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Short communication

# Bile acylcarnitine profiles in pediatric liver disease do not interfere with the diagnosis of long-chain fatty acid oxidation defects

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**Abstract**

*Background:* Plasma acylcarnitine measurement is an important diagnostic tool for inherited disorders of fatty acid and organic acid metabolism. Biliary excretion has been shown to be the primary route of excretion for acylcarnitines and analysis of bile acylcarnitine profiles may provide greater sensitivity for detecting metabolic disorders. Disorders of fatty acid oxidation frequently present with deranged liver function and the effect of hepatic disease on biliary acylcarnitine excretion are unknown.
*Methods:* We measured biliary acylcarnitine levels in pediatric patients aged 6 months to 1 year undergoing open liver biopsy with prospectively determined non-metabolic liver disease in order to determine the effect of the liver disease on acylcarnitine excretion. Bile was collected in syringes and was transported immediately and stored at − 70 °C until the time of testing. The disease patient population consisted of 2 patients with known defects in long- and short-chain fatty acid oxidation (long-chain L-3-hydroxy acyl-CoA dehydrogenase: LCHAD and short-chain L-3-hydroxy acyl-CoA dehydrogenase: SCHAD). The sample from the LCHAD patient was collected at autopsy and the patient with SCHAD deficiency was subsequently diagnosed as part of the prospective study and removed from the unknown etiology group. Acylcarnitine profiles were obtained for each specimen as butylated derivatives using tandem mass spectrometry.
*Results:* The non-metabolic liver disease had no effect on the diagnostic value of bile acylcarnitine levels for detecting LCHAD deficiency. The concentrations of bile long-chain acylcarnitine species analyzed from patients with non-metabolic liver disease were far lower than the levels seen in LCHAD deficiency which also demonstrated a characteristic pattern of 3-hydroxyacylcarnitine excretion. In SCHAD deficiency, for which pathognomonic markers have not yet been established, bile analysis did not improve the diagnostic ability.
*Conclusion:* The analysis of bile acylcarnitines for the diagnosis of long-chain fatty acid oxidation defects will provide unbiased information even in the presence of severe non-metabolic liver disease.


*Keywords:* Acylcarnitines; Bile; Fatty acid oxidation defects; Pediatric liver disease; Tandem mass spectrometry

## 1. Introduction

Plasma and dried blood spot acylcarnitine measurement is an important diagnostic tool for inherited disorders of fatty acid and organic acid metabolism. However, biliary excretion has been shown to be the most likely primary route of excretion for acylcarnitines and analysis of bile acylcarnitine profiles has been shown to provide greater sensitivity for detecting abnormal acylcarnitine profiles,

*Abbreviations:* LCHAD, long-chain L-3-hydroxyacylcoenzyme A dehydrogenase; SCHAD, short-chain L-3-hydroxyacylcoenzyme A dehydrogenase; FAO, fatty acid oxidation.

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particularly in fatty acid oxidation (FAO) defects when plasma acylcarnitine analysis may lack sensitivity and urine organic acid analysis may lack specificity [1].

There is an increased risk of sudden and unexpected infant death in FAO disorders and the diagnosis may require analysis of samples collected post-mortem. Blood spot acylcarnitine analysis has been successfully utilized to identify some cases of FAO defects in post-mortem samples [2]. It has been demonstrated by retrospective analysis that bile analysis is more sensitive in the scenario of sudden unexpected death [1]. At autopsy or upon biopsy in living patients, suspicion of a possible FAO disorder may be raised by the finding of diffuse macro- and microvesicular hepatic steatosis or in the case of LCHAD deficiency, cirrhotic hepatocyte changes that are not distinguishable histologically from other causes of cirrhosis [3]. A final diagnosis can be reached through various biochemical, enzymatic, and molecular methods [3–7], but only if fluid and tissue specimens are collected during autopsy for this purpose. In our experience, this is seldom done, which possibly contributes to an underestimation of metabolic disorders among sudden death cases. To increase the general awareness of these disorders, alternative biochemical methods that are based on the constant availability of a suitable sample other than blood or urine, require simple preparation procedures, and allow the diagnosis of as many disorders as possible on the basis of multiple independent analytical criteria [8]. Rashed et al. [1] postulated that a definitive diagnosis could be obtained by the analysis of acylcarnitines in bile, a specimen easily collected at autopsy, for the post-mortem diagnosis of FAO and other metabolic disorders associated with the accumulation of characteristic acylcarnitines in blood and urine. Shneider et al. recently indicated that increased concentrations of bile esterified carnitine predisposed to a worse outcome in acute pediatric liver failure thus increasing the potential utility of this measurement [9].

