Acta Medica Okayama

Volume 52, *Issue* 3 1998 *Article* 4

JUNE 1998

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Masako Nishimura* Satoru Ikeda†

Kazuhisa Taketa‡

*Okayama University,

†Okayama University,

‡Okayama Univeristy,

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Masako Nishimura, Satoru Ikeda, and Kazuhisa Taketa

Abstract

The clinical implications of a wider distribution of salivary type (S-type) isoamylase activity, as compared with that of pancreatic type (P-type) isoamylase activity in the serum of young female adults of 18-23 years old was studied. A high correlation existed between the S-type isoamylase levels in the initial determination and those in the second determination one year after on the same subjects, indicating that the wider distribution of S-type isoamylase level reflects an individual variation. The serum level of S-type isoamylase was highly correlated with the S-type isoamylase activity in saliva. Among the additional factors studied, a weak positive correlation was present between energy intake and the total and S-type isoamylase activities in serum. However, there was no negative correlation between the S-type isoamylase level and body mass index (BMI), which was reported for young male adults.

KEYWORDS: isoamylase, serum, saliva, young female energy intake

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*PMID: 9661741 [PubMed - indexed for MEDLINE]

ACTA MED OKAYAMA 1998; 52(3): 143-148

Wider Distribution of Salivary-Type Isoamylase Activity as Compared with Pancreatic-Type Isoamylase Activity in Serum: A Study on Young Female Adults

Masako NISHIMURA*, Satoru IKEDA and Kazuhisa TAKETA

Department of Public Health, Okayama University Medical School, Okayama 700-8558, Japan

The clinical implications of a wider distribution of salivary type (S-type) isoamylase activity, as compared with that of pancreatic type (P-type) isoamylase activity in the serum of young female adults of 18-23 years old was studied. A high correlation existed between the S-type isoamylase levels in the initial determination and those in the second determination one year after on the same subjects, indicating that the wider distribution of S-type isoamylase level reflects an individual variation. The serum level of S-type isoamylase was highly correlated with the S-type isoamylase activity in saliva. Among the additional factors studied, a weak positive correlation was present between energy intake and the total and S-type isoamylase activities in serum. However, there was no negative correlation between the S-type isoamylase level and body mass index (BMI), which was reported for young male adults.

Key words: isoamylase, serum, saliva, young female, energy intake

Serum amylase (EC 3.2.1.1) consists of salivary-type (S-type) and pancreatic-type (P-type) isoamylases. The analysis of isoamylase is important for identification of the involved organs when altered serum levels of total isoamylase are found in pathological conditions. Increased P-type isoamylase activities are seen most frequently in acute pancreatitis and increased S-type isoamylase activities in acute parotitis. Decreased serum levels of amylase are also noted in diseases involving the pancreas and salivary gland (1). Obesity is also known to be associated with lower serum levels of amylase (2). Taketa *et al.* (3) demonstrated that decreased serum levels of amylase activity in obese young adult males could be ascribed to decreases in S-type isoamylase.

In this study, serum levels of S-type and P-type isoamylases were analyzed in young female adults in order to see whether the S-type isoamylase activity is also lower in obese females. Contrary to the assumption, no significant reduction in S-type isoamylase activity was found in subjects with increased body mass index (BMI). Instead, a wide distribution of S-type isoamylase levels as compared with P-type isoamylases level was observed. This observation was extended in the present study to characterize the wider distribution of S-type isoamylase.

Subjects and Methods

Total amylase activity was determined with *p*-nitrophenyl maltoheptaoside (Isoamylase EPS, Boehringer Mannheim Yamanouchi Co., Ltd., Tokyo, Japan) as a substrate and P-type isoamylase activity by the identical method with added monoclonal antibodies that specifically inhibit S-type isoamylase activity (Isoamylase EPS, Boehringer Manheim Yamanouchi, Co.) (4, 5). S-type isoamylase activity was calculated by subtracting the P-type isoamylase activity from the total.

