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Reference Interval for Serum α -Amylase Determined with *p*-Nitrophenyl- α -D-Maltoheptaoside as a Substrate

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Summary: The reference interval for the catalytic concentration of α -amylase (EC 3.2.1.1) in serum was determined in 326 blood donors. The analytic method used *p*-nitrophenyl- α -D-maltoheptaoside as substrate, which allows a continuous colorimetric measurement at 37 °C of the catalytic concentration of α -amylase in serum. The limits obtained were 0.7–3.8 μ kat/l (42–228 U/l), which were in agreement with the only previous report found.

*Referenzintervall für α -Amylase im Serum, mit *p*-Nitrophenyl- α -D-maltoheptaosid als Substrat bestimmt*

Zusammenfassung: An einem Kollektiv von 326 Blutspendern wurde das Referenzintervall für die katalytische Konzentration von α -Amylase (EC 3.2.1.1) im Serum bestimmt. Die angewandte Methode mit *p*-Nitrophenyl- α -D-maltoheptaosid als Substrat gestattet die kontinuierliche kolorimetrische Messung der katalytischen Konzentration von α -Amylase im Serum bei 37 °C. Der erhaltene Bereich von 0,7–3,8 μ kat/l (42–228 U/l) stimmt mit dem im einzigen vorherigen Bericht angegebenen überein.

Introduction

According to a report on the workshop conference of the German Society of Clinical Chemistry on α -amylase assay (1), 200 different methods for α -amylase (EC 3.2.1.1) estimation have been described over the past century. The use of a defined substrate for determining the catalytic concentration of the enzyme offers several advantages. One of these substrates, *p*-nitrophenyl- α -D-maltoheptaoside, allows continuous colorimetric measurement. The fact that only a few reports on serum α -amylase reference values estimated with *p*-nitrophenyl- α -D-maltoheptaoside as the substrate have been documented in the literature, prompted us to determine the reference interval for serum α -amylase catalytic concentration with this substrate.

Materials and Methods

Determinations were done with blood samples obtained from 326 healthy volunteer blood donors; 251 men and 75 women with ages ranging from 16 to 65 years. Samples were taken at the time of blood donation. Because of the working conditions of our blood bank, clot and serum could not always be separated on the day of extraction and we therefore studied whether prolonged contact between serum and clot could in any way influence the catalytic concentration of α -amylase in serum. For this purpose, the data comparison method recommended by Strike (2), and based on the Deming's regression model (3), was applied to two groups of 40 results of the catalytic concentration of α -amylase in serum which were obtained from 40 outpatients. The only difference between the two groups of samples was that one group was stored at room temperature for one hour and the other group for 24 hours before analysis. The absence of significant proportional or constant differences or both between the two groups demonstrates that 24 hours of contact between serum and clot does not influence the catalytic concentration of α -amylase in serum.

The catalytic concentration of α -amylase in serum was determined with a continuous method using *p*-nitrophenyl- α -D-maltoheptaoside as substrate (Boehringer Mannheim, "kit" ref. 568651) at 37 °C and with a Hitachi 705 analyser (Boehringer Mannheim, Mannheim, F. R. G.).

The effects of age and sex between reference samples were determined by the *Kolmogorov-Smirnov* two sample test (4, 5). *Gaussian* distribution of data was tested by the *Kolmogorov-Smirnov* method (4, 5).

The reference interval was estimated according to the recommendations of the International Federation of Clinical Chemistry (6).

Results and Discussion

Reference samples were provisionally stratified according to sex, and age in decades (tab. 1), in order to discover any possible age-related differences. Statistical comparisons between paired groups in ascending order were performed by the *Kolmogorov-Smirnov* two sample test, which allows the comparison of small and large samples (4, 5). Comparison results are shown in table 2.

Because the only significant difference was not less than a significance degree of 0.01, data were rearranged according to sex and the two new groups compared; no significant difference was found.

The test for *Gaussian* distribution was applied to the grouped results for men and women, as well as to two transformations of these data ($y = \sqrt{x + 1}$ and $z = \lg(x + 1)$). The best fit of the data to a *Gaussian* distribution occurred with the logarithmic transformation and reference limits were calculated parametrically from this transformation: 0.7–3.8 $\mu\text{kat/l}$ (42–228 U/l).

In addition, imprecision and relative inaccuracy of the analytical method were evaluated using two control sera (Precinorm® and Precipath®; Boehringer Mannheim); results of this control programme are expressed in table 3.

No age-related differences in the catalytic concentration of α -amylase in serum have been documented in adults and the only sex-related differences in the catalytic concentration of α -amylase in serum were reported by Henry et al. (7) who described higher levels in men determined with the *Somogyi* method. In the present study no age- or sex-related differences were observed.

Reference limits obtained in our study, 0.7–3.8 $\mu\text{kat/l}$ (42–228 U/l), were in agreement with the only previous report by Hohenwallner et al. (8) with the same analytical method.

Tab. 1. Description of the reference sample.

Age	Sex	N	$\bar{x}$ $\mu\text{kat/l}$ (U/l)	Range $\mu\text{kat/l}$ (U/l)
(a)				
16–25	♂	7	2.6 (156)	1.1–4.1 (66–246)
16–25	♀	21	2.1 (126)	1.2–3.0 (72–180)
26–35	♂	78	2.4 (144)	0.7–4.1 (42–246)
26–35	♀	44	2.0 (120)	0.5–3.5 (30–210)
36–45	♂	105	2.6 (156)	0.6–4.5 (36–270)
36–45	♀	5	1.9 (114)	1.2–2.6 (72–156)
46–55	♂	47	2.5 (150)	1.0–4.0 (60–240)
46–55	♀	7	2.0 (120)	0.9–3.1 (54–186)
56–65	♂	13	2.6 (156)	0.9–4.2 (54–252)

N: sample size

$\bar{x}$: mean values of α -amylase catalytic concentration in serum.

Tab. 2. Comparison between paired groups with the *Kolmogorov-Smirnov* test.

Group 1			Group 2			Result
Age	Sex	N ₁	Age	Sex	N ₂	
(a)			(a)			
16–25	♂	7	26–35	♂	78	NS
26–35	♂	78	36–45	♂	105	0.01 < p < 0.05
36–45	♂	105	46–55	♂	47	NS
46–55	♂	47	56–65	♂	13	NS
16–25	♀	21	26–35	♀	44	NS
26–35	♀	44	36–45	♀	5	NS
36–45	♀	5	46–55	♀	7	NS

N₁ and N₂: sample size

NS: not significant ($\alpha = 0.05$)

p: significance degree.

Tab. 3. Quality control data.

Between-run imprecision	Relative inaccuracy
CV ₁ = 2.7 ($\bar{x}_O = 7.4 \mu\text{kat/l}$)	$\Delta_1 = -1.7$ ($\bar{x}_C = 7.6 \mu\text{kat/l}$)
CV ₂ = 2.7 ($\bar{x}_O = 14.4 \mu\text{kat/l}$)	$\Delta_2 = -1.8$ ($\bar{x}_C = 14.2 \mu\text{kat/l}$)
CV ₁ and CV ₂ : coefficient of variation (%)	
$\bar{x}_O$: observed mean	
Δ_1 and Δ_2 : $(\bar{x}_O - \bar{x}_C)100/\bar{x}_C$	
$\bar{x}_C$: consensual mean	

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