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Serum insulin & C-peptide responses in individuals with impaired glucose tolerance & diabetes

C. Snehalatha, V. Mohan, A. Ramachandran, R. Jayashree & M. Viswanathan

Diabetes Research Centre & M.V. Hospital for Diabetes, Madras

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Serum concentrations of immunoreactive insulin (IRI) and C-peptide (CP) were measured during oral glucose tolerance test, in individuals with impaired glucose tolerance (IGT) and in non-insulin dependent diabetic (NIDDM) patients. Molar ratios of IRI/CP × 100 (p mol/ml) were also calculated. The results were compared with weight-matched controls. Beta cell function was found to be low in persons with IGT, as shown by low CP concentration. The impairment in B cell function was more marked in individuals with diabetes. The IRI concentrations were disproportionately high for the corresponding values of CP and thus the IRI/CP ratios were elevated. The alteration in the IRI/CP ratios occurred in the non-obese as well as obese NIDDM. However, obese patients had more marked elevation of the ratios. Maximum elevation of the ratios was seen in the group with IGT. The increased IRI/CP ratios probably indicates decreased hepatic extraction of insulin. The study thus showed that decreased hepatic insulin extraction occurred in persons with glucose intolerance and this alteration depended on the severity of hyperglycaemia as well as on the body weight of the patient.

With the advent of radio immunoassays for insulin (IRI) and C-peptide (CP), considerable information has been obtained on the beta cell function in diabetes[1-3]. Recently it has been demonstrated[4-6] in NIDDM patients that the hepatic extraction of insulin is diminished in hyperglycaemic conditions. These observations are supported by similar findings in experimental animals[7,8]. While the CP concentration in plasma measures the B cell secretion, the molar ratio of IRI/CP helps to evaluate the metabolic fate of insulin in blood, especially its hepatic extraction, as a major proportion is removed by the liver[9]. This paper presents the IRI and CP concentrations in individuals with impaired glucose tolerance and overt diabetes mellitus.

Material & Methods

This study was done on 92 individuals with either diabetes or impaired glucose tolerance and in 17 non-diabetic, control subjects with no family history of diabetes. Obese and non-obese patients were compared with weight matched controls. Oral GTT with 75 g of glucose load was performed in all study groups. The patients

stopped all anti-diabetic drugs at least 3 days prior to the GTT. The WHO expert committee criteria were used for diagnosis of impaired glucose tolerance and diabetes[10]. Individuals with impaired glucose tolerance (IGT) were classified as group I. The NIDDM patients were divided into 2 sub-groups *viz.*, patients with 2 h plasma glucose of <250 mg/dl during the GTT were classified as group II and those with 2 h glucose >250 mg/dl as group III. The plasma glucose was estimated by O-toluidine method.

All the study subjects were free from renal and hepatic disease as indicated by normal levels of blood urea, creatinine, creatinine clearance, 24 h proteinuria, SGOT, SGPT, alkaline phosphatase, serum albumin : globulin ratios and thymol turbidity.

The body mass index (BMI) was calculated using the formula: weight in kg/height in metres^2. Those with BMI of 27 or more were considered obese.

The clinical details of the study groups are shown in Table I.

Blood samples were collected in EDTA for hormonal assay and plasma was kept frozen at $-20°C$ for the assays. Immunoreactive insulin (IRI) was estimated by the method of Herbert *et al*[11] and C-peptide (CP) was estimated by the method of Heding[12]. The intra-and inter-assay coefficients of variations for IRI were 6·6 and 10·8 per cent respectively. The respective variations for CP were 5·2 and 11·2 per cent. Recovery of standard C-peptide added to serum samples varied between 85 to 114 per cent. IRI was estimated in all the five samples during the GTT. CP was estimated in the fasting, 60, 90 and 120 min samples of plasma. The

Table I. Clinical details of the study groups
(Data are mean ±SE)

