); there was a tendency for AUC insulin to be greater in non-obese hypertensive compared with non-obese normotensive subjects, but this did not reach statistical significance (2p = 0.14, Figure 2). In NIDDM subjects, there was a trend for AUC insulin to be greater in obese hypertensive compared with non-obese hypertensive subjects, whom in turn tended to have higher AUC insulin than non-obese normotensive subjects; however, these trends did not reach statistical significance (Figure 2).

Glucose profiles

There were no statistically significant differences in fasting glucose concentrations within the non-diabetic and NIDDM groups (Table 1). However, within the non-diabetic group, AUC glucose was higher in obese hypertensive compared with non-obese hypertensive subjects (2p < 0.05), and in non-obese hypertensive compared with non-obese normotensive subjects (2p < 0.05, Figure 2).

Insulin sensitivity

Mean ± SD steady-state insulin concentrations (µU/ml) achieved during the euglycaemic clamp procedures (exogenous insulin infusion) were higher in obese subjects {non-diabetic subjects 113 ± 20.6 (non-obese normotensive), 115 ± 27.2 (non-obese hypertensive), 163 ± 38.0 (obese hypertensive); NIDDM subjects 109 ± 19.0 (non-obese normotensive), 121 ± 27.9 (non-obese hypertensive), and 159 ± 60.6 (obese hypertensive)}, p < 0.01, ANOVA. The insulin sensitivity index (Si), correcting for steady-state insulin concentration, was therefore calculated for each subject (Figure 3). Coefficients of variation of blood glucose during the final 40 min of the clamps were 3.3% (non-diabetic subjects) and 4.7% (NIDDM subjects).

In both the non-diabetic subjects and subjects with NIDDM, insulin sensitivity index (Figure 3) was lower in the sub-groups with higher blood pressure and increased weight (non-diabetic subjects, p < 0.001; NIDDM subjects p < 0.01, ANOVA). When subgroups were compared, with adjustment for multiple comparisons, insulin sensitivity index

![Figure 1](image-url). Assay comparison. a Simple correlation. Log_{10} AUC insulin from OGTT data in all subjects (n = 53, missing data in 3 cases) determined using conventional and specific assays. b Bland-Altman plot. The difference between AUC insulin as measured by the conventional and specific assays plotted against log_{10} specific AUC insulin.
Figure 2. Insulin and glucose concentrations (geometric means). Upper and lower panels show results for the non-diabetic and NIDDM subjects, respectively. Specific and conventional insulin concentrations are shown in the left and middle panels, with the glucose concentrations in the right panel. *$p<0.05$ for comparison of log$_{10}$ AUC between groups. Non-diabetic subjects: ○, non-obese normotensive; □, non-obese hypertensive; △, obese hypertensive. NIDDM subjects: ●, non-obese normotensive; ■, non-obese hypertensive; ▲, obese hypertensive.
independent of assay specificity in both non-diabetic subjects and subjects with NIDDM (Table 2, lower panel).

**Fasting insulin in non-diabetic subjects**

There was a negative correlation (Figure 4) between log_{10} fasting serum insulin and insulin sensitivity index: \( r = -0.59, p < 0.001, 95\% \text{ CI} -0.29, -0.78 \) (specific assay), \( r = -0.64, p < 0.001, 95\% \text{ CI} -0.36, -0.81 \) (conventional assay).

**Fasting insulin in subjects with NIDDM**

There was a negative correlation (Figure 4) between log_{10} fasting serum insulin and insulin sensitivity index: \( r = -0.47, p < 0.05, 95\% \text{ CI} -0.12, -0.71 \) (specific assay), \( r = -0.55, p < 0.01, 95\% \text{ CI} -0.23, -0.76 \) (conventional assay).

**AUC insulin in non-diabetic subjects**

There was a negative correlation (Figure 5) between log_{10} AUC insulin and insulin sensitivity index: \( r = -0.81, p < 0.0001, 95\% \text{ CI} -0.62, -0.91 \) (specific assay), \( r = -0.82, p < 0.0001, 95\% \text{ CI} -0.64, -0.91 \) (conventional assay).

**AUC insulin in NIDDM subjects**

There was no significant relationship between log_{10} AUC insulin and insulin sensitivity index: \( r = -0.24, 95\% \text{ CI} +0.17, -0.58 \) (specific assay), \( r = -0.33, 95\% \text{ CI} +0.07, -0.64 \) (conventional assay).

