JOANNEUM RESEARCH

ISO 9001:2000 certified

A Sensitive HPLC/MS/MS Method for the Determination of Short- and Long Chain Fatty Acyl-CoAs in Biological Tissues

Christoph Magnes[1]
Werner Regittnig[2]
Thomas R. Pieber[1,2]
Oksana Tehlivets[3]
Sepp D. Kohlwein[3]
Frank M. Sinner[1,*]

[1)]JOANNEUM RESEARCH
Forschungsgesellschaft mbH
Institute of
Medical Technologies and Health
Management
Auenbruggerplatz 20
A-8036 Graz, Austria
Phone: +43 316 876-21 03
*corresponding author:
frank.sinner@joanneum.at
http://www.joanneum.at/msg

[2)]Medical University of Graz
Department of Internal Medicine
AUenbruggerplatz 15
A-8036 Graz, Austria

[3)]University of Graz
Institute of Molecular Bioscience
Schubertstrasse 1
A-8010 Graz, Austria

www.joanneum.at/msg

Introduction

Fatty acids and intracellular fatty acid metabolites like coenzyme A activatied long chain fatty acids (LCACoAs), diacylglycerol and ceramide seem to play a critical role in the development of insulin resistance. One hypothesis assumes that increased FFA and LCACoA-levels directly affect insulin stimulated glucose transport. Hereby, the inhibition of glucose transport is caused by intracellular accumulation of LCACoAs and diacylglycerol[1]. One important factor controling LCACoA-levels in cells is the regulative effect of the short chain acyl CoA (SCACoA) malonylCoA on carnitin-palmitoyl-transferase-1, the responsible enzyme for transport of LCACoAs into mitochondrias for β-oxidation. Particullary in muscle cells such a disordered closed loop may lead to insulin resistance, one of the main characteristics of type 2 diabetes[1]. A simple, efficient, and sensitive method for exact LCACoA and SCACoA quantification from biological tissues is crucial to investigate the role of those metabolites. We herein report the expansion of the published LC/MS^2 method[2] for LCACoAs to additionally determine SCACoAs in different biological tissues, like rat liver, mice liver, mice muscle and yeast cells.

Methods

All experiments were carried out on an Ultimate-System (Dionex, LCPackings) coupled to a Quantum TSQ Ultra AM (ThermoFinnigan). Separation was performed on a pH stable column (Zorbax 300 Extend-C18). Elution solvents consisted of acetonitrile, water and 15mM ammonium hydroxide (pH 10.5). Loading and cleaning step solvents contained 0.1% of formic acid (exact compositions of eluents and gradient profile see figures). Positive ESI-MS/MS was performed using a spray voltage of 5.5 kV, and a optimized collision energy for neutral loss of 507 dalton of 40eV. Sample preparation for LCACoAs was performed like described previously[2]. Due to the different chemical nature of these molecular species, a different sample pre-treatment using an adjusted solid phase extraction (SPE) procedure was applied in order to isolate

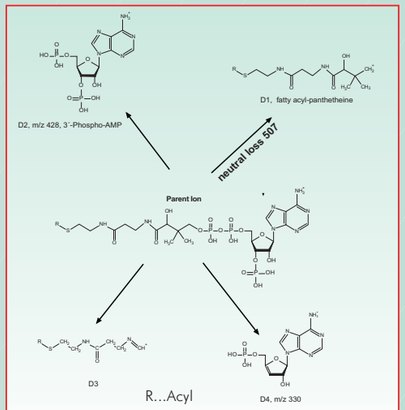

Figure 1. Most abundand ions obtained from parent ion [M-H]$^+$. Ion D1 was chosen for quantitative determinations.

SCACoAs[3].

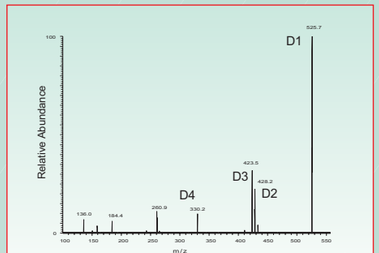

Figure 2. MS/MS of Oleoyl-Coenzyme A. Parent ion [M+H]$^+$ 1032.4 m/z, collision energy 40eV.