Fasting blood samples were collected from 191 females recruited from a woman's college, ranging in age from 18 to 23 years old and having no symptoms or abnormal results upon a physical examination given in April, 1994. Mixed unstimulated saliva was collected for analysis of isoamylase activities after irrigation of the mouth and dietary intakes were taken as detailed below from 146 of them. Protein contents of saliva was determined by the bicinchoninic acid (BCA) method (6) to express the amylase activity in saliva on the protein basis to minimize variation. Among the 191 subjects, sera

* To whom correspondence should be addressed.

were collected again from 121 of them in April, 1995 for analysis of isoamylase activities in order to compare these with the previous activities.

One-week dietary records were obtained after the physical examination to calculate average nutritional intake per day. Body weight and height were recorded at the time of the physical examination and BMI was calculated there from. Statistical analysis of variance and correlation were made with a visual statistics software program, Stat Flex for PC-9800 series (View Flex Co., Osaka, Japan).

Results

Mean serum and salivary activities of total amylase as well as P-type and S-type isoamylases, their standard deviations, coefficients of variations and minimum and maximum values, which were calculated based on the data obtained at the initial physical examination and showing normal distribution are presented in Tables 1 and 2. Mean serum activity of S-type isoamylase was slightly greater than that of P-type isoamylase as reported earlier (7). The coefficient of variation of S-type isoamylase activity was calculated to be 42.1 % and that of P-type isoamylase 28.5 % with a significant difference between them (F test, $P < 0.001$). In fact, the minimum value of the S-type isoamylase was as low as 4 IU/l and the maximum as large as 362 IU/l (Fig. 1), while the corresponding values of the P-type isoamylase were 23 and 119 IU/l, respectively (Fig. 2). Thus, it is apparent that S-type isoamylase activity is distributed over a wider range than P-type isoamylase activity. Incidentally, there was no significant correlation found between BMI and P-type isoamylase activity, nor was there any found between BMI and S-type isoamylase activity.

Table 1 Total, pancreatic type (P-type) and salivary type (S-type) isoamylase activities in serum determined in April 1994

Amylase	Activities				
	Minimum (IU/l)	Maximum (IU/l)	Mean (IU/l)	SD (IU/l)	CV (%)
P-type	23	119	62.1	17.1	28.5
S-type	4	362	108.8	51.2	42.1
Total	5	418	171.0	67.6	39.5

SD: Standard deviation; CV: Coefficient of variation.

Table 2 Total, salivary type (S-type) and pancreatic type (P-type) isoamylase activities in saliva determined in April 1994

Saliva amylase	Activities				
	Minimum (IU/mg protein)	Maximum (IU/mg protein)	Mean (IU/mg protein)	SD (IU/mg protein)	CV (%)
P-type	0.2	12.5	3.4	1.9	55.9
S-type	1.9	749.0	194.6	115.9	59.6
Total	2.2	35.3	198.0	117.8	59.5

SD: Standard deviation; CV: Coefficient of variation.

In saliva most of the amylase activity consisted of S-type isoamylase. The variation of the S-type isoamylase was also large as revealed by a high coefficient of variation of 59.6 % and also the maximum and minimum values of 749 IU/mg protein and 1.9 IU/mg protein, respectively (Table 2).

In order to see the interrelationship among those amylase activities, correlation analyses were performed and results are shown in Table 3. It is apparent from the high correlation coefficient of 0.944 existing between total amylase and S-type isoamylase activities in serum that the serum total amylase activity represents the S-type isoamylase. The correlation between serum S-type and P-type isoamylase activities was significant, although the correlation coefficient was very low. There was also a significant correlation between serum S-type isoamylase and salivary S-type isoamylase activities. Although a similar correlation was found between the serum S-type isoamylase and the salivary P-type isoamylase activities, this was considered to be due to the excessively high activities of salivary S-type isoamylase activities, which were not sufficiently inhibited by the monoclonal antibodies and counted as P-type isoamylase. This was evident from a correlation coefficient of nearly one existing between the P-type and S-type isoamylase activities in saliva.

Since dietary factors are possibly involved in the individual variation, correlation analyses were preformed between amylase activities and the nutrient intakes, and the results are presented in Table 4. There was a significant correlation between energy intake and serum S-type isoamylase activity, although the correlation coefficient was not high. A similar correlation was also present between energy intake and total amylase activity which represents the change in S-type isoamylase activity. The energy intake was correlated with carbohydrate, fat and protein intakes.

