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Scand J Clin Lab Invest 2004; 64: 371–384

Reference intervals for eight enzymes in blood of adult females and males measured in accordance with the International Federation of Clinical Chemistry reference system at 37°C: part of the Nordic Reference Interval Project

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Strømme JH, Rustad P, Steensland H, Theodorsen L, Urdal P. Reference intervals for eight enzymes in blood of adult females and males measured in accordance with the International Federation of Clinical Chemistry reference system at 37°C: part of the Nordic Reference Interval Project. Scand J Clin Lab Invest 2004; 64: 371–384.

As part of the Nordic Reference Interval Project we present reference intervals for alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), lactate dehydrogenase (LD), alkaline phosphatase (ALP), gamma-glutamyltransferase (GT), amylase (AMY) and pancreatic type of AMY in blood of adult males and females. A total of 3036 reference persons, all of whom considered themselves to be in good health, were recruited by 102 Nordic clinical biochemical laboratories. Exclusions were undertaken on the basis of predefined biochemical and clinical criteria. Enzyme activities in serum and plasma were measured in the different laboratories using various commercially available routine measurement systems at 37°C. Only results obtained with the International Federation of Clinical Chemistry (IFCC) compatible measuring systems were selected for estimation of the enzyme reference intervals. The final number of results on each enzyme varied from 459 (LD) to 2300 (ALT). The 2.5 and 97.5 percentile reference limits were calculated by a non-parametric method in accordance with the IFCC recommendations, using the Refval 4.0 data program. Statistical partitioning testing was undertaken to decide whether the reference intervals ought to be partitioned according to gender and/or age. For most of the enzymes, but not for all, the upper reference limits were found to be higher than those that have been in general use until now.

Key words: Age; gender; NORIP; plasma enzymes; reference range; serum enzymes; upper reference limits; URL

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DOI 10.1080/00365510410002742

The catalytic activity concentrations measured for enzymes in blood will depend strictly on the incubation conditions used for their measurements. An international standardization of the methodology has therefore been necessary in an attempt to achieve acceptable comparability of diagnostic enzyme results between medical laboratories. Accordingly, the International Federation of Clinical Chemistry (IFCC) has established extensive reference systems for the measurement of the commonly analysed enzymes in blood [1]. These systems consist of IFCC-approved primary reference methods at 30°C [2–8], five of which have recently been adapted to 37°C [9–13], certified and commutable reference materials [14, 15] and worldwide reference laboratories. By applying these systems the goal of standardization of different commercially available measurement systems, comprising reagent kits, calibrators and instruments in use in medical laboratories is about to be reached. The markedly better inter-laboratory agreement of enzyme measurements thus achieved, ought also to lead to an acceptable harmonization of enzyme reference intervals and decision levels, and finally to an increased diagnostic efficiency of enzyme results.

So far, reports on reference intervals traceable to the IFCC reference systems are limited [16]. Here we present reference intervals on eight commonly analysed serum enzymes: alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), lactate dehydrogenase (LD), alkaline phosphatase (ALP), gamma-glutamyltransferase (GT), amylase (AMY) and pancreatic type of amylase (AMY-P). The values included in the database are all obtained with IFCC-compatible routine measurement systems as a part of the Nordic Reference Interval Project (NORIP) [17]. It should be mentioned that the IFCC-approved reference system for ALP is still only based on 30°C, but the producers have made preliminary adaptations to 37°C. The proposed primary reference method for AMY is as yet not finally approved of by the IFCC, but compatible commercially available routine methods at 37°C for AMY and AMY-P have been used for many years. Results of the last three enzymes obtained with these measurement systems were therefore also included in the project.

MATERIALS AND METHODS

Detailed information about the selection of reference persons and collection and handling of samples is given elsewhere [17, 18]. Here one will also find information about the general clinical and biochemical criteria used for exclusion of reference persons and results.

With respect to enzyme methodology, NORIP applied in addition strict criteria for inclusion of reported results from the laboratories: Only results obtained by assay conditions at 37°C which were compatible with and traceable to the IFCC reference methods were included. Moreover, only complete measurement systems, i.e. reagents, calibrators and instrumentation supplied by the same manufacturer, were accepted. The procedures were considered IFCC compatible when this was explicitly stated by the producer; in other words, that the methods had been standardized and calibrated in accordance with the IFCC reference systems mentioned above. In cases where local adjustment of slopes and intercepts had been used, the reported values were recalculated to the original values. Finally, for each enzyme the individual laboratory mean was calculated and evaluated against the mean and standard deviation (SD) of the individual means. When the individual mean differed by more than 2 SD from the overall mean, all results from this laboratory on this enzyme were excluded.