Group	No.	M/F	Mean age (yr)	Body mass index	Plasma glucose mg/dl, at min				
					Fasting	30	60	90	120
Non-obese :									
Controls	11	8/3	36·2±3·6	21·5±0·6	87±3	124±4	117±6	103±6	79±9
Group I	15	10/5	35·1±2·5	20·8±0·8	102±2	160±3	175±20	164±3	144±6
Group II	13	9/4	34·2±3·0	22·0±0·5	137±8	206±17	259±7	285±9	233±13
Group III	35	22/13	36·4±1·4	21·6±0·3	219±10	298±11	358±11	366±11	364±12
Obese :									
Controls	6	4/2	32·3±4·3	30·1±1·2	89±5	133±8	141±8	125±5	111±4
Group I	6	4/2	36·0±2·2	31·2±0·9	104±7	161±9	188±8	172±11	145±9
Group II	15	10/5	35·6±1·7	33·6±10·7	120±5	206±7	238±7	231±19	214±11
Group III	8	5/3	37·0±2·8	31·9±1·1	169±19	284±15	343±20	332±17	286±26

sample at 30 min was omitted as the peak CP value was always obtained after 60 min. The IRI/CP $\times 100$ ratios[4] (p mol/ml) were calculated at the four time intervals.

Student's 't' test was used for statistical evaluation.

Results

Non-obese individuals : Fig. 1 shows the IRI responses in the non-obese group. A gradual transition from an elevated IRI response to a low response was observed from IGT to severe diabetes. The IRI values were significantly elevated at all time intervals in IGT (group I). In group II, the IRI values were nearly normal except at the fasting and at 120 min when they were higher ($P<0\cdot001$). In group III, the mean IRI response was significantly reduced ($P<0\cdot001$).

The corresponding C-peptide response in the non-obese group is seen in Fig. 2.

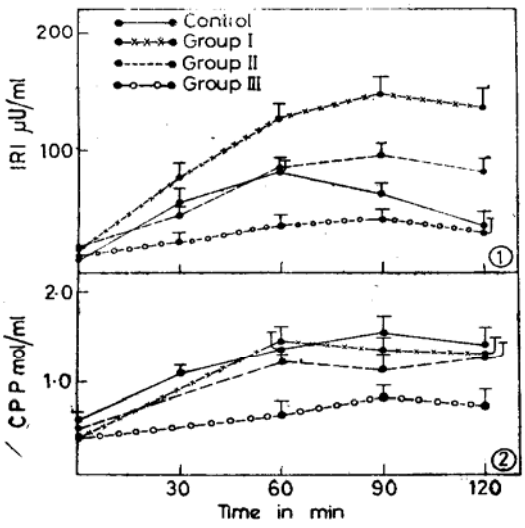

Fig. 1. Insulin response in non-obese study groups.
Fig. 2. C-peptide responses in non-obese groups.

In groups I and II the CP responses were low; but the differences were not statistically significant from control. In group III a significant ($P<0\cdot001$) reduction in CP concentration was seen. Thus the CP responses were lower than controls in all the three groups, but the values decreased from IGT to severe diabetes.

Table II shows the IRI/CP $\times 100$ ratios in these individuals. The ratios were significantly elevated in IGT. In group II also the ratios were elevated but to a lesser degree than in IGT. In group III, the ratios were elevated ($P<0\cdot01$) only at the fasting and at 120 min.

Obese individuals : The obese controls had significantly higher insulin responses than the non-obese controls. In obese IGT, the IRI response was very similar to that in the controls, except for a delayed peak response (Fig. 3). The values gradually decreased with increasing hyperglycaemia and in the group III, the values were significantly low at 30 and 60 min ($P<0\cdot001$).

Fig. 4 shows the CP responses in the obese individuals. Obese controls showed higher CP response compared to the non-obese controls. However, interestingly, all obese hyperglycaemic individuals in groups I, II and III had lower fasting CP concentration than their non-obese counterparts unlike the insulin responses. The CP concentration was low even in individuals with IGT and the reduction was greater with increasing severity of carbohydrate intolerance. Obese and non obese patients in group III with severe diabetes had comparable C-peptide values.