### Table 2: Univariate correlations (Pearson’s \( r \)) between demographic, metabolic and haemodynamic measurements and insulin sensitivity index (\( S_{IP} \))

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic subjects ((n = 30))</th>
<th>NIDDM subjects ((n = 26))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.40*</td>
<td>-0.22</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.77***</td>
<td>-0.69***</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>-0.29</td>
<td>-0.16</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>-0.40*</td>
<td>-0.51**</td>
</tr>
<tr>
<td>Waist–to–hip ratio</td>
<td>-0.75***</td>
<td>-0.66***</td>
</tr>
<tr>
<td>Fasting glucose ((\log_{10}))</td>
<td>-0.23</td>
<td>-0.21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic</th>
<th>NIDDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay</td>
<td>Specific</td>
<td>Conventional</td>
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<tr>
<td>Fasting insulin ((\log_{10}))</td>
<td>-0.59***</td>
<td>-0.64***</td>
</tr>
<tr>
<td>30–min insulin ((\log_{10}))</td>
<td>-0.67***</td>
<td>-0.68***</td>
</tr>
<tr>
<td>120 min insulin ((\log_{10}))</td>
<td>-0.49*</td>
<td>-0.44</td>
</tr>
<tr>
<td>AUC insulin ((\log_{10}))</td>
<td>-0.81***</td>
<td>-0.82***</td>
</tr>
<tr>
<td>HOMA^{37} ((\log_{10}))</td>
<td>-0.59***</td>
<td>-0.63***</td>
</tr>
</tbody>
</table>

Data are \( r \) values.

* \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \)
Blood pressure and insulin sensitivity

Non-diabetic subjects
Diastolic blood pressure was negatively correlated with insulin sensitivity index (Figure 6): $r = -0.40$, $p < 0.05$, 95% CI $-0.05$, $-0.66$.

NIDDM subjects
Diastolic blood pressure was negatively correlated with insulin sensitivity index (Figure 6): $r = -0.51$, $p < 0.01$, 95% CI $-0.15$, $-0.75$.

Insulin concentrations and blood pressure

Non-diabetic subjects
There was a positive correlation between log$_{10}$ AUC insulin and diastolic blood pressure, independent of assay method (Figure 7): $r = 0.40$, $p < 0.05$, 95% CI 0.03, 0.68 (specific assay); $r = 0.38$, $p < 0.05$, 95% CI 0, 0.66 (conventional assay).

NIDDM subjects
No significant relationship was detected with either assay (Figure 7): $r = 0.24$, $p = 0.18$, 95% CI $-0.17$, 0.58 (specific assay), $r = 0.28$, $p = 0.17$, 95% CI $-0.13$, 0.60 (conventional assay).

Predictors of insulin sensitivity

The results of multiple regression analyses were almost identical when insulin concentrations measured by either assay method were entered as predictor variables.

Non-diabetic subjects
The best single predictor of insulin sensitivity index was AUC insulin (adjusted $R^2 = 65\%$, $p < 0.001$); the best two combined predictors were AUC insulin and waist-to-hip ratio together (adjusted $R^2 = 80\%$, $p < 0.001$).

NIDDM subjects
BMI was the best predictor of insulin sensitivity index (adjusted $R^2 = 46\%$, $p < 0.001$).

Discussion

The major finding of this study was that, in the subjects studied, the specificity of the insulin assay used to measure serum insulin concentrations had no detectable effect on the relationships observed amongst serum insulin concentrations, insulin sensitivity, and blood pressure. Serum insulin concentra-
Figure 5. AUC insulin and insulin sensitivity. Log$_{10}$AUC insulin plotted against insulin sensitivity index ($S_p \times 10^4$ dl/(min.kg) per µU/ml) as measured by a 3 h euglycaemic clamp. Pearson’s $r$ is shown together with 95% CIs. Upper panels: Non-diabetic subjects ($n=28$, 2 subjects with missing data). Left panel, specific insulin concentrations; right panel, conventional insulin concentrations. Lower panels: NIDDM subjects ($n=25$, 1 subject with missing data). Left panel, specific insulin concentrations; right panel, conventional insulin concentrations. Symbols as Figure 4.

Figure 6. Blood pressure and insulin sensitivity. Diastolic blood pressure (mmHg) plotted against insulin sensitivity index ($S_p \times 10^4$ dl/(min.kg) per µU/ml). Pearson’s $r$ is shown together with 95% CIs. Upper panel: Non-diabetic subjects ($n=30$). Lower panels: Subjects with NIDDM ($n=26$). Symbols as Figure 4.