In the case of AMY and AMY-P, only results obtained with the EPS method of Roche/Boehringer, which is compatible with the suggested IFCC reference method, were selected. Because the routine methods of ALP and LD used during 1999–2001 were still mostly the Scandinavian recommended methods, LD and ALP were reanalysed. A total of 464 randomly selected samples originating from the five countries were measured in three laboratories using the IFCC compatible methods of the Roche Modular system.

Reference method values for the project calibrator CAL [17] were only established for ALT (18 U/L), CK (119 U/L) and GT (36 U/L). The mean value for ALT in CAL analysed by the selected laboratories differed by +2.0 U/L. For CK the mean deviation was −9.0 U/L and for GT −0.1 U/L. Occasionally the deviations from the expected values (reference method values for ALT, CK and GT and

consensus means for the other five enzymes) were observed. However, we regard these comparisons only as indicative since the enzyme activities in CAL were low.

Whereas the number of results excluded by the general clinical and biochemical criteria amounted to only a few percentages [18], the number excluded owing to incompatibility with the IFCC reference methods varied from 15 to 57% for the enzymes assayed with wet chemistry, and from 5 to 17% for the enzymes assayed with IFCC-compatible dry chemistry methods (OrthoVitros 250/950) (Table I).

The number of laboratories that used methods satisfying the compatibility criteria for the eight enzymes is shown in Table II.

TABLE I. Percentage of totally reported results included in the computation of reference intervals for the eight enzymes.

	Number reported		Percent included	
Enzyme*	Wet chem.	Dry chem.	Wet chem.	Dry chem.
ALT	2079	675	80	95
AST	1886	566	85	94
CK	2043	598	66	83
GT	1962	595	43	90
AMY-P	831	19	60	–
AMY	1345	676	54	0

ALT = alanine aminotransferase; AST = aspartate transaminase; CK = creatine kinase; GT = gamma-glutamyltransferase; AMY-P = pancreatic type amylase; AMY = amylase; LD = lactate dehydrogenase; ALP = alkaline phosphatase.

*LD and ALP: 464 samples were reanalysed on Roche Modular. In addition, 495 ALP results from OrthoVitros 250/950 were included.

TABLE II. Number of laboratories performing IFCC-compatible methods.

Enzyme*	Wet chem.	Dry chem.
ALT	58	22
AST	53	18
CK	55	20
ALP	*	19
GT	27	19
AMY-P	18	–
AMY	23	–

ALT = alanine aminotransferase; AST = aspartate transaminase; CK = creatine kinase; GT = gamma-glutamyltransferase; AMY-P = pancreatic type amylase; AMY = amylase; LD = lactate dehydrogenase; ALP = alkaline phosphatase.

*ALP and LD: Three laboratories reanalysed 464 samples on Roche Modular.

Statistics

Calculation of reference limits. The 2.5 and 97.5 percentile reference limits were calculated by a simple non-parametric method in accordance with IFCC recommendations, using Refval 4.0 [19]. Partitioning by gender and age was evaluated using a modification of Refval 4.0 according to principles outlined by Lahti *et al.* [20] with the possible outcome according to the percentage of the subpopulation outside the common reference limit:

Non-partitioning, "negative": between 1.8% and 3.2%.

Moderate partitioning, "uncertain": between 0.9% and 1.8% or between 3.2% and 4.1%

Partitioning, "positive": less than 0.9% or above 4.1%.

As a general rule we have recommended differentiation of the reference intervals by gender or age only when the partitioning test was "positive".

Outliers. As Refval 4.0 failed to detect two obviously extreme but close CK values (1065 and 1068 U/L), this test was replaced by the following outlier test: data outside a mean of ± 4 SD (logarithmic transformed) were discarded as outliers. The limits of exclusion and the number of results excluded are shown in Table III. These few are in addition to those given in Table I.

Analyse-IT, Z-score. The cumulative distribution of enzyme activity concentrations is presented as a Z-score plot (standard deviate). Whereas a direct plotting of the distribution gives an S-shaped curve for a normal distribution, the Z-score plot results in a straight line. This is due to a transformation of the ordinate scale by "stretching" it at both ends and replacing cumulative frequency (P) with z-values on the ordinate, i.e. 2.5% at -1.96, 50% (median) at zero, and 97.5% at $+1.96$.

The Spearman rank test was performed with Analyse-It for MS Excel (Analyse-It Software, Ltd., P.O. Box 103, Leeds LS27 7WZ, England United Kingdom).

TABLE III. Upper limits of exclusion according to the "mean ±4 SD" test, along with enzyme activity concentrations of the excluded results.

