Specific Insulin and Proinsulin Concentrations in Nondiabetic South Indians

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The study was performed to determine plasma levels of proinsulin (PI) and specific insulin (SI) in normoglycemic (NGT) Asian Indians and to assess the effect of obesity and impaired glucose tolerance (IGT) on these concentrations. Blood samples from 151 adult nondiabetic South Indian subjects were collected during an epidemiological survey of diabetes. Plasma SI and PI levels were measured in fasting and 30-minute and 120-minute samples of a glucose tolerance test (World Health Organization criteria) using monospecific antibodies. The total insulin (TI) level was also measured by the nonspecific assay. The molar ratio of PI to SI (PI/SI) was calculated. Correlations of the peptides with anthropometry, serum lipids, and blood pressure (BP) were studied by univariate and multivariate analyses. Comparisons were also made in NGT versus IGT groups. As expected, TI values were higher than SI values, but the patterns of response were similar for both. SI and PI responses in NGT were similar to the values found in Mexican-Americans who had a higher body mass index (BMI). Asian Indians were thus found to have a high SI response despite a low BMI. Obesity and IGT produced an increased response of both PI and SI, with normal PI/SI ratios thus showing an absence of hyperproinsulinemia in either condition. Fasting PI showed a strong association with serum triglycerides, and proinsulin at 120 minutes was associated with cholesterol. None of the peptides showed a correlation with BP. Using specific assays for insulin and PI, it is shown that Asian Indians with NGT have a hyperinsulinemic response despite a low BMI. Obesity and mild hyperglycemia in IGT produce a simultaneous increase in PI and SI with no alteration in the PI/SI ratio.

PREVIOUS STUDIES OF INDIANS living in India[1] or in foreign lands[2-6] have shown characteristic features of hyperinsulinemia and insulin resistance. Most of the studies used conventional radioimmunoassays (RIAs) that cross-reacted with proinsulin (PI) and its split products. Therefore, there was an overestimation of insulin in those reports.[5] With the availability of RIA procedures using monospecific antibodies, it is now possible to estimate specific insulin (SI) and PI separately. Some studies have also shown that there could be abnormal cleavage of PI resulting in higher blood concentrations of intact PI and/or its split products, accounting for higher total immunoreactive insulin (IRI) levels. Such metabolic changes have been reported in diabetes,[7-11] impaired glucose tolerance (IGT),[10-15] and obesity,[16] and also in the offspring of diabetic parents.[16-18] There have been no population studies on PI and SI in native Indians. Therefore, this study was performed (1) to determine the normal plasma levels of PI and SI in nondiabetic adults, (2) to search for possible alterations in their concentrations in obesity, and (3) to search for changes associated with mild hyperglycemia as found in IGT.

SUBJECTS AND METHODS

Blood samples from 151 adult South Indian subjects aged 40 years and older, collected during an epidemiological survey of diabetes conducted in 1994 to 1995, were used for this study. Glucose tolerance was determined according to World Health Organization criteria by estimation of fasting and 120-minute plasma glucose following a 75-g oral glucose load.[19] The samples for the study were from every fifth nondiabetic subject in the survey. Fasting, 30-minute, and 120-minute plasma samples were collected for estimation of total insulin (TI), PI, and SI. The fasting lipid profile (total cholesterol, high-density lipoprotein [HDL] cholesterol, and triglycerides) was estimated for all subjects. Each subject in this study provided informed consent.

Age and sex were recorded, and anthropometric measurements including height, weight, and waist and hip girth were made. The body mass index ([BMI] kilograms per square meter) and waist to hip ratio (WHR) were calculated. Systolic and diastolic blood pressure (sBP and dBP) were also recorded.

