β cell reserve in cystic fibrosis patients and heterozygotes

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SUMMARY An assessment of β cell and exocrine pancreatic reserve based on fasting C peptide and immunoreactive trypsin (IRT) concentrations was carried out in non-diabetic (fasting blood glucose < 6 mmol/l) patients with cystic fibrosis (CF; $n = 20$) and their parents (heterozygotes; $n = 26$). These indices were also measured in comparable control subjects ($n = 20$) and insulin-dependent diabetics (IDDs). C peptide concentrations in 18 CF patients were markedly lower than those in controls, and in 18 were comparable to those in IDDs ($n = 27$). Fasting C peptide concentration in heterozygotes was also significantly lower than that in controls and was significantly greater than that in IDDs. IRT concentrations in CF patients and heterozygotes were also significantly lower than those in controls. The frequency of subnormal C peptide concentrations was greater in CF patients with non-measurable IRT than in those who had residual IRT in their sera.

These data show for the first time that the diminution in β cell reserve in CF patients may be of a magnitude similar to that in IDDs in spite of their normal blood glucose and HbA_{1c} concentrations. Therefore, these patients may have enhanced insulin sensitivity. CF heterozygotes too have a significant diminution in endocrine and exocrine pancreatic reserve when compared with the controls.

It has been shown recently that the exocrine pancreatic function in insulin dependent diabetics (IDDs) is significantly lower than that in normal subjects and that the degree of loss of exocrine pancreatic reserve is related to the diminution in β cell function in these patients.^{1,2} There have also been data to show that the residual exocrine pancreatic reserve in chronic pancreatitis is related to the residual β cell function.³ We hence undertook a study to establish whether the residual β cell function was related to the exocrine pancreatic reserve in patients with cystic fibrosis (CF). We also took this opportunity to study 26 parents (heterozygotes) of CF patients. Since fasting peptide concentrations have been shown to correlate with the magnitude of C peptide release following a maximal stimulatory challenge with glucagon,⁴ we used this biochemical index as the indicator of β cell reserve in this study. This facilitated the study of a large number of patients and their parents.

Material and methods

Twenty patients (16 males and 4 females; age range: 16 to 46 yr) with CF but no evidence of diabetes mellitus, were included in this study. All had typical clinical features of this condition with chronic pulmonary involvement. All except three had steatorrhoea for which they were on pancreatic enzyme supplements. The diagnosis of CF had been established in all patients following the demonstration of raised concentrations (> 70 mmol/l) of sodium and chloride in the sweat.

Twenty-six parents of the patients included in this study were also investigated. Each parent of a patient with this condition is by definition a heterozygote since it has an autosomal recessive mode of transmission. The age range of heterozygotes was 35-68 yr; 11 heterozygotes were men and 15 were women.

Twenty-seven IDDs (15 men and 12 women; age range: 20-60 yr) and 20 control subjects (12 men and 8 women; age range 20-61 yr) were also included in the study for comparisons to be made.

Fasting blood samples were obtained from all

these patients and allowed to clot. They were then centrifuged and the separated serum was frozen at -20°C . Heparinised samples were used for $\text{HbA}_{1\text{c}}$ measurement.

Serum immunoreactive trypsin (IRT) and C peptide concentrations were measured by specific radioimmunoassays duplicate. Kits were obtained from Hoechst and Novo for IRT and C peptide measurements respectively. IRT was measured according to the methodology previously described⁵ and C peptide according to the methodology of Hed- ing.⁶ Glycosylated haemoglobin ($\text{HbA}_{1\text{c}}$) was measured by an electrophoretic technique (Corning). Plasma glucose concentration was measured by a Beckmann autoanalyser.

Statistical comparisons were made by Student's *t* test and Mann Whitney U tests for parametric ('C' peptide) and non-parametric (IRT) data respectively.

Results

All patients had a fasting blood glucose concentration of less than 6 mmol/l and an $\text{HbA}_{1\text{c}}$ of less than 7% (normal reference range: 5.8 to 8.0%).

C PEPTIDE CONCENTRATIONS

The mean C peptide concentration (0.139 ± 0.037 nmol/l) in CF patients was markedly lower ($p < 0.001$) than that in the controls (0.240 ± 0.04 nmol/l; see Table). In all but three patients the C peptide concentration was less than the lower end of the normal reference range. Although the mean C peptide concentration of CF patients was significantly higher ($p < 0.01$) than that of IDD (0.08 ± 0.04 nmol/l) selected at random, in all except two of them, C peptide concentration was within 2 SD (less than 0.18 nmol/l) of the mean C peptide level of the group with IDD. Therefore, 18 of 20 CF patients had C peptide concentration similar to those of IDD.