Enzyme	Gender	Limits of exclusion	Excluded results (U/L)
ALT	F	104	123,108
	M	162	168
AST	F	58	62
	M	77	81,134
CK	F	515	625,871
	M	885	10,581,065
LD	F+M	289	–
ALP	F+M	188	265
GT	F	124	136,145,248
	M	217	–
AMY-P	F+M	132	–
AMY	F+M	247	286

ALT = alanine aminotransferase; AST = aspartate transaminase; CK = creatine kinase; GT = gamma-glutamyltransferase; AMY-P = pancreatic type amylase; AMY = amylase; LD = lactate dehydrogenase; ALP = alkaline phosphatase.

RESULTS

The enzyme results included in the final database subgrouped to the respective measurement system from which they originated are presented in Table IV.

Ortho Clinical Diagnostics claims compatibility with the IFCC reference systems at 37°C for their Vitros dry chemistry systems (250/950 models) in the case of ALT, AST, CK, GT and ALP. Reference intervals for these enzymes were initially processed separately. However, the medians of results obtained by wet and dry chemistry methods were nearly the same (Table V). For several enzymes the 97.5 percentiles differed more, obviously owing to the skewed distribution at the higher range. But their 90% confidence intervals overlapped (Table V), and the partitioning tests were either "negative" or "uncertain". Consequently,

TABLE IV. Number of enzyme results grouped according to the analysis systems used for the measurements.

Enzyme	1	2	3	4	5	6	7	Wet chem.	Dry chem.
								Sum 1–7	8
ALT	72	175		114	57	574	670	1661	639
AST	65	175	59	120	34	503	655	1633	407
CK	51	36		174	52	511	529	1318	531
LD							459	459	
ALP							459	459	495
GT			59	94	34		659	845	536
AMY-P						105	392	497	
AMY					33	382	304	719	

ALT = alanine aminotransferase; AST = aspartate transaminase; CK = creatine kinase; GT = gamma-glutamyltransferase; AMY-P = pancreatic type amylase; AMY = amylase; LD = lactate dehydrogenase; ALP = alkaline phosphatase.

1. Bayer Axon, Opera.
2. Beckman Coulter.
3. Dade Behring Dimension.
4. Konelab.
5. Olympus.
6. Roche Cobas Integra.
7. Roche Hitachi.
8. Ortho (Vitros 250/950).

TABLE V. Medians and 97.5 percentiles with 90% confidence intervals (CI) for the five enzymes measured by wet and dry chemistry.

		Median		97.5 percentile			
				Wet Chemistry		Dry chemistry	
Enzyme	Gender	Wet chem. (U/L)	Dry chem. (U/L)	U/L	90% CI	U/L	90% CI
ALT	F	18	21	44	40 – 48	50	43 – 62
	M	23	28	72	66 – 83	58	53 – 66
AST	F	21	20	36	33 – 37	40	37 – 44
	M	25	24	45	43 – 50	44	40 – 47
CK	F	77	72	209	188 – 256	178	148 – 295
	M	108	103	371	309 – 435	329	251 – 487
ALP	F + M	62	64	107	103 – 115	106	98 – 113
GT	F	18	18	66	53 – 77	73	48 – 84
	M	27	26	114	99 – 134	86	70 – 110

ALT = alanine aminotransferase; AST = aspartate transaminase; CK = creatine kinase; GT = gamma-glutamyltransferase; ALP = alkaline phosphatase.

the results from both wet and dry chemistry methods were pooled in the further calculations of reference intervals for these five enzymes. The results obtained for LD, AMY and AMY-P using the Vitros systems have been excluded because these methods are not IFCC compatible.

The reference intervals (2.5 and 97.5 percentiles) along with the 90% confidence intervals of the 97.5 percentiles for the eight enzymes calculated on the basis of the final reference material are presented in Table VI. For comparison, the 95 percentiles are also given.

Alanine transaminase (ALT) NPU01121

The distribution of ALT activity concentrations was skewed towards higher levels as shown by the curved line in the Z-score plot of Figure 1. At all percentiles, the concentration was higher in males than females (Fig. 1), and

TABLE VI. Reference limits (2.5 and 97.5 percentiles) along with 95 percentiles for eight serum enzymes.

