Isoamylases in Diabetes Mellitus

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Summary. We have evaluated the relationship between diabetes mellitus and exocrine pancreatic insufficiency in diabetic subjects by measuring a specific isoamylase arising from the pancreas. The pancreatic- and salivary-type isoamylase activity in serum and urine were evaluated and related to duration of disease, sex, age, weight, blood glucose level and glycosuria in 153 diabetics. In the insulin-dependent diabetics diagnosed between 15 and 24 years of age, a significant decrease in pancreatic isoamylase activity was found in serum and urine (means with 2 SD range, 50 U/l, 19–137 U/l, and 47 U/l, 4–607 U/l, respectively), as compared with control subjects (79 U/l, 45–140 U/l, and 183 U/l, 43–789 U/l, respectively). In this group of patients a low stimulated output of amylase into the duodenum was also observed (mean with range, 537 U, 87–1808 U), compared with controls (the lower limit of normal 2183 U). A significant positive correlation was found between stimulated output of amylase into the duodenum and pancreatic isoamylase activity in serum ($r = 0.84$, $p < 0.01$). In the non-insulin dependent diabetics, a significant negative correlation was found between blood glucose levels and pancreatic isoamylase activity in serum ($r = -0.46$, $p < 0.01$). In the diet-treated non-insulin dependent diabetics the pancreatic isoamylase activity in serum was increased compared with controls (mean and 2 SD range, 102 U/l, 49–211 U/l). In patients with blood glucose level above 12 mmol/l, however, a lowered pancreatic isoamylase activity in serum, and no apparent rise in serum immunoreactive insulin in glucose loading test was observed. The results suggest that production of pancreatic amylase is related to endocrine pancreatic function in diabetes mellitus.

Key words: Amylase isoenzymes, serum amylase, urine amylase, insulin dependent diabetes, non-insu-

lin dependent diabetes, exocrine and endocrine pancreatic function.

Abnormal exocrine pancreatic function has been reported in some diabetics [1, 2]. Recently, serum and urinary amylase originating from pancreas have been used as a particularly useful index for pancreatic function [3, 4]. This prompted us to evaluate the significance of amylase isoenzyme determination in diabetes mellitus.

Patients

Measurements were made in 153 diabetics. The group of insulin-dependent diabetics (IDD) consisted of 18 patients with diabetes diagnosed between 15 and 24 years of age (IDD, group a) and 57 patients with diabetes diagnosed after 25 years of age (IDD, group b). The respective doses of insulin, mostly Insulin and Interdep Insulin, SPOFA, ČSSR (mean with range) were 88 (32–320) and 57 (2–120) units in the IDD groups a and b, respectively. Thirty four diabetics were treated by oral agents (sulphonylureas) and 44 patients were treated by diet only (non-insulin dependent diabetes, NIDD). The control group consisted of 30 volunteers, mostly blood donors.

Subjects with elevated creatinine levels, renal stone disease, diseases of the digestive tract, pancreas and hepatobiliary system, endocrine failures or diseases other than diabetes, malignant diseases and patients taking other agent known to interfere with metabolism of pancreas or gut, were excluded from the study.

Informed consent was obtained from all the subjects for the study.

Methods

Fasting sera and random urine samples collected in the morning for amylase activity were examined on the same day. The total activity of amylase (EC 3.2.1.1) in serum and urine was deter-

Table 1. Characteristics of the population with diabetes mellitus

Group	Number of cases	Age (years)	Weight (kg)	Duration of diabetes	Blood glucose (mmol/l)	Glucose output in urine (mmol/24 h)
IDD						
Group a	18	29 17–53	71 55–99	15 6–36	13.6 ^a 4.9–38.2	19 ^a 0–23120
Group b	57	59 23–77	78 53–122	17 1–43	13.2 ^a 7.9–22.2	52 ^a 0–7762
NIDD						
Oral agents	34	57 34–71	77 56–97	7 2–20	12.1 ^a 6.8–21.4	47 ^a 0–6208
Diet	44	63 39–79	77 55–106	3 0–9	8.0 ^b 4.7–13.7	1 ^b 0–393
Control	30	45 16–77	74 54–82	0	4.5 3.6–5.6	