The stability of the individual variation of the S-type

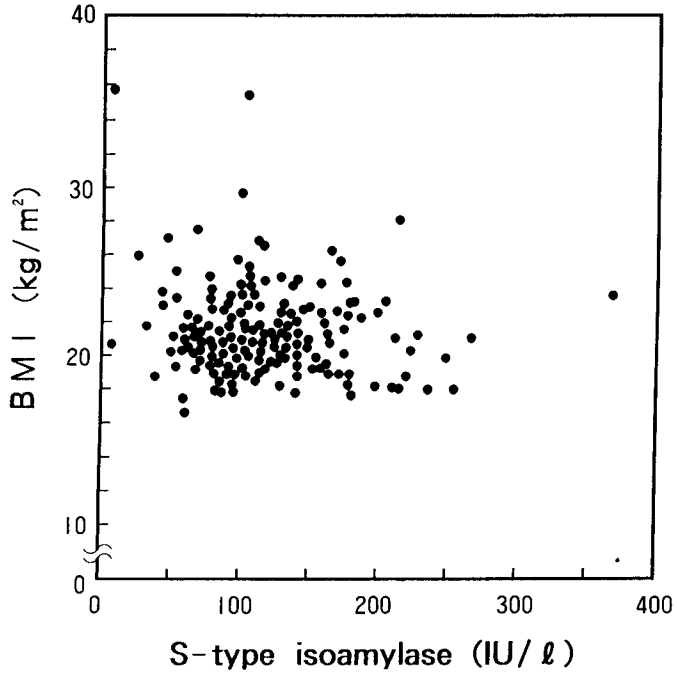

Fig. 1 Scattergram of serum salivary type (S-type) isoamylase activity against body mass index (BMI). BMI, 21.3 ± 2.6 (kg/m^2); Serum S-type isoamylase activity, 108.8 ± 51.2 (IU/l) n = 191. No significant inverse correlation was noted between serum S-type isoamylase activity and BMI.

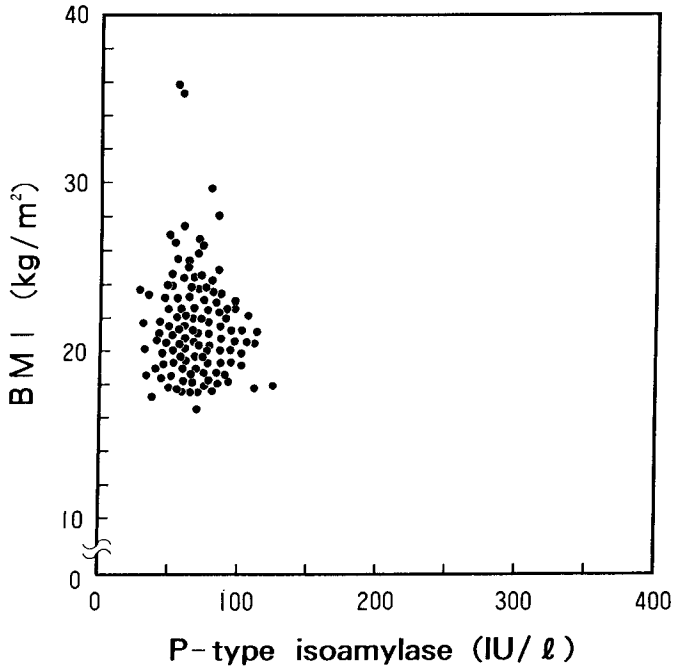

Fig. 2 Scattergram of serum pancreatic type (P-type) isoamylase activity against body mass index (BMI). BMI, 21.3 ± 2.6 (kg/m^2); Serum P-type isoamylase activity, 62.1 ± 17.1 (IU/l) n = 191. No significant correlation was noted between serum P-type isoamylase activity and BMI.