				Percentiles			
Enzyme	Gender	Age (years)	No.	2.5	*95*	97.5	(90% CI*)
ALT	F	≥18	1220	8	*37*	46	(43 – 49)
	M	≥18	1080	10	*56*	68	(63 – 74)
AST	F	≥18	1128	13	*33*	37	(35 – 38)
	M	≥18	1012	14	*40*	45	(43 – 47)
	F	≥18	1048	34	*158*	207	(180 – 233)
CK	M	<50	397	50	*313*	398	(351 – 487)
	M	≥50	404	39	*239*	277	(252 – 415)
LD	F + M	<70	372	103	*195*	204	(198 – 210)
	F + M	≥70	87	114	*219*	255	–
ALP	F + M	≥18	954	37	*97*	106	(101 – 113)
	F	<40	283	10	*34*	42	(34 – 63)
GT	F	≥40	445	11	*55*	77	(64 – 81)
	M	<40	244	12	*55*	78	(56 – 168)
	M	≥40	409	15	*83*	114	(99 – 134)
AMY-P	F + M	≥18	497	12	*50*	64	(4 – 68)
AMY	F + M	≥18	719	27	*101*	118	(113 – 124)

ALT = alanine aminotransferase; AST = aspartate transaminase; CK = creatine kinase; GT = gamma-glutamyltransferase; AMY-P = pancreatic type amylase; AMY = amylase; LD = lactate dehydrogenase; ALP = alkaline phosphatase.

*90% confidence interval of the 97.5 percentile.

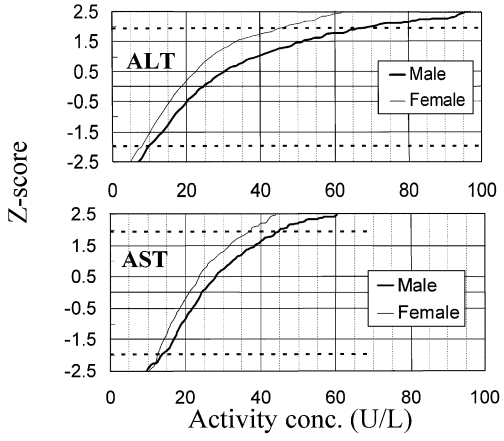

FIG. 1. Cumulative distribution of alanine aminotransferase (ALT)- and aspartate transaminase (AST) activity concentrations presented as Z-score plots. The results are presented in Table VI. The two dashed horizontal lines denote the 2.5 and 97.5 percentiles.

the partitioning test for gender was "positive". The activity levels were slightly higher in males below 60 years of age than in those above that age [18], but at the 97.5 percentile the partitioning test was "uncertain" for males and "negative" for females.

There was a positive correlation between ALT activity concentration and body mass index (BMI), giving a higher 97.5 percentile for both males and females with a BMI above 28 kg/m2 compared with that below this level (Table VII). However, we do not suggest separate limits related to BMI because the increase was modest and gradual through the whole age range. The Spearman rank nonparametric test showed a significant correlation between ALT and cholesterol, triglycerides and glucose in serum. No striking differences in results from the various countries were found.

TABLE VII. Percentiles of ALT related to body mass index (BMI).

		Percentiles				
Gender/BMI (kg/m2)	No.	2.5	25	50	75	97.5
Women BMI < 28	1114	8	14	18	23	44
Women BMI ≥ 28	104	10	17	21	31	56
Men BMI < 28	955	10	19	24	31	63
Men BMI ≥ 28	120	11	23	32	46	97

We suggest separate reference intervals for males and females (Table VI).

Aspartate transaminase (AST) NPU01324

The distribution of AST activity concentrations was slightly skewed towards higher levels (Fig. 1). Concentrations were on average slightly higher in males than in females, particularly at the higher percentiles, and the partitioning test was "positive" [18]. No correlation with age or with BMI was found.

We suggest separate reference intervals for males and females (Table VI).

Creatine kinase (CK), NPU01796

CK was measured using IFCC-compatible methods in 1169 females and 1051 males. Of these, 121 females and 250 males were excluded owing to extraneous physical activity during the previous week. The distributions of CK activity concentrations of the remaining 1048 females and 801 males were highly skewed towards higher levels (Fig. 2). Concentrations were on average higher in males than in females at all percentiles with a "positive" partitioning test for gender. In males, the 97.5 percentile decreased with increasing age [18]. The partitioning test was "uncertain" when using 50 years as the breakpoint. Nevertheless, because

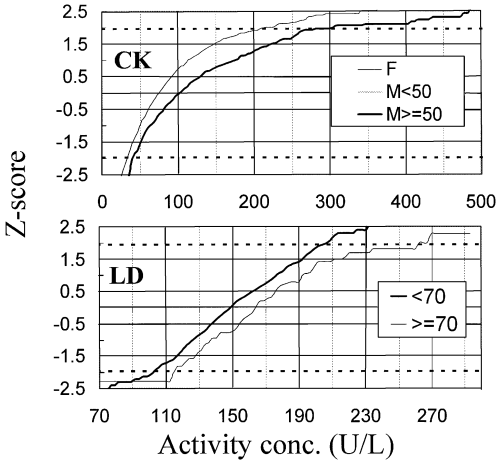

FIG. 2. Cumulative distribution of creatine kinase (CK) and lactate dehydrogenase (LD) activity concentrations presented as Z-score plots. The results are presented in Table VI. The two dashed horizontal lines denote the 2.5 and 97.5 percentiles.

the difference was considerable at the upper limit, we considered it appropriate to suggest different limits below and above 50 years of age. It should be emphasized that the high levels were found particularly in the age group from 18 to 40 years. In females, no significant correlation with age was found. No correlation between BMI and CK was found in either males or females [18].