C peptide concentrations in heterozygotes (0.172 ± 0.024 nmol/l) were significantly lower ($p < 0.001$) than those in controls and significantly greater than those in CF patients ($p < 0.01$). In seven heterozygotes, C peptide concentration was within 2 SD of

Distribution of IRT concentration in sera of CF patients, heterozygotes and controls. IRT in CF patients and heterozygotes was significantly lower than that in controls ($p < 0.001$ and < 0.05 respectively). Also see reference 7 for analysis of larger CF population.

the mean for the IDD group. Therefore, seven of 26 CF heterozygotes had C peptide concentrations comparable to those of IDDs. The C peptide concentrations in male and female heterozygotes were similar.

IRT CONCENTRATIONS

The concentration of IRT in the sera of CF patients and other groups, was non-parametrically distributed. IRT in CF patients (median: 0; range: 0–90 $\mu\text{g/l}$) was significantly lower ($p < 0.001$) than that in the controls (median: 260; range: 160–330 $\mu\text{g/l}$) heterozygotes (median: 199; range: 89–349 $\mu\text{g/l}$) and IDDs (median: 163; range: 72–230 $\mu\text{g/l}$). IRT in CF heterozygotes was significantly lower than that

Serum C peptide and IRT concentrations in cystic fibrosis (mean $\pm$ SD)

	IRT ($\mu\text{g/l}$) (median and range)	C peptide (nmol/l) (mean $\pm$ SD)
Controls (n = 20)	260 (160–340)	0.240 ± 0.048
CF homozygotes (n = 20)	0 (0–90)	$0.139 \pm 0.037^{*}\ddagger$
CF heterozygotes (n = 26)	199 (89–349) $\ddagger$ §	$0.172 \pm 0.024^{*}$
Insulin-dependent diabetics (n = 27)	163 (72–230) $\ddagger$ §	$0.08 \pm 0.04^{*}$

* $p < 0.001$ when compared with controls.

$\ddagger p < 0.05$ when compared with controls.

$\ddagger p < 0.01$ when compared with CF heterozygotes.

§ $p < 0.01$ when compared with CF homozygotes.

in controls ($p < 0.05$) but was similar to that in IDD. There was no difference in IRT concentration of males and females and IRT in IDDs was significantly lower than that in controls ($p < 0.001$).

RELATION OF C PEPTIDE AND IRT CONCENTRATIONS

The frequency of subnormal C peptide concentrations (<0.144 nmol/l) was significantly greater ($p < 0.02$) in those CF patients who had no detectable IRT in their sera (10 of 14) than in those who still had detectable IRT (1 of 6). There was however, no linear correlation between IRT and C peptide concentrations in either CF patients or heterozygotes.

Discussion

Our assessment of β cell reserve in these patients and their parents is based on fasting C peptide concentrations.

That this measurement is a valid indicator of β cell reserve is supported by the demonstration that fasting C peptide concentrations are significantly related to the concentrations of C peptide generated by a supramaximal glucagon challenge.⁴

Three important facts emerge from our study. Firstly, in CF patients serum C peptide concentrations were significantly lower than those in controls. In all but two of these patients, C peptide concentrations were comparable to those found in IDDs, this in spite of the fact that a majority of these patients had normal fasting blood glucose concentrations and had no clinical features of diabetes. Although there have been reports in the past that β cell reserve is diminished in patients with CF^{8,9} our observation that this diminution is often comparable to that in IDDs is a new one. Furthermore, we have shown that in spite of the marked lowering of the β cell reserve, the fasting blood glucose and HbA_{1c} remain normal.

These data are of interest since they suggest that these patients may have an enhanced insulin sensitivity. Malnutrition due to malabsorption may also account for the diminished requirements of insulin such that the impaired β cell function does not result in diabetes. In this respect, they are similar to patients with chronic pancreatitis and steatorrhoea.³ Further studies are being undertaken to establish whether the insulin receptor population on the cells of these patients is altered, as is known to occur in patients with anorexia nervosa with profound weight loss.¹⁰

Secondly, low C peptide values are more frequent in patients with no exocrine pancreatic reserve although there is no direct correlation between serum IRT and C peptide concentrations. There is thus a destruction of pancreatic islets in parallel with

that of pancreatic acini although the damage to pancreatic acini is much greater than that to pancreatic β cells. The absence of a direct correlation between C peptide and IRT concentrations may reflect the extremely skewed distribution of IRT concentrations: 14 of 20 patients had no detectable IRT in their serum.

Thirdly, the most intriguing observation is that the serum C peptide concentration in heterozygotes of CF was significantly lower than that in the normal population and greater than that in patients (homozygotes) with CF. It is also noteworthy that the median IRT concentration in heterozygotes was lower than that in normal subjects. Therefore the heterozygote state is associated with some loss of endocrine and exocrine pancreatic reserve. In view of the considerable overlap of both IRT and C peptide concentrations of heterozygotes in comparison with normal controls, it is unlikely that either of these two indices measured could be developed into a test for heterozygosity. However, the abnormality of these two indices in heterozygotes of CF, hitherto unreported, point to a subtle alteration in their pancreas. This area requires further critical assessment.

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