Table 3 Correlation matrix of serum total, salivary type (S-type) and pancreatic type (P-type) isoamylases and salivary total, S-type and P-type isoamylases

	Serum			Saliva		
	Total	S-type	P-type	Total	S-type	P-type
Serum						
T-type	1.000	0.944***	0.517***	0.295***	0.294***	0.296***
S-type		1.000	0.205*	0.324***	0.324***	0.328***
P-type			1.000	0.032	0.031	0.028
Saliva						
T-type				1.000	1.000	0.986***[a]
S-type					1.000	0.985***
P-type						1.000

T-type: Total type; $*P < 0.05$; $***P < 0.001$.

[a]: The high correlation between total and P-type isoamylase activities in saliva was probably due to insufficient inhibition of the excess S-type isoamylase by its monoclonal antibody and may have no practical implication.

Table 4 Correlation matrix of serum total, pancreatic type (P-type) and salivary type (S-type) isoamylase activities and nutritional factors

	Dietary intake			
	Energy	Carbohydrate	Fat	Protein
Total amylase activity	0.212*	0.125	0.099	0.068
P-type isoamylase activity	0.064	0.065	−0.008	−0.050
S-type isoamylase activity	0.219**	0.108	0.116	0.097
Energy	1.000	0.730***	0.843***	0.558***
Carbohydrate		1.000	0.544***	0.709***
Fat			1.000	0.586***
Protein				1.000

$*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Calorie in takes per dey, 1299 ± 289 Cal/day; carbohydrate, 198 ± 61.2 g; fat, 46.5 ± 13.6 g; protein, 50.6 ± 19.4 g (Mean and standard deviations).

isoamylase level in serum was studied by comparing the isoamylase activities in 1994 and those in 1995 on 121 subjects with available data. The correlation coefficient in the S-type isoamylase was as high as 0.90 ($P < 0.001$) (Fig. 3), although there was also a high correlation between the two assays ($r = 0.82$, $P < 0.001$) in the P-type isoamylase (Fig. 4), indicating that the variation is stable in the same individuals.

Discussion

In contrast to our assumption that there might be a negative correlation between BMI and serum S-type isoamylase activity, no such correlation was observed in the population analyzed in this study. The subjects analyzed had a markedly low mean BMI for young females of this age group (8). This was considered to be due to abnormal weight reduction for cosmetic purposes and may account for the lack of negative correlation which was observed between the S-type isoamylase and BMI in young male adults (3).

On the other hand, the S-type isoamylase activity in serum was found to be distributed more widely than the P-type isoamylase activity, the coefficient of variation in the former, 42.1%, being significantly greater than the latter, 28.5%. Since the S-type isoamylase activity was obtained by subtracting P-type isoamylase activity from total isoamylase activity, the determination of S-type isoamylase would have an intrinsic error, resulting in a potentially wider distribution. In order to eliminate this

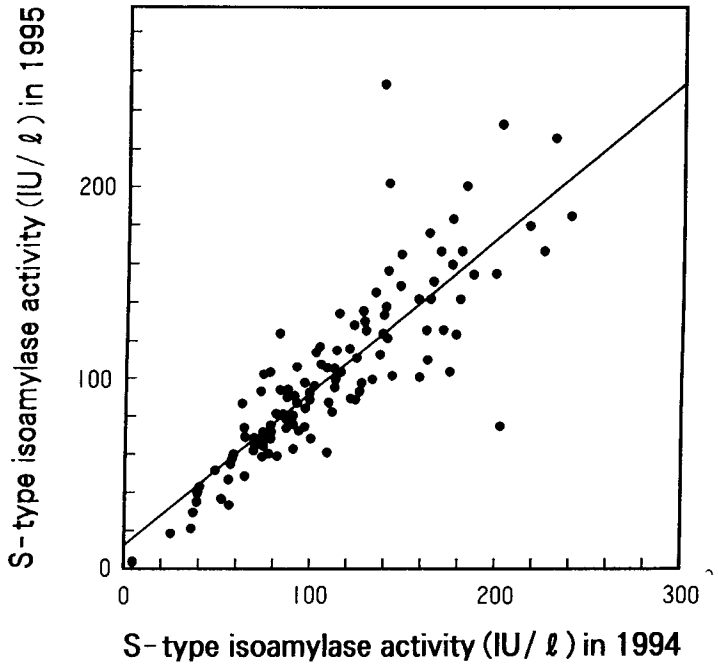

Fig. 3 Comparison of salivary type (S-type) isoamylase activities in 1994 and 1995. Serum S-type isoamylase activity in 1994, 107.3 ± 44.9 (IU/l); coefficient of variation, 42.0%. Serum S-type isoamylase activity in 1995, 102.1 ± 44.7 (IU/l); coefficient of variation, 43.7%. $r = 0.90$ ($P < 0.001$), $n = 121$.