There were comparatively many high CK activity concentrations reported among males from Finland, 9 out of the 13 values above 400 U/L originated form Finland, and 5 out of 9 values from men <50 years of age. Altogether, 24 out of the 397 values in this age group were above 280 U/L, range 282–726 U/L. Excluding the Finnish CK values from the calculation in this case decreases the 97.5 percentile from 397 U/L to 326 U/L.

When the CK values were subgrouped according to the day of sampling, we found that in males the 97.5 percentile was highest on Tuesday and thereafter declined gradually until Friday, the Monday percentile being higher than the Friday percentile (Fig. 3). A gradual but much less pronounced decrease was found in females from Monday to Friday. There was no change in the medians in either gender (Fig. 3).

We suggest separate reference intervals for males and females, and separate intervals for males below and above 50 years of age (Table VI).

Lactate dehydrogenase (LD), NPU02546

The LD activity concentrations of persons below 70 years of age showed a near normal distribution, whereas those comparatively few values from persons above 70 were skewed towards higher values (Fig. 2). There were no gender-related differences in the 97.5 percentiles. The activities were higher at all percentiles for persons older than 70 years, whereas the 97.5 percentiles for those below 70 and below 60 were the same, 204 and 202 U/L, respectively. The partitioning test was positive at both ages [18]. No relation to BMI was found.

We suggest separate reference intervals for those below and above 70 years (Table VI).

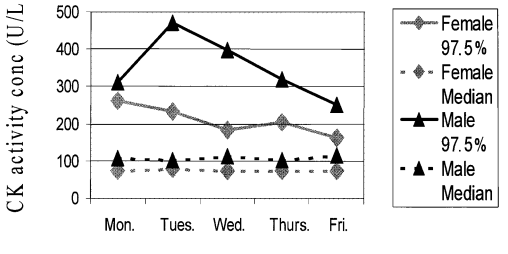

FIG. 3. Medians and 97.5 percentiles of creatine kinase (CK) results from males and females as calculated for each weekday of blood sampling. The results each day varied for females from 114 to 288, and for males from 80 to 218.

Alkaline phosphatase (ALP), NPU01144

The ALP activity concentrations showed a near normal distribution in the range above median (Fig. 4). The average values were slightly higher for males than females, and they were higher for those above 50 years of age than for those below that age. But the partitioning test was "uncertain" in both cases. There was a slight gradual increase related to BMI. No difference between countries was found [18].

We suggest common reference intervals for males and females (Table VI).

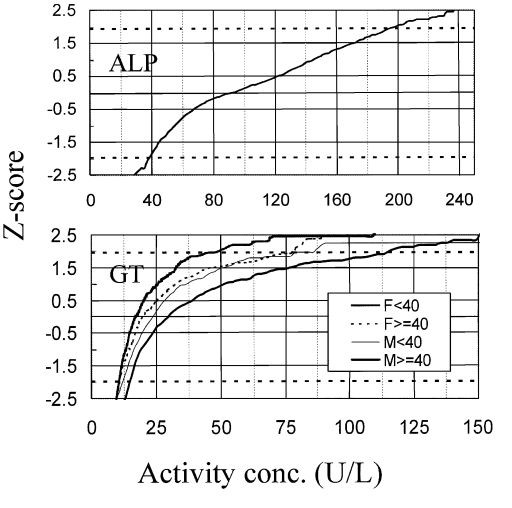

FIG. 4. Cumulative distribution of alkaline phosphate (ALP)- and gamma-glutamyltransferase (GT) activity concentrations presented as Z-score plots. The results are presented in Table VI. The two dashed horizontal lines denote the 2.5 and 97.5 percentiles.

Gamma-glutamyltransferase (GT), NPU02251

The distribution of GT activity concentrations was markedly skewed towards higher levels (Fig. 4). The GT activity concentrations were higher in males than in females, particularly at the higher percentiles. The 97.5 percentile showed a steep increase in both genders at the age of about 40 years [18]. The partitioning test was "positive" for gender and also for age in both sexes, using a breakpoint of 40 years. There was a weak positive correlation between GT activity concentration and BMI [18] with an average increase in the 97.5 percentiles of about 10 U/L for persons with a BMI above 28 compared to those below. We do not suggest separate limits related to BMI. The Spearman rank test showed a significant correlation of GT activity also to cholesterol, triglycerides and glucose in serum.