Many of the large-scale studies which have examined the relationship between insulin concentrations and blood pressure in non-diabetic subjects measured insulin using conventional radioimmunoassays with a high degree of cross-reactivity with proinsulin and its partially-processed intermediates. Three of these studies used the same commercially available conventional assay kit (Pharmacia RIA 100) that was used in this study (62% molar cross-reaction with intact proinsulin and 5% cross-reactivity with split-proinsulin) reported particularly weak and inconsistent relationships between insulin concentra-
Specific insulin assays

Figure 7. Blood pressure and AUC insulin. Diastolic blood pressure (mmHg) plotted against log_{10} AUC insulin concentrations. Pearson’s r is shown together with 95% confidence intervals. Upper panel: Non-diabetic subjects (n = 28, 2 subjects with missing data). Left panel: specific insulin concentrations; right panel: conventional insulin concentrations. Lower panels: Subjects with NIDDM (n = 25, 1 subject with missing data). Left panel: specific insulin concentrations; right panel: conventional insulin concentrations. Symbols as in Figure 4.

tions and blood pressure. This data, taken together with observations of adverse events during the clinical use of proinsulin and the associations between serum concentrations of proinsulin-like molecules and both birth weight and angiographic severity of coronary atherosclerosis, led us previously to speculate that cross-reacting proinsulin-like molecules (rather than insulin itself) might play a role in the pathogenesis of insulin resistance and/or hypertension/atherosclerosis. Since that time, two further large epidemiological studies using specific insulin assays have been reported. One of these, suggested that specific insulin concentrations were more highly correlated with blood pressure in a general population than were those measured using a conventional assay. The other, conducted in non-diabetic subjects from the San Antonio study, found no difference in the relationship; however, confidence in this finding was diminished somewhat by the finding of higher insulin concentrations with the specific than with the conventional assay.

The detailed nature of the present study was such that only a relatively small number of subjects (n = 56) could be studied. Insulin sensitivity was measured by a 3-h euglycaemic clamp technique, which is the acknowledged current ‘gold standard’, and the patients studied were not taking potentially-confounding drug treatment. In non-diabetic subjects, the relationship between serum insulin concentrations (as determined by either assay method) and blood pressure was almost identical to that between euglycaemic clamp-derived insulin sensitivity and blood pressure. Taken with the findings from the San Antonio group, our data suggest that assay specificity is unlikely to account for discrepancies between epidemiological studies in the strength of the relationship observed between serum insulin concentrations and blood pressure. It is likely that such discrepancies are accounted for either by differences in the populations studied, or by lack of standardization of insulin assays between centres. We did not measure proinsulin and partially-processed proinsulin products in the present study, but the relationships examined were almost identical when insulin concentrations were measured with insulin assays of both relatively high and no cross-reactivity with these molecules. Disproportionately elevated insulin concentrations measured with the conventional as opposed to specific insulin assay, suggesting high levels of proinsulin-like molecules, were observed mainly in obese (NIDDM or non-diabetic glucose intolerant) subjects. However, this does not explain the lack of a univariate relationship between serum insulin concentrations and blood pressure in these
subjects, as similar correlation coefficients and confidence intervals were observed when insulin concentrations were measured using the specific assay.

Insulin sensitivity was negatively correlated with blood pressure in both non-diabetic subjects and subjects with NIDDM. However, positive correlations were detected between insulin concentrations and blood pressure only in non-diabetic subjects. This finding was independent of assay method, and is likely to reflect the close physiological relationship between insulin concentrations and insulin sensitivity in non-diabetic subjects, and the failure of the pancreatic β-cell to compensate for insulin resistance in NIDDM. It is in keeping with previous reports suggesting that insulin concentrations per se are unlikely to have an important influence on blood pressure in NIDDM.7

In summary, this study showed no differences attributable to insulin assay specificity in the relationships among serum insulin concentrations, clamp-derived insulin sensitivity, and blood pressure in a group of 56 diabetic and non-diabetic subjects with widely-varying degrees of blood pressure and body mass index. Use of specific assays which have recently become commercially available in future epidemiological studies is unlikely to alter the relationships detected between serum insulin concentrations, insulin sensitivity and blood pressure.

Acknowledgements

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References