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Urinary Isoamylases in Juvenile Diabetics

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Summary: An anomalous ratio of salivary to pancreatic amylase activities has been observed in urine from juvenile diabetics. Decreased pancreatic amylase activity in urine from these subjects appears to be a characteristic trait.

Isoamylasen im Harn bei juvenilen Diabetikern

Zusammenfassung: Im Harn juveniler Diabetiker wurde ein von Gesunden abweichendes Verhältnis von Speichel- zu Pankreasamylase-Aktivität beobachtet. Die erniedrigte Pankreasamylase-Aktivität im Harn gesunder Diabetiker scheint ein für diese Patienten charakteristisches Merkmal zu sein.

Introduction

Ben Abdeljlil & Palla (1) were the first to describe the action of insulin on the biosynthesis of α-amylase (α-1,4-glucan 4-glucanohydrolase EC 3.2.1.1) and some other enzymes of rat pancreas. *Couture* et al. (2) confirmed these findings but they did not show any direct action of insulin on secretion and synthesis in vitro. *Söling* et al. (3) obtained similar results and supposed that the insulin regulates amylase synthesis in the pancreas mainly at the level of transcription. Exocrine pancreatic function has been shown to be often impaired in diabetic subjects. *Chey* et al. (4) reported low amylase contents in duodenal juice from juvenile diabetics. The reported findings of reduced amylase activity in duodenal juice are convincing and indicate an impaired exocrine pancreatic function. Unfortunately amylase activity determination in duodenal juice did not yield reliable results, and it is difficult to obtain this biological material, particularly from children. We have therefore tried to estimate the pancreatic amylase level from the ratio of salivary to pancreatic amylase activities in urine from healthy controls and juvenile diabetics.

Materials and Methods

Subjects

We examined 20 children – juvenile diabetics between ages 9 and 16 years and a control group of 20 healthy children of the same average age. The sick children were admitted to the hospital with the diagnosis of various states of decompensated juvenile diabetes mellitus, with polyuria, polydipsia and weight loss, and all had elevated blood glucose levels and impaired glucose tolerance. In five individuals from the group of randomly selected juvenile diabetics the renal clearance of total, salivary and pancreatic amylase was also determined. As a control, we examined amylase renal clearance in ten young adults (range 19 to 36 years). *Skude* (5) reported recently that the amylase clearances values in young healthy adults did not exhibit any significant differences in comparison with those found in healthy children (range 6–16 years).

Procedure

Urine and sera collected for amylase activity determinations were examined on the same day, or after storage at 4 °C for 3–6 days. During this time there are no changes in the amylase activities.

Salivary and pancreatic isoamylases were separated by means of agar gel electrophoresis in veronal buffer pH 8.4. After detection and iodine staining, it is possible to evaluate the salivary and pancreatic amylase activities as relative fractions by densitometry (600 nm) of the zymograms. The method has been already reported elsewhere (6). The total amylase activities in blood serum and urine were determined by the method of *Street & Close* (7). Creatinine clearances were determined as an index of glomerular filtration rate.

Results

Densitometry of urinary amylase zymograms from 20 healthy subjects showed mean values of 0.363 and 0.637 for the relative activities of salivary and pancreatic amylases, respectively (tab. 1). In the group of 20 juvenile diabetics the relative activities of salivary and pancreatic isoamylases represented average values of 0.540 and 0.460, respectively. In all examined juvenile diabetics the relative pancreatic isoamylase activity

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Tab. 1. Salivary and pancreatic isoamylases in urine and blood serum from healthy subjects and juvenile diabetics.

	Urine		Serum		
	Salivary amylase	Pancreatic amylase	Total amylase	Salivary amylase	Pancreatic amylase
	Activity fractions		*Street-Close* U/l	Activity fractions	
	n = 20 Healthy controls n = 20				
$\bar{x} \pm s$	0.363 0.083	0.637 0.083	182 43	0.514 0.153	0.486 0.150
	n = 20 Juvenile diabetics n = 10				
$\bar{x} \pm s$	0.540 0.066	0.460 0.073	213 63	0.708 0.107	0.292 0.112
	$P < 0.005$	$P < 0.005$	$P > 0.1$	$P < 0.005$	$P < 0.005$