We suggest different reference intervals for males and females and different intervals for both genders below and above 40 years of age (Table VI).

Amylase, pancreatic type (AMY-P)

The AMY-P activity concentrations were skewed towards higher values (Fig. 5). The activity concentrations were lower in females than in males, particularly in the upper part, the 97.5 percentiles being 52 and 68 U/L, respectively. A similar difference was found with respect to age, the 97.5 percentile being 47 U/L in persons below and 69 in those above 50 years of age. The partitioning test was "positive" with respect to both gender and age [18]. Nevertheless, we do not consider these differences sufficiently pronounced to propose gender- or age-related upper reference limits to be used as guidelines in the diagnostic work. No relation to BMI was found. AMY-P was almost exclusively performed in Sweden and Norway.

We suggest common reference intervals for males and females (Table VI).

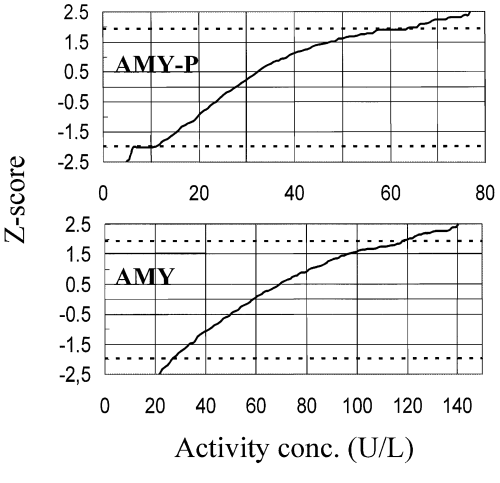

FIG. 5. Cumulative distribution of pancreatic type amylase (AMY-P)- and AMY activity concentrations presented as Z-score plots. The results are presented in Table VI. The two dashed horizontal lines denote the 2.5 and 97.5 percentiles.

Total amylase (AMY), NPU01238

The AMY activity concentrations were skewed towards higher values (Fig. 5). In contrast to AMY-P, the total AMY activities showed no marked age- or gender-related differences in the higher part of the distribution curve [18]. No relation to BMI was found.

We suggest common reference intervals for males and females (Table VI).

Relation between serum and plasma

All the measurements were routinely done on frozen and thawed sera. Except for LD, the enzymes were also measured in a number of samples of simultaneously drawn fresh, unfrozen plasma and serum. In Tables VIII and IX we present the regression constants for the slopes and intercepts found in thawed serum vs. fresh plasma samples, and in thawed serum vs. fresh serum samples. We consider the correlations sufficiently good to allow common reference intervals for these seven enzymes regardless of whether the material is fresh plasma, fresh serum or frozen and thawed serum.

DISCUSSION

The upper reference limits for ALT, AST, CK, and GT presented here are markedly higher than those recently reported by Schumann & Klauke [16] on behalf of the IFCC expert panel. Our limits for these enzymes are also somewhat higher than those currently in common use in

TABLE VIII. Relationship* between activity concentrations measured in frozen/thawed serum and fresh plasma.

Enzyme	No.**	Slope	95% CI	Intercept	95% CI
ALT	811	1.001	0.969 to 1.035	−0.7	−1.2 to −0.1
AST	871	0.995	0.974 to 1.016	−0.0	−0.5 to 0.4
CK	714	0.991	0.981 to 1.000	1.0	−0.1 to 1.9
ALP	91	1.013	0.976 to 1.052	−1.6	−4.0 to 1.0
GT	522	0.993	0.980 to 1.008	−0.3	−0.7 to 0.0
AMY-P	193	1.002	0.986 to 1.019	−0.6	−1.1 to −0.1
AMY	307	1.017	1.001 to 1.032	−2.3	−3.1 to 1.0

ALT = alanine aminotransferase; AST = aspartate transaminase; CK = creatine kinase; GT = gamma-glutamyltransferase; AMY-P = pancreatic type amylase; AMY = amylase; ALP = alkaline phosphatase.
*As tested by regression analysis.
**Number of persons from whom the two samples were drawn simultaneously.

TABLE IX. Relationship* between activity concentrations measured in frozen/thawed serum and fresh serum.

