We suggest four possible explanations for the observed increased "cardiac" troponin T values:

First, the unspecific "cardiac" troponin assay shows 1–2% cross-reactivity with skeletal muscle troponin T [6]. Therefore, possible skeletal muscle injury in uremic patients or after liver transplantation may be involved.

Second, possible minor myocardial damage cannot be discarded, neither by normal creatine kinase activity, normal creatine kinase MB activity in blood, nor normal electrocardiogram. Therefore, like Wu et al. [9], we cannot rule out some minor myocardial injury in our patients. As Zimmermann et al. [10] showed, "cardiac" troponin T is detectable in heart transplant patients for at least 3 months after transplantation without evidence of ischemic myocardial injury. Their findings were thought to result from a prolonged leakage of cytosolic "cardiac" troponin T from viable myocytes as well as prolonged disintegration of myofibers from irreversibly damaged myocytes.

Third, the expression of cardiac troponin T possibly is upregulated in chronically damaged skeletal muscle [11], as has been demonstrated for the phylogenetically older B-isoform of creatine kinase [12].

Fourth, possible interfering substances may be the reason for increased "cardiac" troponin T in our patients, given the lack of correlation (r = 0.19) between increased "cardiac" troponin T and serum creatinine (Fig. 1). Additionally, in some serum samples with excessively high serum creatinine concentrations (>884 μmol/L), no "cardiac" troponin T is measurable, whereas in serum samples with moderately increased serum creatinine, high amounts may be detectable. Stepwise dilution of these samples showed that "cardiac" troponin T was linear in the range examined (data not shown).

We therefore conclude that findings of increased "cardiac" troponin T values by the unspecific first-generation assay in uremic patients should be considered very cautiously and not interpreted as indication of acute myocardial damage without corroboration evidence.

Note added: Preliminary studies with the second-generation assay of cardiac troponin T also show increased "cardiac" troponin T values in some serum samples with increased creatinine but normal creatine kinase activity (unpublished data).

We thank Boehringer Mannheim for providing the reagents for the first-generation troponin T assay and the ES 300 analyzer, and Sharon Page for reading the manuscript.

Reference Method for Serum Total Cholesterol Measurement: Does Substituting Enzymatic Step for Liebermann–Burchard Reaction Improve Specificity? Annalisa Modenesi,1,* Anna Carobene,1 Carlo Ferrero,1 Ferruccio Ceriotti,1 Carlo Franzini,1 Elena De Giorgi,1 Michela Franzini,1 Angela Giorgio,1 and Lucia Baldo2 (1 Istit Scientifico H. S. Raffaele, Lab. Analisi, Via Olgettina 60, 20132 Milano, Italy; 2 Istit Scienze Biomed., Univ. di Milano, Ospedale L. Sacco, Milano, Italy; * author for correspondence: fax Int +39-2-2643-2640, e-mail modenesa@imghsra.hsr.it)

A small but consistent bias (~1.6%) exists between the Reference and the Definitive Method for total cholesterol measurement [1]. Part of this bias (~0.6%) has been attributed to interference from sterols other than cholesterol normally present in serum [2], and it is caused by the low specificity of the Liebermann–Burchard (LB) reaction. Franzini and Besozzi [3] several years ago proposed a modification of the reference method, substituting the LB reaction with the one catalyzed by cholesterol oxidase (EC 1.1.3.6). Franzini and Luraschi [4] demonstrated a small systematic negative bias of the extractive/enzymatic procedure (0.8%) vs the Reference Method and
hypothesized that this bias could be attributable to a higher specificity of the enzymatic reaction. Several authors have investigated the specificity of cholesterol oxidase, as reported in the reviews of Smith and Brooks [5] and Richmond [6]. The degree of specificity found was quite low [7-9], but all the studies were performed with only the enzyme from Nocardia and often included sterols present in serum at negligible amounts. Thus we decided to verify the correctness of the hypothesis of Franzini, testing the ability of five commercially available cholesterol oxidases of microbial origin to oxidize the five noncholesterol sterols most commonly found in normal serum under homogeneous experimental conditions, similar to those proposed for serum cholesterol measurement [3, 4].

