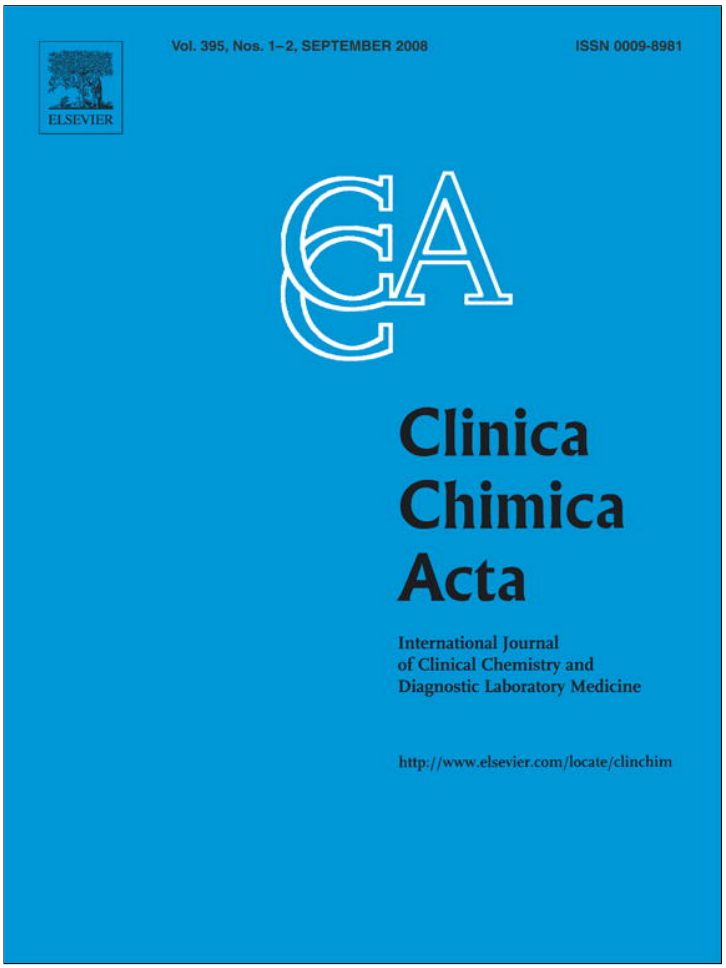

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Letter to the editor

Fractions of plasma gamma-glutamyltransferase in healthy individuals: Reference values

Dear Editor,

Gamma-glutamyltransferase (GGT) activity, long used as a biomarker of liver function and excessive alcohol use [1], has been recently recognized to be a biomarker of future occurrence of diabetes and cardiovascular events [2–3], either in unselected populations [4], or in patients with ascertained ischemic heart disease [5]. Although the assay is widely used, highly reproducible and sensitive, it holds a poor specificity [1].

We recently set up a novel, highly reproducible and sensitive method for differentiating and quantifying four GGT activity fractions ("big", "medium", "small" and "free": b-, m-, s-, and f-GGT, respectively) in human blood [6], by means of molecular exclusion chromatography. We aimed to assess the clinical potential of the method by establishing reference values in healthy subjects.

Fasting blood samples were obtained between 8:00 and 9:00 from 200 healthy individuals [blood donors, 100 males, mean ± SD, age 44 ± 10 years (range, 21–64), 100 females, age 40 ± 11 years (range 20–64)]. The presence of acute or chronic diseases, and of metabolic syndrome (according to World Health Organisation criteria [7]) was excluded by history, clinical examination and comprehensive laboratory testing. Subjects on pharmacological treatment for whatever reasons or with alcohol consumption >45 g/day for men and >30 g/day for women were previously excluded from the study. The study was approved by the Institutional Ethics Committee and all subjects gave informed consent.