The plasma glucose level was measured by the glucose oxidase method and lipid levels by enzymatic procedures using Boehringer (Mannheim, Germany) reagents and an Hitachi (Boehringer) 704 autoanalyzer within 5 hours of blood collection. Plasma samples for TI, PI, and SI were stored at −70°C till the time of assay. The TI level was measured by a RIA kit (IRI) from Bhabha Atomic Research Centre (Bombay, India). The method was a modified procedure of Herbert et al[20] with double antibody and polyethylene glycol precipitation.[20] Intraassay and interassay coefficients of variation were less than 5% and 7%, respectively; the sensitivity of the assay was 14.4 pmol/L (2 μU/mL). PI and SI were assayed at the Department of Medicine, University of Texas Health Science Centre at San Antonio, by RIA kits supplied by Linco (St Louis, MO). Monospecific antibodies were used in the assays. The kit for SI uses an antibody that reacts with the free NH_2 terminal of the A-chain of insulin. Intact human PI and des 31,32 human PI do not cross-react significantly (<0.2%). Although the cross-reactivity with des 64,65 PI is much higher (~76%), this product of PI comprises less than 5% of all PI. For SI, the lowest detection limit was 14.4 pmol/L. The Linco human PI RIA uses an antibody made specifically against human PI that recognizes a specific epitope formed by intact A-chain C-peptide junction. Under nonequilibrium conditions, A-chain C-peptide junctional cleaved forms of PI are less than 1% as potent as intact PI, whereas B-chain C-peptide junctional cleaved forms such as des 31,32 PI have a cross-reactivity greater than 95%. Because des 31,32 is the major circulating form of split PI (~95%), the PI RIA used in this study provides an estimate of the total concentration of PI (intact + B-C junctional cleaved forms) in plasma. The lowest detection limit for PI was 2 pmol/L, and intraassay and interassay variations were less than 7% to 11% for both assays.

Statistical Analysis

Group means were compared by unpaired *t* test. Due to skewness, TI, SI, and PI values were logarithmically transformed before analysis. Pearson's correlations for TI, SI, and PI with the anthropometric, metabolic, and hemodynamic variables were calculated. Multiple linear regression analyses were performed to determine the contributory

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parameters for SI and PI responses. The contributions of SI and PI to plasma lipids and BP were also analyzed by separate multiple regression analyses.

RESULTS

Clinical and biochemical characteristics of the study groups are shown in Table 1. Subjects with IGT were older and had higher sBP and dBP. The mean triglyceride level was also higher in IGT, but the difference versus NGT was statistically nonsignificant.

Table 2 shows the TI activity measured by the nonspecific assay, SI measured by the Linco assay, PI, and the molar ratio of PI to SI (PI/SI). Insulin and PI concentrations are expressed in picomoles per liter.

NGT

As expected, TI values were higher than SI values due to cross-reactivity of the antibodies with insulin-like components. However, both showed a similar pattern of response with glucose challenge. SI at 30 minutes was higher than the 120-minute value. PI increased with glucose intake, and the highest value was at 120 minutes. The lowest PI/SI ratios were at 30 minutes.

IGT

Insulin responses (TI and SI) were delayed in IGT, with peak values at 120 minutes, in contrast to the peak at 30 minutes in NGT. TI values were higher in IGT, but a statistically significant difference was present at 120 minutes only. Fasting and 120-minute values for SI and PI were also significantly higher in IGT compared with NGT. PI was highest at 30 minutes, as in NGT. No significant differences were seen in PI/TI ratios in NGT and IGT. The PI/SI ratio at 120 minutes was lower in IGT.

Effect of Obesity

The BMI was significantly correlated with insulin and PI concentrations (Table 3). The WHR correlated with fasting TI and fasting PI only.

PI/SI ratios were similar in non-obese NGT ($\leq$25 kg/m^2, n = 82) and obese NGT (>25 kg/m^2, n = 40) subjects. Fasting PI/SI ratios (mean ± SD) in non-obese and obese subjects were 0.115 ± 0.11 and 0.118 ± 0.08, respectively, and the corresponding 2-hour ratios were 0.11 ± 0.08 and 0.16 ± 0.18 (P = .127).