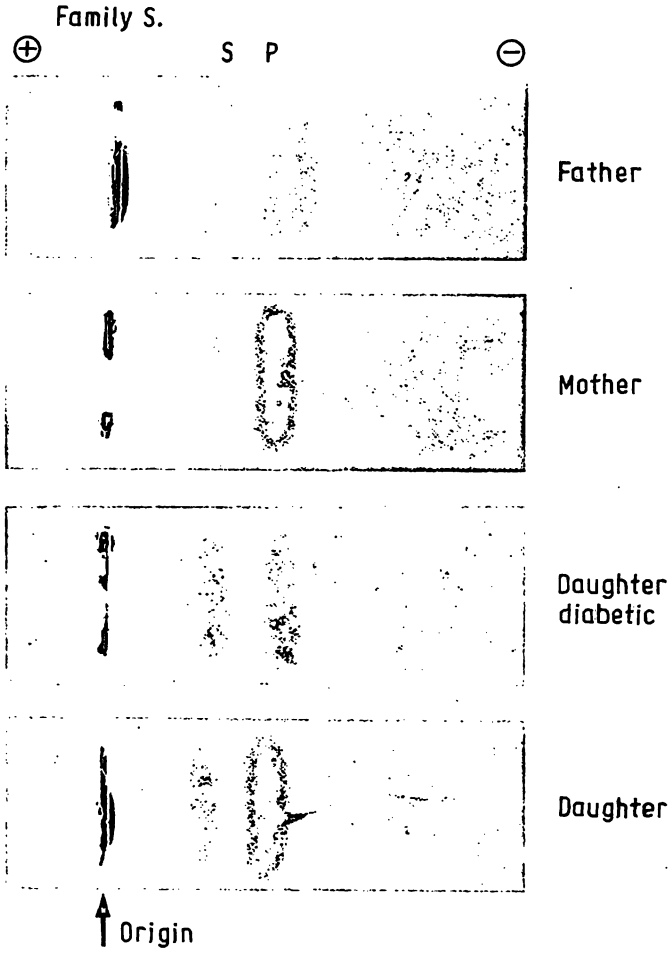

Fig. 1. Zymogram showing isoamylases in urine from members of a family with juvenile diabetes. Isoamylase activities as relative fractions.

	Salivary amylase (S)	Pancreatic amylase (P)
father	0.155	0.405 + 0.440
mother	0.214	0.786
propositus	0.503	0.497
healthy sister	0.368	0.632

Note: father carries the genetically determined heterozygous variant of amylase polymorphism with two pancreatic fractions (Amy 1A1A Amy 2A2B).

fraction in urine was lower than in nondiabetic subjects ($P < 0.005$). Though there were no significant differences between total serum amylase activities in diabetics and healthy controls ($P > 0.10$), the ratio of both isoamylase activities were still significantly shifted ($P < 0.005$) due to the higher activity of salivary and the lower activity of pancreatic isoamylase. The anomalous salivary to pancreatic amylase ratio in urine from diabetics was very marked especially in comparison with zymograms of the diabetic's parents or his healthy sister, respectively (fig. 1). The mean values of total and particularly pancreatic amylase clearances in juvenile diabetics are considerably reduced in comparison with the corresponding values in the nondiabetic controls ($P < 0.01$; $P < 0.002$) (tab. 2). The salivary amylase clearance in juvenile diabetics was not significantly changed ($P > 0.10$). The values of total amylase clearance to creatinine clearance ratios did not indicate any differences between the group of juvenile diabetics and healthy controls ($P > 0.10$). Small but not statistically significant differences were obtained between the ratios of salivary amylase clearance to creatinine clearance, and the ratios of pancreatic amylase clearance to creatinine clearance ($P > 0.10$). However the wide range of all these ratios are evident in the group of juvenile diabetics.

Discussion

In healthy subjects the average ratio of salivary to pancreatic amylase activities appears admirably stable: in blood serum approximately 1:1, in urine 1:2. Higher pancreatic isoamylase activity in urine is due to the higher renal clearance of this isoenzyme (8). The regular lower activities of pancreatic amylase in the urine of juvenile diabetics, due to the lower production of the enzyme by the pancreas, may be related to either the partial or total deficiency of insulin secretion in this disorder (9).

Tab. 2. Renal clearances of total, salivary and pancreatic amylase and their ratio to creatinine clearance in healthy young adults and juvenile diabetics.

	Total amylase		Salivary amylase		Pancreatic amylase	
	Clearance (μl/s)	Clearance ratio	Clearance (μl/s)	Clearance ratio	Clearance (μl/s)	Clearance ratio
			Healthy controls n = 10			
$\bar{x} \pm s$	41.3 9.7	0.0198 0.0061	25.7 5.3	0.0124 0.0034	59.3 14.8	0.0284 0.0082
			Juvenile diabetics n = 5			
$\bar{x} \pm s$	22.8 9.2	0.0203 0.0106	20.2 10.3	0.0175 0.0091	28.8 7.5	0.0268 0.0121
	$P < 0.01$	$P > 0.10$	$P > 0.10$	$P > 0.10$	$P < 0.002$	$P > 0.10$

The results obtained in our laboratory agree with the above mentioned findings reported for experiments in animals (1, 2, 3), i. e., that the insulin plays a permissive role in pancreatic amylase synthesis.

The decreased amylase production by pancreas, together with higher amylase secretion from salivary glands (compensatory mechanism?), results in adjustment of the total blood serum amylase activity within a normal range in juvenile diabetics. The shifted isoamylase ratio in urine appears as a consequence of these mechanisms. Even if the changed ratio of salivary to pancreatic amylase in urine from juvenile diabetics appears as a characteristic trait, the proper cause of this phenomenon remains unclear. Further investigations aimed to elucidate this problem are desirable.

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