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Quantification of the concentration and ^{13}C tracer enrichment of long-chain fatty acyl-coenzyme A in muscle by liquid chromatography/mass spectrometry

Dayong Sun, Melanie G. Cree, Robert R. Wolfe *

Metabolism Unit, Shriners Burn Hospital, University of Texas Medical Branch, 815 Market Street, Galveston, TX 77550, USA

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Abstract

Recent diabetes and obesity research has been focused on the role of intracellular lipids in insulin resistance. Fatty acyl-coenzyme A (CoA) esters play a central role in the trafficking of intracellular lipids, but there has not previously been a method with which to quantify their kinetics using tracer methodology. We have therefore developed a high-performance liquid chromatography (HPLC)–mass spectrometry method to simultaneously measure the ^{13}C stable isotopic enrichment of palmitoyl-acyl-CoA ester and the concentrations of five individual long-chain fatty acyl-CoA esters extracted from muscle tissue samples. The long-chain fatty acyl-CoA can be effectively extracted from frozen muscle tissue samples and baseline separated by a reverse-phase HPLC with the presence of a volatile reagent—triethylamine. Negative ion electrospray mass spectrometry with selected ion monitoring was used to analyze the fatty acyl-CoAs to achieve reliable quantification of their concentrations and ^{13}C isotopic enrichment. Applying this protocol to rabbit muscle samples demonstrates that it is a sensitive, accurate, and precise method for the quantification of long-chain fatty acyl-CoA concentrations and enrichment.

Keywords: Stable isotope enrichment; Fatty acyl-coenzyme A; Muscle tissue; HPLC; Mass spectrometry

Fatty acids must be activated by conversion to coenzyme A (CoA)[1] esters or acyl-CoAs, with the fatty acid group linked to the terminal thiol moiety before they can be metabolized. The thiol ester is a highly energetic bond that permits a facile transfer of the acyl group to receptor molecules. This is true for the simplest fatty acid of all, acetic acid (i.e., as acetyl-CoA), and for long-chain fatty acids. Fatty acyl-CoA is therefore the breakdown product of fatty acids and is the direct precursor for β-oxidation of fat by mitochondria. Fatty acyl-CoA is also a signaling molecule with metabolic effects in muscle tissue and in pancreatic islet cells [1,2]. Recent research has focused on the role of fatty acyl-CoA in insulin resistance, both as an active metabolite and as a marker for fat oxidation [3]. Several studies have measured the total concentration of fatty acyl-CoA at rest and following exercise or hyperinsulinemia [4–6]. However, results based on total tissue concentrations of fatty acyl-CoA have been conflicting and inconclusive. Some have suggested that more conclusive information on the role of fatty acyl-CoA could be gained if the profile of the concentrations of individual fatty acids in the CoA could be quantified [5].

Tracer studies with stable and radioactive isotopes to elucidate the fates of glucose, fat, and protein have been conducted for over 50 years [7]. Use of tracer methodology to quantify the kinetics of fatty acyl-CoA esters could help to clarify the fate of plasma free fatty acid (FFA) within tissues that can be sampled in vivo, including muscle. For example, measurement of the stable isotopic tracer

* Corresponding author. Fax: +1 409 770 6825.
E-mail address: rwolfe@utmb.edu (R.R. Wolfe).

[1] *Abbreviations used:* CoA, coenzyme A; FFA, free fatty acids; SPE, solid-phase extraction; SIM, selected ion monitoring; TEA, triethylamine; TIC, total ion chromatograms; SIC, selected ion chromatograms; TTR, tracer/tracee peak area ratio.

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enrichments of fatty acyl-CoA would be useful to calculate the rate of fatty acid oxidation more accurately than current methods. Use of the acyl-CoA as a precursor enrichment, rather than plasma FFA would control for any dilution of the plasma enrichment by intracellular unlabeled products [7]. Further, by comparing the measured enrichment of fatty acyl-CoA and the corresponding plasma FFA, it is possible to distinguish other intracellular sources of fatty acids from plasma FFA as precursors for fatty acid oxidation. Thus, a method to measure both the individual fatty acyl-CoA concentration and the tracer enrichment would be useful for a variety of reasons.

Previous fatty acyl-CoA analysis has utilized high-performance liquid chromatography (HPLC) coupled with UV [8–14], photodiode array detection [15,16], fluorescence [17], on-line immobilized enzyme reactor detection [18], and mass spectrometry techniques, such as negative chemical ionization mass spectrometry [19], fast atom bombardment mass spectrometry [20], and recently electrospray tandem mass spectrometry [21–25]. These methods usually involve complicated sample purification by solid-phase extraction (SPE) or liquid–liquid extraction, and chemical transformation and derivatizations, which usually cause the loss of extraction recoveries and analytical sensitivity. Relatively larger amounts of sample are commonly required, making analysis of small amounts of tissue available by needle biopsy impractical. Another major drawback of previous methods is the difficulty in identifying impurities and measuring isotopic enrichment in specific molecules.