Table 1. Characteristics of the Study Groups (mean ± SE)

Characteristic	NGT (n = 122)	IGT (n = 29)
Age (yr)	42.7 ± 1.1	49 ± 1.5*
BMI (kg/m^2)	23.7 ± 0.4	23.3 ± 0.6
WHR	0.85 ± 0.01	0.88 ± 0.13
Glucose (mg/dL)		
Fasting	101 ± 1	109 ± 2.4
120 min	106 ± 1.5	162 ± 2.8
Cholesterol (mg/dL)	189 ± 3.2	198 ± 7.6
HDL cholesterol (mg/dL)	43.8 ± 0.9	42.0 ± 1.4
Triglycerides (mg/dL)	135 ± 7.2	169 ± 21.4
sBP	119 ± 1.3	124 ± 2.5†
dBP	77.3 ± 0.8	84 ± 1.0*

*P < .002.
†P < .05.

Table 2. Insulin and PI Profiles in NGT and IGT (mean ± SE, pmol/L)

Profile	NGT (n = 122)	IGT (n = 29)
TI		
Fasting	98 ± 6	110 ± 12
30 min	695 ± 35	748 ± 59
120 min	442 ± 29	846 ± 49*
SI		
Fasting	70 ± 5	81 ± 7†
30 min	399 ± 20	476 ± 40
120 min	258 ± 18	581 ± 49*
PI		
Fasting	7.0 ± 0.52	9.6 ± 1.4†
30 min	15.5 ± 1.0	20.5 ± 3.2
120 min	25.2 ± 1.7	41 ± 5.3*
PI/TI		
Fasting	0.088 ± 0.008	0.102 ± 0.02
30 min	0.026 ± 0.0014	0.029 ± 0.005
120 min	0.070 ± 0.005	0.056 ± 0.009
PI/SI		
Fasting	0.121 ± 0.009	0.117 ± 0.011
30 min	0.043 ± 0.003	0.045 ± 0.006
120 min	0.125 ± 0.011	0.083 ± 0.013†

NOTE. Insulin and PI values were logarithmically transformed before statistical comparison.
*P < .001.
†P < .05.

Correlations With Other Parameters

Table 3 shows the correlations of insulin and PI with anthropometric, metabolic, and hemodynamic variables in NGT subjects.

Table 4 shows multiple linear regression analyses in NGT with SI or PI as the dependent variable. Age, BMI, WHR, and fasting and 120-minute plasma glucose were the independent variables. Fasting SI did not show a significant association with any of the tested parameters. Plasma glucose showed an association with the 120-minute SI. Fasting PI was associated with BMI and 120-minute plasma glucose, and 120-minute PI was associated with BMI.

Table 3. Correlation of Insulin and PI With Clinical and Biochemical Variables in NGT Subjects

Variable	TI		SI		PI	
	Fasting	120 min	Fasting	120 min	Fasting	120 min
Age	−.259†	.074	−.145	.166*	−.196*	−.045
BMI	.418‡	.304‡	.280†	.135	.406‡	.310‡
WHR	.258†	.112	.103	.045	.185*	.139
Fasting glucose	−.097	.130	−.029	.096	−.068	.061
120-min glucose	−.023	.330‡	−.079	.402‡	−.302‡	.137
Cholesterol	−.006	.246†	−.035	.248†	.056	.249†
HDL cholesterol	−.288†	.005	−.209*	.056	−.142	.010
Triglycerides	.207*	.269†	.135	.132	.463‡	.383‡
sBP	.032	.201†	.090	.075	.189*	.071
dBP	.059	.156	.123	.038	.078	−.052

*P < .05.
†P < .01.
‡P < .001.

Table 4. Multiple Linear Regression Analysis (forward method) in NGT

Parameter	β	SEβ	*P*	R^2 (%)
SI 120 min				
2-h plasma glucose	4.6	1.03	.0000	18.8
PI fasting				
BMI	0.48	0.15	.0017	10.4
2-h plasma glucose	−0.104	0.036	.0056	8.0
PI 120 min				
BMI	1.64	0.54	.0032	8.7

NOTE. Variables significantly associated with SI and PI are shown. Independent variables tested: age, BMI, WHR, and fasting and 2-hour plasma glucose.

Abbreviations: β, coefficient; SEβ, standard error of β.