Enzyme	No.**	Slope	95% CI	Intercept	95% CI
ALT	608	0.985	0.958 to 1.012	1.3	−0.8 to 1.9
AST	586	0.987	0.970 to 1.005	0.4	−0.1 to 0.8
CK	482	0.994	0.981 to 1.006	2.5	1.3 to 3.8
ALP	62	0.994	0.946 to 1.023	0.4	−1.5 to 2.2
GT	295	1.007	0.994 to 1.020	−0.3	−0.5 to 0.0
AMY-P	65	0.988	0.954 to 1.021	0.2	−0.8 to 1.1
AMY	252	1.019	1.009 to 1.028	−0.7	−1.2 to −0.1

ALT = alanine aminotransferase; AST = aspartate transaminase; CK = creatine kinase; GT = gamma-glutamyltransferase; AMY-P = pancreatic type amylase; AMY = amylase; ALP = alkaline phosphatase.
*As tested by linear regression.
**Number of persons from whom the two samples were drawn simultaneously.

the Scandinavian countries and in other parts of the world.

These marked discrepancies are most likely related to the different methods by which the reference persons have been selected in the two studies. Our reference material consists of persons who considered themselves to be in good health. Additional exclusions were undertaken on the basis of predefined biochemical and clinical criteria [17, 18]. The reference persons were, on the other hand, recruited from five different countries, which could tend to broaden the reference intervals. Indeed enzymes such as CK and GT showed differences in the 97.5 percentiles between the Nordic countries. For instance, excluding CK values of males younger than 50 years from Finland decreased the 97.5 percentile from 397 to 326 U/L. But even the latter value is higher than what is commonly used as the upper reference limit. Schumann & Klauke [16], on the other hand, selected their reference persons among in-hospital patients and examined their samples by one of three enzyme profiles routinely performed in the hospital on admission. The profiles consisted of 3 to 7 serum enzymes, one of which was always the target enzyme in question. The measurement methods used to select their reference persons were not necessarily IFCC compatible. The patient was included in the study only provided all enzyme levels of the profile were below the respective upper reference limits, presumably the 97.5 percentile limits. This means that the data for each enzyme must have been strongly truncated at the upper part, which inevitably tends to decrease the 97.5 percentiles. In accordance with this, Schumann & Klauke found narrower 90% confidence intervals at the respective 97.5 percentiles [16] than we did (Table VI), particularly for the two enzymes with a long tail at the higher levels, i.e. CK and GT.

Another reason for the discrepancies may be related to the fact that Schumann & Klauke [16]

used carefully standardized IFCC reference measurement procedures at 37°C, and all analyses were done in one and the same medical laboratory, whereas our samples were measured in many different laboratories using various commercially available routine measurement systems. The latter procedures had all been standardized in accordance with the IFCC reference systems by the producers and thus they ought to be compatible with the IFCC recommended methodology. Still, one might expect that the diversity of systems used in this study would tend to broaden the reference intervals. Two lines of evidence suggest that this cannot be a major reason for the broad intervals presented. First, the overall external reproducibility of the IFCC compatible enzyme methods in general use in Scandinavia shows CVs in the order of 5–10%, as generally revealed by data from the Nordic External Quality Assessment Surveys. Secondly, the distribution of results obtained by one closed, dry chemistry system (Ortho Vitros) compared well with the combined distribution of results from seven wet chemistry systems (Table V).

A third reason for the discrepancies may be that we, despite the precautions taken in the selection of a reference population, may have included a significant number of diseased persons without clinical symptoms and biochemical signs, e.g. diseases like hepatic steatosis, non-alcoholic steatohepatitis or chronic hepatitis C [21].

Among the enzymes discussed so far, reference limits and decision limits for ALT have frequently been the subject of studies, obviously because ALT also serves as an important safety parameter in blood banking. In a recent study based on a material from 6835 blood donors, Prati *et al.* [22] suggested upper reference limits of 19 and 30 U/L for women and men, respectively. The paper initiated an engaged debate on the pros and cons in the journal [21, 23, 24]. It should be emphasized, however, that their limits corresponded to the 95 percentiles of the material. Apparently these researchers were speaking about suitable decision levels rather than conventional upper reference limits. But their 95 percentiles were still markedly lower than our 95 percentiles (Table VI). It was not stated whether pyridoxal-5-phosphate was included in the reagents, but even omitting this would not explain the large differences. Like Prati *et al.* [22] and others [21], we found correlations between ALT and BMI, triglycerides, cholesterol, and glucose.

Reference limits for ALT suggested by producers of *in vitro* diagnostic reagents such as Roche, Dade/Behring, Olympus and Ortho/Vitros suggest upper reference limits from 31 to 52 U/L for females, and from 35 to 72 U/L for adult males. The higher limits are concordant with our findings.

Our upper limits for AST are in fairly good agreement with those of others [25], including producers of reagents, recommending upper limits from 35 to 37 U/L for females, and from 45 to 59 U/L for males.