A few tracer studies with short-chain fatty acyl-CoAs have been reported with the CoA alkaline hydrolyzed and derivatized prior to GC/MS analysis [26–28]. However, this method cannot be applied to long-chain fatty acyl-CoA because the intact molecules of long-chain fatty acyl-CoA esters are too large and thermally unstable and lack the necessary volatility to be directly analyzed by GC/MS. Additionally, the alkaline hydrolysis and derivatization procedure may introduce contaminates and thus the measured isotopic enrichments and concentrations are not accurate. In this paper, we report a new and effective method to readily extract long-chain fatty acyl-CoA from muscle tissue samples using a simplified extraction procedure and the use of reverse-phase HPLC/MS with SIM analysis to provide sensitive, accurate, and precise simultaneous quantification of the stable isotopic tracer enrichments and the concentration of individual long-chain fatty acyl-CoA esters.

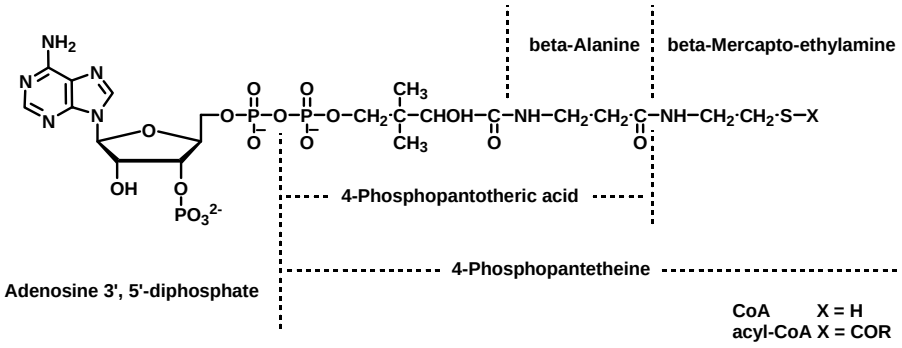
Materials and methods

The chemical structures of the long-chain fatty acyl-coenzyme A esters analyzed and the ions used for SIM monitoring are shown in Table 1.

Reagents

Multiple long-chain fatty acyl-CoA standards were used: myristoyl-coenzyme A, palmitoyl-coenzyme A, stearoyl-coenzyme A, oleoyl-conyzeme A, linoleoyl-conyzeme A, and internal standard heptadecanoyl-conyzeme A were purchased from Sigma Chemical (St. Louis, MO, USA) as lithium salts and stored at −20 °C as received. ^{13}C uniformly labeled palmitoyl-coenzyme A was purchased from Isotec (St. Louis, MO, USA). Internal standard heptadecanoyl-conyzeme A was prepared to the stock solution by dissolving in 1:1 100 mM KH_2PO_4 buffer (pH 4.9)/2-propanol to obtain a concentration of 1 ng/μl and stored at −80 °C until use. Triethylamine (TEA; purity 99.5%) was purchased from Aldrich (Milwaukee, WI, USA). All solvents used were of HPLC grade from Fisher (Fair Lawn, NJ, USA).

Table 1
Chemical structure of long-chain fatty acyl-coenzyme A esters analyzed, and their SIM ions used for quantification



X	Name	Abbreviations	SIM ions (*m/z*)
$OC–(CH_2)_{12}CH_3$	Myristoyl-coenzyme A	C14:0-CoA	976.4
$OC–(CH_2)_{14}CH_3$	Palmitoyl-coenzyme A	C16:0-CoA	1004.4
$O^{13}C–(^{13}CH_2)_{14}{}^{13}CH_3$	U-^{13}C-Palmitoyl-coenzyme A	U-^{13}C-C16:0-CoA	1020.4
$OC–(CH_2)_{15}CH_3$	Heptadecanoyl-coenzyme A	C17:0-CoA	1018.4
$OC–(CH_2)_{16}CH_3$	Stearoyl-coenzyme A	C18:0-CoA	1032.4
$OC–(CH_2)_{14}(CH)_2CH_3$	Oleoyl-coenzyme A	C18:1-CoA	1030.4
$OC–(CH_2)_{12}(CH)_4CH_3$	Linoleoyl-coenzyme A	C18:2-CoA	1028.4