Figures for age, weight, duration of diabetes are averages with range. Figures for blood glucose and glucose output are averages and 2 SD range, statistical analysis of results was performed on the assumption of log-normal distribution of the values

^a Probability by use of one-way analysis of variance and covariance, as compared with diet treated NIDD and controls, $p < 0.005$

^b Probability as compared with controls, $p < 0.005$

Table 2. Total amylase activities and activities of pancreatic- and salivary-type isoamylases (U/l) and of amylase clearance ratios (ACCR) in diabetics and in control subjects

	Control subjects n = 30	IDD group a n = 18	IDD group b n = 57	NIDD oral agents n = 34	NIDD diet n = 44
Serum amylase					
Total	154 94–252	112 ^c 48–261	137 ^c 70–270	133 55–322	165 ^{b,c} 86–319
Pancreatic	79 ^{b,e} 45–140	50 ^{a,d,e} 19–137	73 ^c 35–152	78 ^b 32–200	102 ^{a,b,c} 49–211
Salivary	71 32–159	57 19–173	61 24–155	52 17–155	59 23–151
Urine amylase					
Total	290 ^{b,c,d} 82–1035	88 ^{a,e} 11–676	141 ^a 36–545	150 ^a 27–830	200 ^b 42–957
Pancreatic	183 ^b 43–789	47 ^{a,e} 4–607	86 18–423	91 12–710	140 23–851
Salivary	86 ^b 18–402	33 ^a 4–286	48 13–186	52 13–209	51 12–209
ACCR	1.4 0.5–3.4	0.9 ^{c,d} 0.3–2.2	1.4 0.5–4.5	1.4 0.5–3.5	1.3 0.5–3.4

Figures are means and 2 SD range

^a Probability by use of one-way analysis of variance and covariance, as compared with control subjects, $p < 0.005$

^{b,c,d,e} Probability as compared with the denoted group (^bIDD group a, ^cIDD group b, ^dNIDD oral agents, ^eNIDD diet), $p < 0.005$

Statistical analysis of results was performed on the assumption of log-normal distribution of the values

mined by the method of Ceska [5] with blue starch substrate (Phadebas, Pharmacia Diagnostics, Uppsala). The activity of the pancreatic- and salivary-type isoamylase was determined after their separation by ion-exchange chromatography, using DEAE-cellulose minicolumn [6]. The control sera (Precipath E, Boehringer, Mannheim) were used as a day-to-day control. The coefficients of variation of the assays for total activity and pancreatic- and salivary-type isoamylases were 2.3%, 4.4%, and 5.8%,

respectively. The total amylase and isoamylases activities, together with serum and urine creatinine concentration [7] were used to calculate the amylase/creatinine clearance ratio (ACCR), expressed as a percentage [8].

Glucose in plasma and 24 h urine collections was determined by the glucose oxidase method on a Beckman glucose analyser.

A glucose loading test was performed in 17 diet treated NIDD patients. Blood samples were collected just before IV administra-

tion of glucose (0.33 g/kg) as well as 10 and 20 minutes later. Serum insulin levels were determined by radioimmunoanalysis as described previously [9], coefficient of variation for the interassay < 10%.

Pancreatic secretion was stimulated by secretin and pancreozymin (100 units, Boots), and total volume, bicarbonate, lipase and amylase output into the duodenum were evaluated in duodenal fluid collected during the 80-minute period, as described previously [10]. The duodenal aspirate was assayed for bicarbonate by the van Slyke manometric method, and for amylase and lipase using photometric methods [11, 12]. The lower limit of normal for our laboratory for stimulated secretion is total volume of 110 ml, bicarbonate output of 9 mmol, and amylase and lipase output of 2183 U and 5 U, respectively.