Table 5. Multiple Linear Regression Analysis (forward method): Effect of SI and PI on Metabolic Parameters

Parameter	β	SEβ	*P*	R^2 (%)
Triglycerides				
Fasting PI	6.76	1.45	.0000	21.8
Cholesterol				
120-min PI	0.48	0.2	.018	6.9

NOTE. Independent variables tested: age, BMI, WHR, fasting and 2-hour plasma glucose, fasting and 120-minute SI, and fasting and 120-minute PI.

The serum triglyceride concentration was associated only with fasting PI, as shown by a multiple linear regression analysis in which age, BMI, WHR, plasma glucose, SI, and 120-minute PI also were included as independent variables (Table 5). A similar analysis for cholesterol showed that only 120-minute PI contributed to its variation. None of the tested parameters showed an independent contribution to the variation in sBP and dBP.

DISCUSSION

In NGT subjects, plasma PI increased together with SI after a glucose load, but its peak was seen later at 120 minutes. This could be mostly due to a slower metabolic clearance of PI as compared with insulin.[21]

SI and PI responses in nondiabetic Indians were similar to values found in Mexican-Americans.[18] The assay procedure was the same in Indians and Mexican-Americans. Asian Indians have high SI values during fasting and in response to glucose, like Mexican-Americans, despite lower BMI values. Previous studies had shown that nondiabetic Asian Indians had hyperinsulinemic responses compared with Europeans.[1-5] Results of this study showed that hyperproinsulinemia did not contribute to hyperinsulinemia, and Asian Indians therefore had a true hyperinsulinemic response. Nagi et al[5] reported a similar finding in Asian Indians in the United Kingdom. The assay procedures used in our study differed from the procedures used in their study. However, SI and PI values obtained in the two studies were comparable.

The BMI had a significant positive correlation with all of the peptide concentrations. PI/SI ratios were similar in obese and non-obese NGT subjects, indicating that hyperinsulinemia in obesity was due to hypersecretion of both SI and PI. These findings agreed with reports by Shiraishi et al[15] and Duckworth et al[22] that obesity alone did not produce a disproportionate increase in PI circulation. Saad et al[9] reported that fasting PI to insulin ratios decreased with increasing body mass in Pima Indians. A similar observation was made by Haffner et al[18] in nondiabetic Mexican-Americans. The BMI of study subjects in these reports was significantly higher than the BMI of subjects in our study.

We did not observe an increased PI/SI ratio in IGT also. PI and SI showed a simultaneous increase in IGT, as reported previously by Saad et al[9] in Pima Indians. Most previous reports had shown higher PI/SI ratios in IGT, including studies by Haffner et al[11,18] in the San Antonio population, Yoshioka et al[12] and Shiraishi et al[15] in Japanese, and Reaven et al[13] and Davies et al[14] in a white population.

SI and PI showed statistically significant associations with several parameters such as BMI and 2-hour plasma glucose. However, it should be mentioned that the noted correlations were weak, as shown by the R^2 values in Table 4. SI and PI had a positive association with BMI, but no association with WHR. In an earlier population survey, we did not observe an association of TI with WHR.[1] This finding also was different from reports in Mexican-Americans[23] showing strong correlations between the WHR and the insulin responses. In Asian Indians, overall adiposity appeared to influence insulin secretion more than regional fat distribution.

Fasting PI showed a strong association with triglyceride levels. Similar results were found in Mexican-Americans by Haffner et al.[23] However, in contrast to Mexican-Americans, PI values were not correlated with BP in Asian Indians. PI at 120 minutes showed a positive correlation with total cholesterol.

To our knowledge, this is the first population study on SI and PI responses in native Asian Indians. It is confirmed that Asian Indians have true hyperinsulinemic responses despite a low BMI. Hyperproinsulinemia is not a feature of normoglycemic hyperinsulinemic subjects. Obesity produces higher secretion of both of the peptides with no change in their ratios. Mild hyperglycemia found in IGT is not associated with altered PI secretion. Changes in diabetic individuals need to be studied.

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