Results, Discussion

In the positive mode for both, LCACoAs and SCACoAs, the predominant ion in MS^2-spectra is the fatty acyl-pantetheine fragment D1 (m/z 525.7), which derives from cleavage between the ADP and pantetheine residues with charge retention on the fatty acyl portion[4] (see Figure 1 and Figure 2). The abundant Ion D2 corresponds to the protonated 3´-phospho-AMP (m/z 428). The lesser abundant Ion D4 (m/z 330) corresponds with the loss of the 3´-phosphate group from m/z 428. Ion D3 (m/z 423.5) contains the fatty acyl portion and results from a neutral loss of 609. We chose the fatty acyl-pantetheine for quantification of both LCACoAs and SCACoAs due to its specifity to CoA activated substances and the substance itself and for its high abundance. Chromatographic conditions are different for SCACoA and LCACoA determinations. For SCACoAs it is necessary to perform an acidic loading step to get sharp peaks for polar SCACoAs like SuccinylCoA or MalonylCoA (Figure 4). For LCACoAs it necessary to perform an additional cleaning step with acidic eluent to get long time stability for separation performance[2]. Sensitivity in "wrong way round" positive electrospray for SCACoAs is excellent and comparable to the sensitivity for LCACoAs. The method was successfully applied to different biological tissues like rat liver [2], mice muscle, mice liver(figure 3) and yeast extracts (figure 5) for determinations of LCACoAs and SCACoAs.

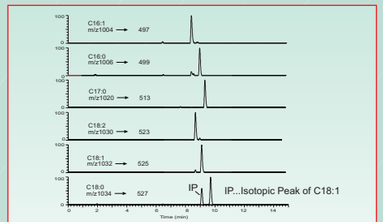

Figure 3. MRM scans of five LCACoAs and internal standard (C17:0 Acyl CoA) in 50mg mice liver. Column: Zorbax 300 extend-C18 150x2.1mm: flow rate 200µl/min, room temperature. Eluent A: 15mM NH_4OH in 10% aqueous actonitrile; eluent B: 15mM NH_4OH in acetonitrile; eluent C: 0.1% formic acid in 70% aqueous acetonitrile. Gradient: 0%B to 45%B/55%A in 8 min; 8-10min, isocratic 45%B/55%A; 45%B/55%A to 100%C in 2min; 100%C for 5min, 100%C to 100%A in 2min. Equilibration time with A was set to 8 minutes. 100µl of 2ml extract was injected.

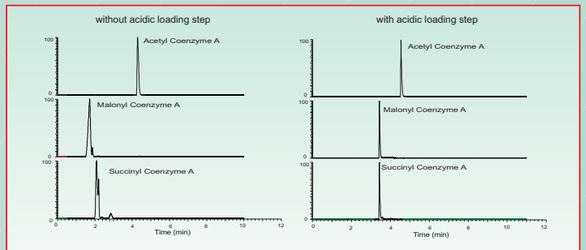

Figure 4. Acidic loading step (right panel) leads to sharp peaks for polar SCACoAs. HPLC conditions: column: Zorbax C18 extend 150x2.1mm; flow rate 200µl/min, room temperature; eluent A: 0.1% formic acid in water; eluent B: 15mM NH_4OH in 1% aqueous acetonitrile; eluent C: 15mM NH_4OH in 5% aqueous acetonitrile. Left panel: gradient from 100% B to 100% C in 1 min, 100%C for 6 min. Right panel: gradient from 100%B to 100% C in 1min, 100%C for 6 min, 5 min conditioning and loading with eluent A.

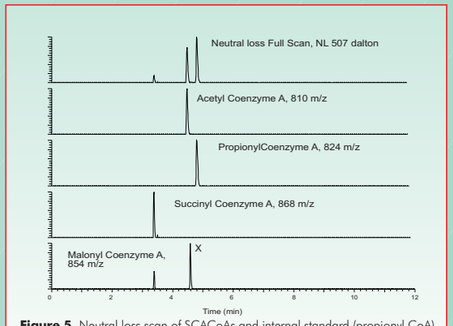

Figure 5. Neutral loss scan of SCACoAs and internal standard (propionyl CoA) in yeast extract. Column: Zorbax C18 extend 150x2.1mm; flow rate 200µl/min, room temperature. Eluent A: 0.1% formic acid in water; eluent B: 15mM NH_4OH in 1% aqueous acetonitrile; eluent C: 15mM NH_4OH in 5% aqueous acetonitrile. Gradient from 100%B to 100% C in 1min, 100% C for 6 min, conditioning and loading with eluent A. X: not identified CoA with same mass as malonyl CoA.

[1]McGarry JD; *Diabetes* **51** (2002) 7 - 18
[2]Magnes C; Sinner FM, Regittnig W, Pieber RT; *Anal. Chem.* **77** (2005) 2889-2894
[3]Bian F, Kasumov T, Thomas KR, Jobbins A, David F, Minklers PE, Hoppel CL, Brunengraber H; *J. Biol. Chem.* **280** (2005) 9265 - 9271
[4]Hankin JA, Murphy RC; *Int. J. Mass Spec. Ion Proc.***165/166** (1997) 467-474

a TRADITION of INNOVATION