Total and fractional GGT analysis was performed on plasma–EDTA samples as described previously [9], by using an FPLC system (AKTA purifier, GE Healthcare) equipped with a gel-filtration column (Superose 6 HR 10/300 GL, GE Healthcare) and a fluorescence detector (Jasco FP-2020). GGT activity determination performed on plasma–EDTA samples showed a correlation coefficient of 0.99 (n=10) with values obtained either in serum or in plasma–heparin samples. Inter-assay CV% was 4.65 for total GGT, 6.19 for b-GGT, 5.13 for m-GGT, 4.66 for s-GGT, 4.77 for f-GGT. Fractional GGT area was calculated with the aid of a computer program (MATLAB Version 7 MathWorks, Inc.) to resolve overlapping peaks, the curve fitting was conducted with a nonlinear least-squares minimization algorithm using four exponentially modified Gaussian (EMG) curves; upper and lower bounds for the curve amplitude, width, position and asymmetry were set for each peak.

Our original procedure allowed the analysis of four GGT fractions in all plasma samples examined, overcoming the low sensitivity of previously proposed methods, which cannot give homogeneous nor reproducible results [8–10]. All four GGT fractions showed significantly higher values in males than in females (Table 1). In both genders total plasma GGT, as well as b-, m- and s-GGT fractions, showed a right-skewed distribution, while f-GGT showed a normal distribution (P>0.10, Kolmogorov–Smirnov test with Dallal–Wilkinson–Lillie for P value).

Plasma GGT fraction analysis might help to specify the complex interaction between GGT activity and cardiovascular risk factors. We have found in healthy subjects of both genders that f-GGT fraction, with a molecular weight of 70 kDa, is the prominent one for total GGT values in the low-normal range (below median; males: <25 U/l, females: <14 U/l) (Fig. 1). For total GGT levels above median values, known to be associated with cardiovascular risk factors such as BMI, DAP, serum triglycerides and LDL cholesterol [4], we observed a consistent logarithmic increase in b-, m- and s-GGT fractions (Fig. 1), whose molecular weights are respectively in the range of VLDL, LDL and HDL [9]. Nevertheless, the relationship between fractional GGT, lipoproteins and cardiovascular disorders needs further characterization.

In conclusion, GGT fraction analysis by high performance molecular exclusion chromatography could improve the low specificity of current GGT assay, allowing the discrimination of underlying risk and/or pathologic conditions.

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Table 1
Total and fractional plasma GGT activities in healthy humans

	Males (n=100)		Females (n=100)		
	Median	5th–95th percentile	Median	5th–95th percentile	P
Total GGT*	25.3	12.3–60.5	14.4	8.4–30.9	<0.001
b-GGT*	2.4	0.7–10.7	1.1	0.4–5.2	<0.0001
m-GGT*	1.0	0.2–3.3	0.5	0.2–1.2	<0.0001
s-GGT*	9.2	2.8–33.7	3.9	1.5–11.6	<0.0001
f-GGT	13.2	8.3–19.6	8.9	5.9–12.5	<0.0001

Data are expressed as U/l. *Student's t-test performed on ln transformed data. N.S: not significant.

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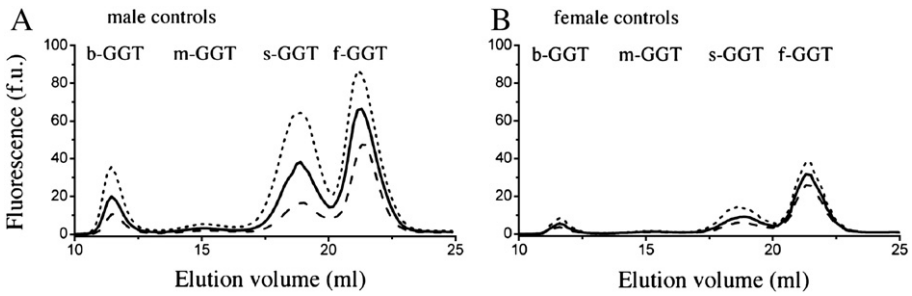

Fig. 1. Elution profile of fractional GGT activity corresponding to the 25th (dashed line), 50th (solid line) and 75th (dotted line) percentile of total GGT in healthy males (A, $n=100$) and healthy females (B, $n=100$). Fractional GGT analysis was performed on plasma–EDTA samples by high performance gel-filtration chromatography, GGT activity was specifically detected by an on-line post-column reaction with a fluorescent substrate, γGluAMC [6].

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