Statistical description of data, one way analysis of variance and covariance, and stepwise regression analysis was performed using BMDP 7D, 1V, and 2R programme, respectively, of the Health Science Computing Facility, University of California, Los Angeles [13]. In diabetes mellitus, the distributions of observed values of plasma glucose and activity of serum amylases were skewed to the right, but this was corrected in each case by logarithmic transformation of the values.

Results

Clinical and biochemical data for IDD and NIDD patients and controls are shown in Tables 1 and 2.

In the whole population of diabetics, a significant negative correlation was found between blood glucose (x) and activity of P-isoamylase in serum (y), $\log y = -0.55 \log x + 2.47$, $r = -0.45$, $p < 0.01$. Similarly, a significant negative correlation was found between blood glucose levels and activity of urinary pancreatic isoamylase, and between glycosuria and pancreatic isoamylase activity in serum ($r = -0.34$, $p < 0.01$, and $r = -0.38$, $p < 0.01$, respectively). This reflects known relationships between pancreatic isoamylases in serum and urine, and between blood glucose levels and glycosuria (in our patients $r = 0.54$, $p < 0.01$, and $r = 0.69$, $p < 0.01$, respectively). The serum activity of pancreatic isoamylase, however, behaved differently in various types of diabetes.

In the IDD diagnosed between 15 and 24 years of age, the pancreatic isoamylase activity was significantly decreased, as compared with the sex, age, and weight matched controls. This can explain the tendency for the total amylase activity and ACCR to be lowish in this group. In IDD, however, no significant correlation was found between blood glucose levels (x) and serum activity of pancreatic isoamylase (y), $\log y = -0.27 \log x + 2.12$, $r = -0.20$. In this group, the salivary-type isoamylase activity was decreased in urine, as compared with controls (Table 2).

In order to evaluate the exocrine pancreatic function in IDD group a, we compared the serum activity of pancreatic isoamylase with stimulated amylase output into the duodenum in ten patients. After

Fig. 1. Relationship between the simulated amylase output into the duodenum and pancreatic isoamylase activity in serum in ten insulin-dependent diabetes diagnosed between 15 and 24 years of age. Dotted lines indicate the lower limit of normal. $\log y = 0.44 \log x + 0.42$, $r = 0.84$, $p < 0.01$

stimulation, the duodenal volume and output of bicarbonate, lipase, and amylase was subnormal in 10, 9, 4, and 10 patients, respectively, and the respective means with ranges were 70 (34–102) ml, 4.0 (0.7–14.6) mmol, 5.3 (1.2–18.3) U, and 537 (77–3737) U. Figure 1 shows a significant positive correlation between stimulated amylase output into the duodenum and serum activity of pancreatic isoamylase ($r = 0.84$, $p < 0.01$). In these patients a significant positive correlation was also found between stimulated lipase output into the duodenum and serum activity of pancreatic isoamylase ($r = 0.64$, $p < 0.05$). The activity of serum pancreatic isoamylase did not correlate with duodenal volume and/or with bicarbonate output into the duodenum in these patients.

In diet-treated NIDD the activity of pancreatic isoamylase in serum was significantly increased, as compared with that in properly matched set of controls. As in the other groups of diabetics, statistical analysis for variables in the diet-treated patients did not reveal any significant influence of the duration of disease, sex, age, and weight of patients.

