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Differences between acylcarnitine profiles in plasma and bloodspots

Monique G.M. de Sain-van der Velden [a,*], Eugene F. Diekman [b], Judith J. Jans [a], Maria van der Ham [a], Berthil H.C.M.T. Prinsen [a], Gepke Visser [b], Nanda M. Verhoeven-Duif [a]

[a] Department of Medical Genetics, UMC Utrecht, The Netherlands Wilhelmina Children's Hospital, University Medical Centre (UMC) Utrecht, Utrecht, The Netherlands
[b] Department of Metabolic Diseases, UMC Utrecht, The Netherlands Wilhelmina Children's Hospital, University Medical Centre (UMC) Utrecht, Utrecht, The Netherlands

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ABSTRACT

Quantification of acylcarnitines is used for screening and diagnosis of inborn error of metabolism (IEM). While newborn screening is performed in dried blood spots (DBSs), general metabolic investigation is often performed in plasma. Information on the correlation between plasma and DBS acylcarnitine profiles is scarce. In this study, we directly compared acylcarnitine concentrations measured in DBS with those in the corresponding plasma sample. Additionally, we tested whether ratios of acylcarnitines in both matrices are helpful for diagnostic purpose when primary markers fail.

Study design: DBS and plasma were obtained from controls and patients with a known IEM. (Acyl)carnitines were converted to their corresponding butyl esters and analyzed using HPLC/MS/MS.

Results: Free carnitine concentrations were 36% higher in plasma compared to DBS. In contrast, in patients with carnitine palmitoyltransferase 1 (CPT-1) deficiency free carnitine concentration in DBS was 4 times the concentration measured in plasma. In carnitine palmitoyltransferase 2 (CPT-2) deficiency, primary diagnostic markers were abnormal in plasma but could also be normal in DBS. The calculated ratios for CPT-1 (C0/(C16 + C18)) and CPT-2 ((C16 + C18:1)/C2) revealed abnormal values in plasma. However, normal ratios were found in DBS of two (out of five) samples obtained from patients diagnosed with CPT-2.

Conclusions: Relying on primary acylcarnitine markers, CPT-1 deficiency can be missed when analysis is performed in plasma, whereas CPT-2 deficiency can be missed when analysis is performed in DBS. Ratios of the primary markers to other acylcarnitines restore diagnostic recognition completely for CPT-1 and CPT-2 in plasma, while CPT-2 can still be missed in DBS.

1. Introduction

As the mitochondrial membrane is impermeable to long chain fatty acids, the carnitine shuttle is used to import acyl-CoA's. Acyl-CoA's can cross the mitochondrial membrane via carnitine acylcarnitine translocase (CACT) after conversion to acylcarnitines by carnitine-palmitoyl CoA transferase 1 (CPT-1). Reconversion of acylcarnitines to acyl-CoA's by carnitine-palmitoyl CoA transferase 2 (CPT-2) provides very-long chain acyl-CoA dehydrogenase (VLCAD) with the degradable acyl-CoA's to ensure energy supply. In addition, potentially toxic acyl-CoA's can be removed via the same route. Accumulation of specific acyl-CoA's due to a metabolic block leaves the cell as acylcarnitines [1,2].

In body fluids, the acylcarnitine profile is not only a diagnostic test for inherited disorders of fatty acid metabolism, but also for defects in branched-chain amino acid catabolism [2]. Patients with these types of metabolic disorders accumulate disease-specific acylcarnitines, since degradation of amino acids produces, in many cases, odd-chain acyl-compounds that are esterified with carnitine.

While in biochemical genetic laboratories plasma is routinely used for acylcarnitine analysis, newborn screening programs use whole blood dried on filter paper (DBS) as the standard specimen. Newborns who show abnormal screening results are referred to the clinical unit for diagnosis and treatment. The workflow in our department primarily involves confirmation by biochemical testing (by measurement of plasma acylcarnitine profile) followed by additional tests (e.g. enzymatic assays or DNA mutation analysis). While cut-off points for free carnitine and acylcarnitine esters have been published for both DBS [3,4] and plasma [5,6] only limited information is available on the correlation between plasma and DBS.

Comparison between free carnitine in plasma and DBS from patients with organic acidurias and fatty acid oxidation disorders were reported by Primassin and Spiekerkoetter [7]. Data on comparison between acylcarnitine concentrations in the different matrices is scarce.

Abbreviations: DBSs, dried blood spots; CPT-1, carnitine palmitoyltransferase 1; VLCAD, very-long-chain acyl-CoA dehydrogenase; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; PA, propionic acidemia; MMA, methylmalonic acidemia; GA-I, glutaric acidemia I; βKT, β-ketothiolase; CPT-2, carnitine palmitoyltransferase 2; IEM, inborn error of metabolism.

* Corresponding author at: Wilhelmina Children's Hospital, University Medical Centre (UMC) Utrecht, PO Box 85090, 3508AB Utrecht, The Netherlands. Fax: +31 887554295.
E-mail address: m.g.desain@umcutrecht.nl (M.G.M. de Sain-van der Velden).

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The use of absolute concentrations may lead to be potential interpretative problems. In newborn screening programs several ratios between different acylcarnitines have been reported that could help as a discriminate factor [4]. Such information is widely available for DBS but only limited for plasma.

This study examines acylcarnitines profiles in plasma and DBS simultaneously in samples from patients with well-defined inborn errors of metabolism (IEM). Subsequently, we evaluated whether ratios of acylcarnitines in plasma are just as helpful as these ratios are in DBS when primary markers fail to be conclusive.