The cholesterol oxidases used were from Nocardia erythropolis (Boehringer Mannheim, Mannheim, Germany), Streptomyces spp., Cellulomonas spp., Brevibacterium spp., and Pseudomonas fluorescens (Sigma, St. Louis, MO).

First, the conditions for the enzymatic reaction were optimized. pH dependence of each cholesterol oxidase was verified by measuring at 500 nm the initial rate of the reaction using two types of buffer: piperazine-N,N-bis-(2-ethanesulfonic acid) (PIEPS) from pH 6.5 to 7.0 and phosphate from pH 7.0 to 7.5. The activities determined with the two buffers were somewhat different only for the cholesterol oxidase from Brevibacterium, with two peaks of maximal activity at pH 6.7 (PIEPS) and 7.2 (phosphate). In any case, between these two pH values (6.7 and 7.2), the activities of all five oxidases were within 90% of the maximum. For this reason we chose PIPES buffer at pH 7.0 for all the oxidases. The amount of enzyme necessary to reach the end point within 15 min for a cholesterol concentration of 12.93 mmol/L was established by testing several amounts of each enzyme. The final activities in the reaction mixture chosen were 127, 73, 208, 208, and 111 U/L for the cholesterol oxidases from N. erythropolis, Streptomyces, Brevibacterium, P. fluorescens, and Cellulomonas, respectively. Concentrations of all the other reagents were as proposed by Cooper et al. [10].

Hexane solutions of β-sitosterol [stigmaster-5-en-3-ol(3β)], lathosterol [cholest-7-en-3-ol(3β,5α)], desmosterol [cholest-5,22-dien-3-ol(3β)], campesterol [ergost-5-en-3-ol(3β,24R)], and cholesterol [cholestan-3-ol(3β,5α)] (Sigma) were prepared at three concentrations ranging from 0.060 to 0.503 mmol/L, equivalent to the concentrations attainable with samples with sterol concentrations of 1.2-10.6 mmol/L submitted to the extraction phase. Aliquots (1 mL) of these solutions were dried under reduced pressure; the residue was dissolved in 0.5 mL of absolute ethanol and processed with the different types of enzymatic reagents. Alternatively, 2 mL aliquots of the same solutions were tested with the LB reagent, according to the CDC protocol [11]. In the case of cholesterol, for the LB reaction, we prepared 10-fold concentrated solutions because of the known low reactivity of this compound.

The results of the specificity testing of the cholesterol oxidases are shown in Fig. 1. Each bar represents the mean of the ratios between sterol and cholesterol signals obtained for the three concentrations tested. The results obtained were not concentration dependent; in fact we obtained almost identical ratios at the three concentrations tested. The results obtained confirm substantially identical specificity patterns of at least three enzymes (from N. erythropolis, Streptomyces, and Cellulomonas) and some very small differences for the enzyme from P. fluorescens, whereas the oxidase from Brevibacterium seems to show some higher specificity, oxidizing lathosterol, cholestanol, and sitosterol at a lower rate than cholesterol.

Fig. 1. Specificity of the five cholesterol oxidases and of the LB reaction: Each bar represents the mean of the ratios of sterol vs cholesterol signals obtained measuring the three concentrations of the various sterols.

data obtained with the Abell–Kendall method are in very good concordance with those presented by Bernert et al. [2] and confirm the nonreactivity of cholesterol and the high reactivity of lathosterol. The results obtained with the cholesterol oxidase from N. erythropolis confirmed the data presented by Smith and Brooks [5]; the only difference was seen with sitosterol, but the authors evaluated the interference by measuring the initial rate, whereas we measured the end point reaction.

We wanted to have a better idea of the actual level of interference due to endogenous sterols with the extraction/enzymatic method (with the various oxidases) and with the Abell–Kendall method on cholesterol measurement in normal serum. Thus we assumed the concentrations of these sterols measured by Bernert et al. [2] in SRM 909 as representative of the content of a normal serum (8.2 μmol/L campesterol, 7.2 μmol/L sitosterol, 2.1 μmol/L desmosterol, 3.6 μmol/L lathosterol, and 9.0 μmol/L cholesterol) and calculated the expected “cholesterol error” using the ratios shown in Fig. 1. Performing this calculation, the total cholesterol error (sum of the errors due to the five sterols) was 28.57, 28.83, 19.34, 27.17, and 29.00 μmol/L with oxidase from N. erythropolis, Streptomyces, Brevibacterium, P. fluorescens, and Cellulomonas, respectively; it was 17.30 μmol/L with the LB reaction.

