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Discussion

Comments on quantitation of carnitine esters by high performance liquid chromatography

Reply to E. Schmidt-Sommerfeld and D. Penn

P.E. Minkler, S.T. Ingalls, C.L. Hoppel*

Medical Research Service, Department of Veterans Affairs Medical Center, Cleveland, OH 44106, USA, and Departments of Pharmacology and Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA

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Until recently, investigators interested in identification or determination of carnitinyl esters (acylcarnitines) in biological matrices were limited to two established procedures. These are the FAB-MS–MS methodology developed and used for this purpose by Millington *et al.* [1], and the radioisotope exchange/HPLC methods developed and applied by Kerner and Bieber [2] and Schmidt-Sommerfeld *et al.* [3]. Due to perceived or real limitations or problems with these methods, several investigators independently have developed alternate approaches to acylcarnitine determination. GC–MS techniques were developed by Huang *et al.* [4] and Lowes *et al.* [5]. Chemical derivatization HPLC techniques have been developed by several workers [4–14].

Schmidt-Sommerfeld and Penn have published two thorough studies which emphasize the strengths of the radioisotope exchange/HPLC method [15,16]. They determined small amounts of hexanoylcarnitine and octanoylcarnitine in samples from patients with medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. They demonstrated that the radioisotope exchange method, when practiced by technical experts, can distinguish specimens obtained from MCAD deficient patients and control samples. However, Schmidt-Sommerfeld and Penn have stated previously that caution must be exercised in using this approach: "It was found that the specific radioactivity of total or acylcarnitine could not be used to calculate the concentrations of individual carnitine esters since a major fraction of the acylcarnitine did not reach isotopic equilibrium. These esters which include medium chain acylcarnitines were indirectly estimated..." [17]. Although the more recent work by these authors includes changes which seem to correct this and other possible problems, the perception of radioisotope exchange/HPLC acylcarnitine determination as troublesome and narrowly applicable largely follows from reports by these authors in which assay conditions were modified to accommodate some newly identified problematic instance of sample composition or concentration. The number of independent investigators who are developing acylcarnitine determination methods which are technically unrelated to radioisotope exchange/HPLC indicates that there remains in the scientific community a

* Corresponding author. Address for correspondence: Medical Research 151 W, VA Medical Center, 10701 East Blvd., Cleveland, OH 44106, USA.

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sense of inadequacy and disappointment in the radioisotope exchange/HPLC method.

Chemical derivatization HPLC methods for carnitine and carnitinyl ester determination offer several advantages over radioenzymatic HPLC methods. Our chemical derivatization HPLC can determine sample concentrations of free and total carnitine in addition to acylcarnitines. Since radioisotope exchange/HPLC cannot accomplish these analyses, an additional analytical procedure is required to provide these data. We designed the procedure for use with common UV detectors operating at 254 nm. No special HPLC detector is required. No radioactivity is required. The chromatographic run time is one quarter that of the radioisotope exchange/HPLC method. It is easily adapted to automated HPLC sampling and data analysis. We have demonstrated the application of the chemical derivatization HPLC procedure to quantification of acylcarnitines in patients with methylmalonic aciduria, propionic acidemia, isovaleric aciduria, and MCAD deficiency, consistent with each patient's diagnosis [13,14]. Last, our HPLC procedure is as sensitive as the radioisotope exchange/HPLC method. We have published a chromatogram of a plasma specimen from a patient with MCAD deficiency which contained 1.5 nmol/ml of octanoylcarnitine and 0.3 nmol/ml of acetylcarnitine [14].

Schmidt-Sommerfeld and Penn commented [18] that chemical derivatization HPLC procedures require "extensive sample clean-up". All analytical procedures require some sample preparation effort. All analysts measure sample preparation effort in relation to relevant criteria of data quality. We isolate carnitine, short-chain and medium-chain acylcarnitines from plasma and urine specimens by small column chromatography. This procedure requires inexpensive materials, and it can be applied conveniently to 60 samples in a single episode of sample preparation. The column chromatographic procedure is generally applicable to samples which vary in carnitine and carnitinyl ester concentrations throughout the range found for normal subjects and for patients with metabolic disease. The chemical derivatization procedure succeeds without sample by sample adjustment of reagent concentrations and reaction conditions. The time required to prepare samples for our HPLC analysis actually is less than or equal to sample preparation time required for radioisotope exchange/HPLC determination [12].

We agree with Schmidt-Sommerfeld and Penn that blinded trials are one measure of the diagnostic applicability of an analytical method. However, our work has been directed toward a context and audience which extend beyond medical diagnosis of inborn metabolic errors. We began our development of HPLC methods for the determination of carnitine, its biosynthetic precursors, and carnitinyl esters 20 years ago. At that time, we decided against use of enzymatic reactions as a basis of our method development effort. We recognized that the substrate selectivity inherent in enzyme-based procedures would impede our biosynthetic studies, and thus it represented to us a methodological dead end. Therefore, we chose to develop chemical derivatization HPLC methods applicable to the full set of compounds involved in carnitine biosynthesis and carnitine metabolism. This work has led to procedures for determination of the carnitine biosynthetic precursors trimethyllysine [19], butyrobetaine [20], carnitine itself [12], and various acylcarnitines [13,14].

We believe that every approach to carnitine and acylcarnitine determination has comparative advantages and disadvantages related to its cost, convenience, reliability and selectivity. We expect that eventually each of these procedures will find application to problems to which it is suited best. For example, based on the refining work of Schmidt-Sommerfeld and Penn, the radioisotope exchange/HPLC method may emerge as the preferred method for detection MCAD deficiency in newborns. FAB-MS–MS probably is the present method of choice for identification and determination of unusual acylcarnitines. We anticipate that chemical derivatization HPLC will be used for determination of free and total carnitine and for monitoring organic aciduria patients who are receiving carnitine therapy.

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the opportunity to respond to them. We look forward to future conversations concerning this important area of investigation.

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