Of the eight upper limits presented here, those for CK deviate the most from those commonly used, particularly the limit for males <50 years of age. But several others have previously reported similar high limits for young males [26–28]. Like these researchers, we cannot offer a definite explanation for the high levels found. However, we believe that in our case the high upper limits might be related to the custom of comparatively high physical activity among Scandinavians, who often participate in outdoor and indoor sports and aerobics, and go on hikes in forests and mountains, including cross-country skiing. The last-mentioned activity takes place particularly during weekends, mostly on Sundays, and includes also less well-trained persons. In accordance with this we find typical profiles for the 97.5 percentiles calculated for each weekday of sampling (Fig. 3). The highest values were found on Tuesdays for males and on Mondays for females, and were higher for males than for females. CK activity concentrations are known to peak 1 to 4 days after strenuous activity; later the higher it peaks [29, 30]. It should be stressed in this connection that all results on samples from reference persons who notified that they had "participated in strenuous sports during the previous week (e.g. run more than 10 km in one go, trained in fighting sports, such as karate, boxing or equivalent or been active in body building" [18], in other words in activities considered to be unusually strenuous, were excluded from the CK calculations. Moreover, careful examination of the information questionnaire of the 24 males with CK above 280 U/L revealed nothing that could explain the high values. The person

with the highest CK activity (725 U/L) was a man from Somalia. Africans are known to have on the average higher CK activities than Caucasians, but not in this order of magnitude [31]. Removing his value would only decrease the 97.5 percentile to 383 U/L.

In the case of LD, we found an upper limit that was in fact lower than that reported by Schumann & Klauke [16], i.e. 204 vs. 248 U/L. Their limit is the same as the limit we found for persons above 70 years of age. Schumann & Klauke did not report on age differentiation. Their material included all persons ≥ 17 years of age, with an average of 50 years. The preliminarily value suggested by the IFCC Committee on Enzymes is 225 U/L [32]. Since all the LD analyses were done on thawed sera, it should be recalled that particularly LD5 and LD4 are liable to inactivation upon deep freezing. LD in the blood of healthy individuals is dominated by LD1, LD2 and to a lesser extent LD3. Therefore the loss in activity in our sera is assumed to be of minor importance. Nevertheless, the upper reference limits found may be slightly underestimated compared with those that would have been found if fresh serum had been analysed instead.

The upper reference limits for ALP found by Tietz & Shuey [33] as well as those proposed by Abicht *et al.* [34] are in good agreement with our findings, although they reported gender and age differences that were more pronounced than those we found.

The upper reference limits reported for GT vary considerably, probably because they often have been based on small reference populations. Schumann & Klauke [16] in their large patient material found upper limits of 38 and 55 U/L. Abicht *et al.* [34] recently proposed similar limits, i.e. 39 and 66 U/L for females and males, respectively. Several previous health studies have demonstrated increasing values with increasing age, particularly in males [35] but also in females [36]. Furthermore, we report on higher 97.5 percentiles for males than for females, the limits being unusually high in both genders. In addition, we found pronounced age differences.

In accordance with previous reports [35, 36], in the present material there was also a statistically significant correlation between GT and cholesterol, triglycerides and glucose in serum, but only a weak correlation to BMI. We did not find any obvious relationship between high GT levels and high ALT values.

Junge *et al.* [37] recently presented reference intervals for AMY and AMY-P that were common to both genders, being 28 – 100 and 13 – 53 U/L, respectively. These intervals are in fairly good agreement with ours, although the upper reference limits are somewhat lower than ours.

Until now, many medical laboratories and clinicians, even in different countries, often apply the same reference limits despite significant variations in the assay conditions used for enzyme measurements by the respective laboratories. It is also a fact that samples from one and the same patient are frequently forwarded to different laboratories for analysis, even across borders. Therefore, the international standardization of the methodology for enzyme measurements that has now occurred, and which in turn allows for an adequate harmonization of the respective enzyme reference limits, is indeed highly desirable. But applying too narrow reference intervals, as obtained under strictly optimized conditions both with respect to analytical refinement and selection of reference persons, may in ordinary health practice cause unnecessary trouble for the patients because of a high percentage of false positives. This in turn also initiates unnecessary use of laboratory resources. Perhaps, therefore, reference intervals determined in the way that has been done in the present study are more suitable to be used as general diagnostic guidelines. However, we must stress that reference limits are not equivalent to diagnostic decision limits, perhaps particularly in the case of serum enzymes. The latter ought to be quite different from the former in a number of specific clinical situations.

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Received: 8 January 2004
Accepted: 21 April 2004