These data are clearly in contrast to those reported by Allain et al. [8], but the discrepancies can be explained mainly by the data on 7-dehydrocholesterol and cholesterol. The plasma concentration of 7-dehydrocholesterol was clearly overestimated, since it is practically absent from plasma; cholesterol does not react with our Abell–Kendall procedure.

In conclusion, even with the most specific enzyme (from Brevibacterium), no interference is obtained as far as the specificity vs the noncholesterol sterols is concerned. In view of the very small amounts of such sterols normally found in serum, this aspect has a limited practical impact. However, although studies on this kind of interference were carried out with serum pools [1, 2], large variations of noncholesterol sterol concentrations in individual sera have been reported [12, 13]. Therefore, it cannot be excluded that, on individual serum samples, significant interference from sterols may take place, whether the enzymatic or the LB reagent is used.
In a previous paper [4], cholesterol concentrations were measured with the enzymatic and the LB reagent in split aliquots of hexane extracts from several individual sera. Statistically the enzymatic values showed a small systematic constant negative bias vs the LB values (−0.052 mmol/L, corresponding to 0.9% at 6.0 mmol/L). Assuming equivalent interference by the noncholesterol sterols in the two reactions, it is tempting to speculate that the enzymatic reaction is less sensitive to the remaining unidentified sources of interference [2], thus being in position to generate results closer to the definitive isotope dilution mass spectrometric method. Again, this appears to have a negligible practical impact.

References

Determination of Organic Acids by Capillary Electrophoresis in Screening of Organic Acidurias, Carla M. Jariego and Angel Hernanz* (Servicio de Bioquímica, Hospital La Paz, Castellana 261, 28046 Madrid, Spain; *author for correspondence: fax 34-1-3582733)

Pathologic alterations of normal catabolism of amino acids, fatty acids, carbohydrates, cholesterol, biogenic amines, and steroids frequently result in abnormal excretion of urinary organic acids. These metabolic disorders manifest themselves either by excretion of abnormal organic acids in the urine that are usually not detected in healthy individuals or by excretion of massive amounts of certain acids that are normally present in the urine in small amounts [1,2]. Neonatal screening of urine for inherited and acquired organic acidurias is important for preventing severe neurologic disease, mental retardation, and death [3,4].

Organic acids are quantified in urine by gas chromatography in combination with mass spectrometry [2,5–7]. This method is accurate and precise but is slow and requires expensive equipment. We propose a simple, fast, and reliable capillary electrophoresis method for detecting and measuring 10 organic acid markers—methy lactonate, glutarate, 3-methylglutarate, N-acetylaspartate, 2-amino adipate, propionate, lactate, 2-oxoisovalerate, isovalerate, and homogentisate—in the screening of organic acidurias. The method comprises direct diluted urine application, electrophoretic separation at 29 kV, and ultraviolet absorption at 185 nm. Complete separation was achieved within 10 min. This method is applicable for use in routine clinical chemistry laboratories.

Upon approval by the Ethics Committee of La Paz Hospital, urine samples were collected from 65 control children ranging from 2 months to 10 years old and from two children with glutaric aciduria type I. Samples were filtered through Ultrafree Millipore (Bedford, MA) filters (M, 10,000), centrifuged 15 min at 2000 g, and then diluted with MilliQ water (Millipore) to obtain a creatinine concentration ~1 mmol/L.

The organic acid separation was performed on a Waters AccuSep polyimide-coated 100 cm × 75 μm fused-silica capillary using a C18® Capillary Electrophoresis instrument (Waters, Milford, MA) equipped with a Millennium® Chromatography Manager (Waters). The electrolyte was a solution of 20

![Fig. 1. Electropherograms of (A) a mixture of 250 μmol/L of each of the following organic acids: methy lactonate (1), glutarate (2), 3-methylglutarate (3), N-acetylaspartate (4), 2-amino adipate (5), propionate (6), lactate (7), 2-oxoisovalerate (8), isovalerate (9), and homogentisate (10); (B) a normal urine sample; and (C) urine from a patient with glutaric aciduria type I, showing the high amount of glutarate in the sample.](image-url)