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Discussion

Comments on quantitation of carnitine esters by high-performance liquid chromatography

Reply to E. Schmidt-Sommerfeld and D. Penn

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This letter is written in response to that submitted by Schmidt-Sommerfeld and Penn [1] concerning our paper entitled "Quantitation of carnitine esters by high-performance liquid chromatography" [2]. Schmidt-Sommerfeld and Penn elaborate on several potential problems associated with the radioisotopic exchange method for measurement of carnitine esters by radio-HPLC [3,4] that were previously mentioned in our paper and in the paper from Minkler and Hoppel [5]. The primary concerns relate to the activity of the carnitine acetyl transferase (CAT) enzyme (EC 2.3.1.7) used to catalyze the radioisotopic exchange. Our response to these concerns are as follows:

(1) Limited substrate specificity of CAT: Schmidt-Sommerfeld and Penn [1] reiterate the limited substrate specificity of the commercial CAT used in their assay, but suggest that this does not pose a problem for the analysis of most metabolic diseases. We disagree in that while a number of disorders may be characterized using the radio isotopic exchange/HPLC method, others may go undetected. For example, we have identified [2] a potentially new metabolic disorder involving limited synthetic capacity for carnitine, resulting in low carnitine levels but elevated γ-butyrobetaine levels. Measurement of the latter is possible using carboxy-derivatizing agents. Furthermore, Roe *et al.* [6] discussed several metabolic disorders including long- and very-long-chain acyl-CoA dehydrogenase deficiencies. To our knowledge, the effects of these disorders on carnitine metabolism have not been well characterized. To study these effects using a method which limits the measurement of acylcarnitines to those containing acyl groups of ≤ 10 carbons could potentially miss several important long-chain carnitine esters affected by these disorders. Although the occurrence of these disorders is not well known (although expected to be low), their presence calls for the most comprehensive methodology to be applied for clinical applications.

(2) Possible inhibition of CAT by unusual carnitine esters: This point was not raised in our paper. However, because of this potential problem, the radio isotopic exchange/HPLC method requires evaluation of standards for each carnitine ester to be tested. If standardization is performed routinely, we agree with Schmidt-Sommerfeld and Penn [1] that this potential

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limitation indeed has insignificant implications or carnitine analysis by radio-HPLC.

(3) Presence of acylcarnitine hydrolase activity within the commercial CAT enzyme: We have no reason to disbelieve the findings of Schmidt-Sommerfeld and Penn [1] regarding hydrolase activity. Our comment was based solely on the concerns expressed by Delisa *et al.* [7].

We agree, as mentioned in our paper [2], that the radio isotopic exchange/HPLC method employed by Schmidt-Sommerfeld and Penn [3,4] is extremely sensitive and can yield reliable results for the short and medium-chain carnitine esters if properly standardized. The method does require a dedicated β-flow monitor which could limit implementation in some laboratories. We also agree that a disadvantage of our procedure is that the sample clean-up and preparation steps can be tedious, and recovery of carnitine esters can be influenced by the isolation buffers employed. There is also a limit in the degree to which a sample can be concentrated without affecting the derivatization efficiency and chromatographic baseline, such that sensitivity is limited compared to the radio isotopic exchange/HPLC procedure. To suggest, however, that these shortcomings preclude the application of our method to the identification and/or study of metabolic disorders, is unfounded. We agree that any analytical method should be tested for sensitivity and specificity as well as repeatability and recovery prior to adoption for use in general clinical diagnosis.

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