However, to date there have been few published studies of acylcarnitine measurement in bile samples from patients suspected of having metabolic disorders. The effects of non-metabolic liver disease on acylcarnitine excretion into bile have also not been evaluated. In this study, we report the biliary acylcarnitine profiles in a prospective series of pediatric patients with non-metabolic liver disease. We compare the data to that of the bile in 2 patients with genetically confirmed disorders of fatty acid oxidation.

## 2. Materials and methods

### 2.1. Materials

We used ethyl alcohol USP, absolute, 200 proof (Aaper Alcohol and Chemical Co, #071603), butanolic hydrochloric acid (Regis Technologies Inc, #201007), acetonitrile, optima grade (Fisher, # A996-1), methanol, omnisolv (EMD Chemicals Inc., #MX0482-6), and stable isotope-labeled acylcarnitine standards (Cambridge Isotope Laboratories, Inc. #NSK-B). Electrospray MS/MS was performed on an API2000 model tandem mass spectrometer (Applied Biosystems, Foster City, CA). Acylcarnitine internal standards included trideuterated ($d_3$) acetyl, propionyl, butyryl, hexanoyl, octanoyl, dodecanoyl, and palmitoyl carnitines which were purchased from Dr. HJ ten Brink (VU Medical Center, Amsterdam, The Netherlands).

### 2.2. Subjects

The bile used in this study was collected prospectively from patients undergoing open liver biopsy for hepatic disease of unknown etiology (the subjects were aged 6 months to 1 year). Bile samples from a total 28 patients were collected after informed consent was obtained following guidelines set by the Institutional Review Board at the University of Texas Southwestern Medical Center. Bile was collected in syringes and was transported immediately and stored at −70 °C until the time of testing. The disease patient population consisted of 2 patients with known defects in fatty acid oxidation (long-chain L-3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency and short-chain L-3-hydroxy acyl-CoA dehydrogenase (SCHAD) deficiency. The sample from the LCHAD patient was collected at autopsy from a previously diagnosed patient [10] and the patient with SCHAD deficiency was subsequently diagnosed as part of the prospective study and removed from the unknown etiology group [11]. The comparison group (non-fatty acid oxidation defects) had significant liver pathology, including cholestasis, steatosis and fibrosis, where liver failure was moderate to severe and which required liver transplantation in 4 patients. Routine metabolic investigations including urine organic acid, plasma amino acid, and plasma acylcarnitine analysis failed to identify likely metabolic cause. One patient had abnormal tyrosine levels but no evidence of tyrosinemia, and 3 patients had abnormal electron transport enzyme activities when measured in liver, which was deemed to be secondary to the liver damage as control enzymes were also diminished in activity in these subjects. The final diagnosis in all cases was idiopathic hepatitis.

### 2.3. Acylcarnitine measurement

Acylcarnitines were extracted from plasma and analyzed quantitatively as their butyl esters using the stable isotope-labeled internal standards described under materials. They are identified as the parent compounds containing the fragment of *m/z* 85, which is unique to the carnitine molecule.

Bile was thawed at room temperature and a 50 μl aliquot was mixed with 300 μl of internal standard solution consisting of 100 μg/ml each of the listed acylcarnitine internal standard species. Fifty microliters of water was added to each sample and vortexed for 10 s to reduce the