The IRI/CP ratios in obese controls were significantly higher than in non-

Table II. IRI/CP×100 molar ratios in the different study groups
(Data are mean±SE)

Group	Non-obese IRI/CP×100 at min				Obese IRI/CP×100 at min			
	Fasting	60	90	120	Fasting	60	90	120
Control	17±2	43±4	32±4	23±3	22±2	48±4	46±5	39±6
Group I	41±11[a]	102±21[b]	102±25[b]	114±23[c]	49±4[c]	142±54	216±39[d]	131±53
Group II	47±17[b]	65±20	74±13[c]	63±11[c]	84±12[c]	148±22[c]	140±21[c]	115±22[c]
Group III	31±5[b]	49±7	42±6	43±7[c]	122±21[c]	176±37[c]	134±18[c]	130±13[c]

P values, [a]<0·05; [b]<0·01; [c]<0·001; [d]<0·002. Others not significant

Fig. 3. Insulin response in obese groups. Fig. 4. C-peptide responses in obese study groups.

obese controls ($P<0·001$ at all time intervals except at 60 min $P<0·05$).

In the obese patients, the IRI concentrations were disproportionately high for the low CP values. Hence IRI/CP ratios were very much elevated in all the 3 groups ($P<0·001$; Table II).

Discussion

This study shows that beta cell secretion is lower in all individuals with hyperglycaemia. In those with IGT, both the

fasting and stimulated C-peptide responses are diminished in obese and non-obese individuals. The impairment in B cell function increases with increasing severity of carbohydrate intolerance. Obesity appears to aggravate this defect.

Using IRI as a measure of the beta cell secretion it would appear that hyperinsulinaemia is a feature of mild hyperglycaemia[13,15]. In marked hyperglycaemia, hypoinsulinaemia is present. Evaluating the B cell secretion in the light of the IRI and CP responses, the fallacies in the previous observations become apparent. The IRI concentrations are high in IGT and nearly normal in mild diabetes in spite of the corresponding CP concentrations being low. This phenomenon, highlighted by the elevated IRI/CP ratios, probably indicates a compensatory mechanism to maintain normal insulin levels. This is most likely brought about by decreased hepatic extraction of insulin. This compensatory mechanism appears to break down eventually as severe diabetes sets in. The concentrations of IRI and CP are low in patients with severe diabetes and the IRI/CP ratios are nearly normal.

Decreased hepatic extraction of insulin in diabetes is suggested by the results of our study as well as others[5,6]. Our study, though similar to that of Bonora *et al*[6] differs from it in certain respects. We observed diminished B cell secretion even in IGT whereas Bonora *et al*[6] have noticed higher CP concentration in obese and non-obese IGT. In their study, altered ratios of the two peptides at the fasting state was present only in the obese patients as against our observation of this feature in all hyperglycaemic individuals. Jayyab *et al*[4] also observed that the IRI/CP ratios were elevated in the fasting state in the obese and non-obese NIDDM compared to the weight matched controls. However, they observed no changes in the IRI/CP ratios during iv glucose administration whereas iv glucagon administration produced further changes in the ratios in obese patients. The hepatic extraction of insulin appears to be lower in the obese controls as the IRI/CP ratios are significantly higher than in non-obese controls. However, the hyperinsulinism in simple obesity is mostly pancreatic in origin as the C-peptide concentrations are also much higher than in the non-obese.

The IRI/CP ratios are elevated to a greater extent in the obese hyperglycaemic individuals than in the non-obese. Whether this is related to a lower hepatic extraction of insulin and/or also related to the lower number of insulin receptors available in the obese individuals, for binding and degradation of the hormone[16] at the periphery remains to be clarified. The apparent hyperinsulinism seen in IGT is not pancreatic in origin as the IRI/CP ratios are elevated. The difference in the pattern of IRI and CP responses and the IRI/CP ratios in the obese and non-obese patients indicate heterogeneity in the metabolism of insulin in NIDDM patients.

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Reprint requests : Dr M. Viswanathan, Director, Diabetes Research Centre and M.V. Hospital for Diabetes, 5 Main Road, Royapuram, Madras 600013