In NIDD, a significant negative correlation was found between blood glucose levels and the activity of pancreatic isoamylase in serum ($r = -0.46$, $p < 0.01$) (Fig. 2). This was evident in the diet-treated patients ($r = -0.45$, $p < 0.01$), as well in oral agents-treated NIDD ($r = -0.34$, $p < 0.05$). In stepwise regression analysis for variables in the diet-treated patients, with pancreatic isoamylase as a

Fig. 2. Relationship between the blood glucose levels and pancreatic isoamylase activity in serum in 34 diet-treated non-insulin dependent diabetics (Δ), and in 44 oral agent-treated non-insulin dependent diabetics ($\circ$). The solid bars indicate the normal ranges (± 2 SD). $\blacktriangledown$ and $\triangledown$, diet-treated non-insulin dependent patients in which an increase and no rise, respectively, was observed in serum immunologically measureable insulin levels in the glucose loading test. $\log y = -0.59 \log x + 2.54$, $r = -0.46$, $p < 0.01$

dependent variable, the blood glucose gave a significant increase in multiple R-square of 0.21.

In order to evaluate further the relationship between blood glucose levels and the pancreatic isoamylase activity in serum, the glucose loading test was performed in 17 diet-treated NIDD. None of the four patients in whom the fasting blood glucose was above 12 mmol/l and pancreatic isoamylase below 90 U/l, responded to IV glucose with an increase in serum immunologically measureable insulin levels. On the other hand, in nine of 13 of the remaining patients (fasting blood glucose below 8 mmol/l, pancreatic isoamylase above 100 U/l), an apparent increase in serum insulin was observed (average with range, 208 pmol/l, 72–359 pmol/l). The patients of the former group were not overweight, as compared with the latter.

Discussion

The results demonstrate that in IDD diagnosed before 25 years of age the serum activity of pancreatic isoamylase is significantly decreased as compared with controls. This is in agreement with the findings of Kamarýt et al. [14], who observed a low pancreatic isoamylase in urine of these patients.

Our results in the IDD group demonstrate that the pancreatic isoamylase activity reflects the activity of amylase stimulated output into the duodenum. However, normal pancreatic isoamylase activity in serum cannot confirm the abnormal exocrine pancreatic function in these patients. This is in good agreement with the findings of Johnson and Levitt [4], who observed a significant positive correlation between serum activity of pancreatic isoamylase and stimulated pancreatic lipase and amylase output into the duodenum in 19 consecutive alcoholic patients. Johnson and Levitt concluded that the finding of a subnormal serum activity of pancreatic isoamylase provides strong evidence for pancreatic exocrine insufficiency, and that a normal or elevated serum pancreatic isoamylase cannot be used as evidence for normal pancreatic exocrine function. However, the genetic variations in serum amylase activity might be also of importance [15].

The cause of exocrine pancreatic dysfunction in some diabetics is not clear. In our study, production of pancreatic amylase was low in insulin-dependent diabetes diagnosed between 15 and 24 years of age, i. e. with an apparent lack of endogenous insulin. In IDD diagnosed after 25 years of age (group b), the patients were adequately replaced with insulin and a normal pancreatic isoamylase activity was observed. In NIDD, the patients with higher blood glucose levels and with low pancreatic isoamylase showed no rise in the radioimmunological level of insulin following administration of glucose, whereas in most patients with an apparent rise of the insulin level in glucose loading test and with only slightly increased blood glucose levels the pancreatic isoamylase was high. These results are in agreement with the findings of Ben Abdeljlil et al. [16], who described the role of insulin in amylase biosynthesis. Sölling and Unger [17] have proposed that insulin regulates amylase synthesis at the level of transcription. Recently, the potentiated effect of insulin on amylase synthesis in parotid glands was observed [18]. In keeping with this, in our IDD group a, the salivary-type isoamylase was decreased in the urine.

The present results suggest that production of pancreatic amylase is related to endocrine pancreatic function. The role of insulin as well as other pancreatic hormones [19] in the changes of exocrine pancreatic function, and evaluation of exocrine pancreatic function after improvement of metabolic control in some diabetics remains to be investigated.

Acknowledgements. We would like to thank Professor L. Krall, Joslin Diabetes Clinic, Boston, and Professor D. W. Moss, Royal Postgraduate Medical School, London, for their interest and valuable discussions.

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Received: January 20, 1980,
and in revised form: August 27, 1980

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