2. Materials and methods

2.1. Study population

Blood was collected (for therapeutic control) from patients with confirmed (enzymatic or molecular) inherited metabolic diseases. These included plasma and DBS from patients diagnosed with different enzyme or transporter deficiencies: CPT-1 deficiency (n = 6 samples, 2 patients), CPT-2 deficiency (n = 5 samples, 4 patients), VLCAD deficiency (n = 12 samples, 11 patients), long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency (n = 2), and medium-chain acyl-CoA dehydrogenase (MCAD) deficiency (n = 27 samples, 21 patients).

Patients with organic acidemias such as propionic acidemia (PA) (n = 12 samples, 5 patients), methylmalonic acidemia (MMA) (n = 9 samples, 6 patients), glutaric acidemia I (GA-I) (n = 4 samples, 2 patients) and β-ketothiolase (βKT) (n = 3 samples, 2 patients) deficiency were also included. All values were compared with age related (<1 month, >1 month and <18 years and >18 years) reference values. Reference values for plasma were determined using 281, 2835 and 393 samples respectively. Reference values for DBS were determined using 39, 24 and 63 samples respectively.

Additionally, 54 patients without metabolic defect were investigated for acylcarnitine profiles in plasma and DBS simultaneously.

2.2. Sampling

Blood samples were collected by venous puncture in heparin containing tubes. Aliquots of whole heparinized blood were aspirated and spotted onto Guthrie card filter papers (Whatman no. 903 Protein Saver TM cards, formerly Schleicher & Schuell, Keene, USA). The blood tubes were then centrifuged and the resulting plasma was stored at −20 °C until further analysis. The Guthrie card filter papers

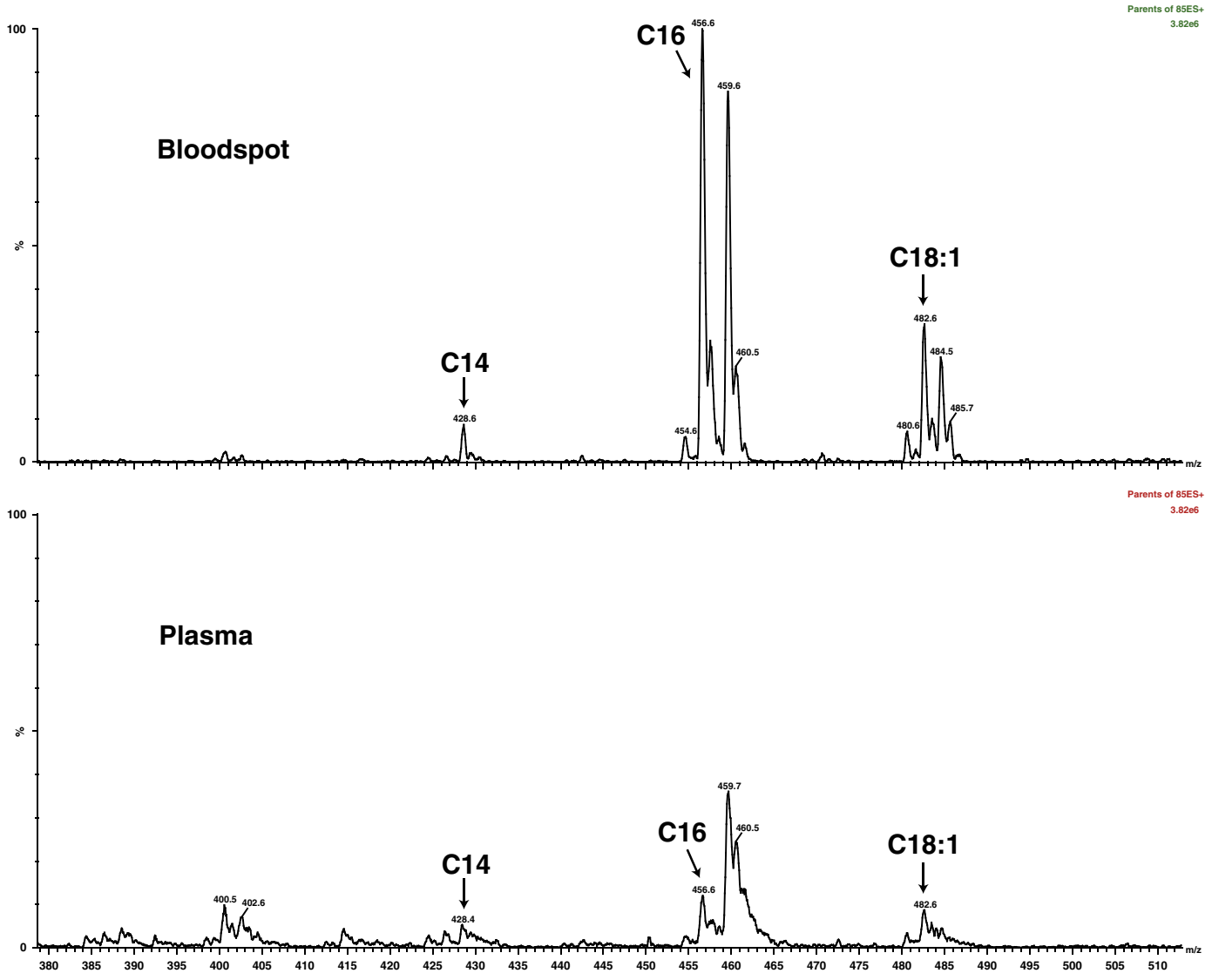

Fig. 1. Acylcarnitine profile in plasma and its corresponding DBS in a neonatal control subject.

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