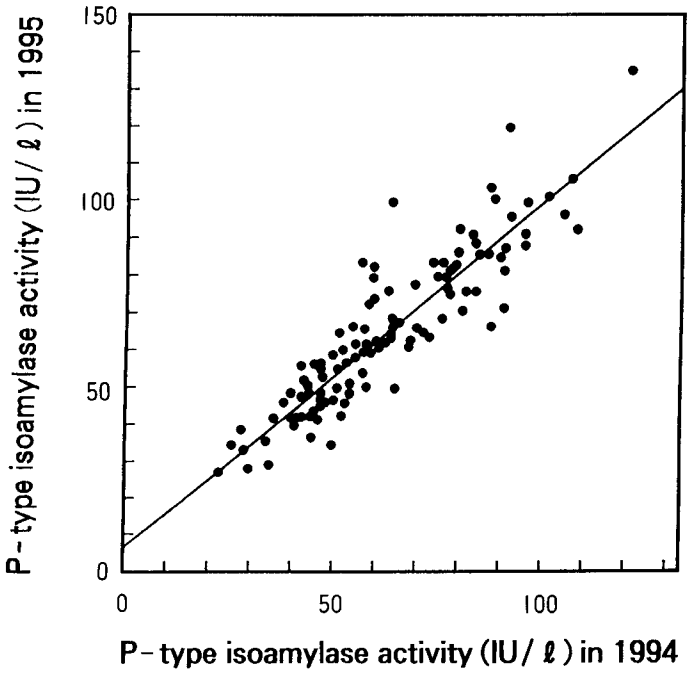

Fig. 4 Comparison of pancreatic type (P-type) isoamylase activities in 1994 and 1995. Serum P-type isoamylase activity in 1994, 61.6 ± 19.4 (IU/l); coefficient of variation, 31.4%. Serum P-type isoamylase activity in 1995, 63.3 ± 20.6 (IU/l); coefficient of variation, 32.5%. $r = 0.82$ ($P < 0.001$), $n = 121$.

possibility, S-type isoamylase activity was determined on the same subjects one year later and the correlation between the two determinations was analyzed. There was a highly significant correlation between them, eliminating the possible error in determination of S-type isoamylase activities as a cause of the wider distribution. It is, therefore, evident now that the large individual variation is reproducible or characteristic of the individuals.

The observation was further consolidated by the finding that a significantly high correlation was present between serum S-type isoamylase activity and salivary total and S-type isoamylase activity. Thus, the wider distribution of serum S-type isoamylase activity appeared to refect that of salivary S-type isoamylase activity.

It would be interesting to know whether the variation in S-type isoamylase activity is related to the dietary intake or not. Although there was a positive correlation between energy intake and S-type isoamylase activity in serum, the interpretation of this result is not easy, because it is contradictory to our previous observation that obese subjects, who are presumed to take in more energy, had lower levels of S-type isoamylase (3). Since the S-type isoamylase activity had no correlation with BMI, the subject with the high S-type isoamylase activity may have consumed an excess energy probably by physical exercise, although this remains to be clarified in future studies (8).

The subject with extremely low S-type isoamylase activity of 4IU/l should be followed-up for possible development of Sjögren's disease, although she had no signs or symptoms of Sjögren's disease at the time of present examination.

A hadditional factor possibly pertaining to low or high S-type amylase activities, genetic variants or polymorphism should also be considered as several genetic variants of S-type isoamylase have been reported by Ikemoto *et al.* (9, 10). This should also be an interesting research area for future studies.

In conclusion, researchers should be aware of the statistically significant wide variation of serum S-type isoamlyase distribution found in this study. Future research and clinical interpretation of these results will obviously shed greater light on this anomalous result and add to understanding of the phenomenon as a whole.

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Received November 20, 1997; accepted January 21, 1998.