An improved method for tissue long-chain acyl-CoA extraction and analysis

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Abstract **We report an extensively modified method for the extraction, solid-phase purification, and HPLC analysis of long-chain acyl-CoAs from tissues. Tissue samples were homogenized in a glass homogenizer in KH_2PO_4 buffer (100 mM, pH 4.9) and again after the addition of 2-propanol. Acyl-CoAs were then extracted from the homogenate with acetonitrile (ACN). The acyl-CoAs in the extract were bound to an oligonucleotide purification column and eluted using 2-propanol. This eluent was concentrated and then loaded onto a C-18 column and eluted using a binary gradient system in which solvent A was KH_2PO_4 (75 mM, pH 4.9) and solvent B was ACN containing 600 mM glacial acetic acid. Initial flow rate was 0.5 or 0.25 ml/min depending upon the tissue used. The HPLC eluent was monitoring at 260 nm. Our modifications increased the recovery of the extraction procedure to 70–80%, depending upon tissue, with high reproducibility and significantly improved separation of the most common unsaturated and saturated acyl-CoAs. We also report, for the first time, the mass (nanomoles per gram wet weight) of the most common polyunsaturated acyl-CoAs in rat heart, kidney, and muscle tissues. The modifications and high recovery permit the use of tissue samples of less than 100 mg, making this method useful for the analysis of small tissue amounts associated with mice.**—Golovko, M. Y., and E. J. Murphy. **An improved method for tissue long-chain acyl-CoA extraction and analysis.** ***J. Lipid Res.*** **2004.** 45: **1777–1782.**

Supplementary key words rat • lipids • brain • heart • kidney • muscle • liver • coenzyme A

Acyl-CoAs have a central role in many basic pathways as allosteric regulators of several enzymes and as key metabolites of biochemical reactions (1–6). With the increased use of mouse models and tissue- and cell culture-based model systems, there is the need for a method that can extract, separate, and quantify small amounts acyl-CoAs using limited amounts of biological material.

Numerous methods have been developed to measure individual long-chain acyl-CoA mass in mammalian tissues (7–20). However, most of the reported methods demonstrate low recovery of the acyl-CoA extraction, with recovery ranging from 10% to 45% (7, 12, 16, 21). Furthermore, these methods demonstrate poor resolution of docosahexaenoyl-CoA (22:6-CoA), arachidonoyl-CoA (20:4-CoA), and linoleoyl-CoA (18:2-CoA) (12, 17, 22). Some authors have quantified acyl-CoAs by gas chromatography after alkaline hydrolysis (7, 21); however, these methods are extremely dependent upon the purity of the long-chain acyl-CoA extracts and are very laborious. The most rapid method is the solid-phase extraction method, but the recovery of this method ranges from 12% to 40%, depending upon the tissue, making this method difficult for acyl-CoA analysis in small amounts of tissue.

In this paper, we describe modifications of an existing method (17, 19) using solid-phase extraction and subsequent HPLC separation of long-chain acyl-CoAs for use with small amounts of tissue (>25 mg). Our modifications increase the recovery of the extraction procedure in excess of 80%, with high reproducibility and significantly improved separation of the most common polyunsaturated and saturated acyl-CoAs. We also report, for the first time, the mass of the most common polyunsaturated acyl-CoAs in rat heart, kidney, muscle, and liver tissues.

MATERIALS AND METHODS

Materials

Long-chain acyl-CoAs were purchased from Sigma Chemical Co. (St. Louis, MO), except 22:6-CoA, which was obtained from Moravek Biochemical (Brea, CA). Other chemicals and solvents of analytical or higher quality were from Merck KGaA (Darmstadt, Germany).

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Abbreviations: ACN, acetonitrile; GAC, glacial acetic acid; 16:0-CoA, palmitoyl-CoA; 17:0-CoA, heptadecanoyl-CoA; 18:0-CoA, stearoyl-CoA; 18:1-CoA, oleoyl-CoA; 18:2-CoA, linoleoyl-CoA; 20:4-CoA, arachidonoyl-CoA; 22:6-CoA, docosahexaenoyl-CoA.

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Several alternative procedures and conditions were tested for the acyl-CoA analyses and are presented in Results and Discussion. The final recommended methods are described below.

Tissue extraction

In these experiments, tissue was prepared from Sprague-Dawley male rats (~350 g; Charles River, St. Louis, MO) that were maintained on Purina laboratory rat chow with free access to food and water. Rats were anesthetized with 4% halothane and killed by decapitation. Their tissues were rapidly removed (<1 min) and immediately frozen in liquid nitrogen. Tissues were then pulverized to a powder under liquid nitrogen conditions and stored at −80°C before extraction. Tissue powders were prepared from whole brain, heart, kidney, skeletal muscles of hind leg, and posterior segment of liver.

The tissue extraction procedure was modified from a previously described method (17). Pulverized tissue was homogenized using a Tenbroeck tissue grinder (Kontes Glass Co., Vineland, NJ) in 2 ml of 100 mM KH_2PO_4 [pH 4.9 to decrease the activity of acyl-CoA hydrolase (23)] containing 16 nmol of heptadecanoyl-CoA (17:0-CoA) as an internal standard. Then, 2.0 ml of 2-propanol was added, the sample was homogenized again in a glass homogenizer, and 0.25 ml of saturated NH_4SO_4 and 4.0 ml of acetonitrile (ACN) were added. This mixture was vortexed for 5 min. This solution was subjected to 5 min of centrifugation (1,900 *g*), and the upper phase containing the acyl-CoA was removed and diluted with 10 ml of 100 mM KH_2PO_4 (pH 4.9). All operations were done quickly over a 10-12 min period before centrifugation in ice-cold conditions. Acyl-CoA were reextracted from the tissue residue according to the described procedure.

Solid-phase extraction

To concentrate and partially purify the acyl-CoA fraction, solid-phase extraction was used (17). The oligonucleotide purification cartridge (Applied Biosystems, Foster City, CA) was activated by prewashing with 5 ml of water, then by 5 ml of ACN, and finally by 25 mM KH_2PO_4 (pH 4.9). Between washings, the cartridge was gently flushed with air. The tissue extracts (after extraction and reextraction; 18 ml each) were loaded separately with a propylene syringe onto activated cartridges using a syringe pump (A-99-E; Razel Scientific Instruments, Stamford, CT). The eluent was then reloaded through the cartridge a second time to bind any residual, unbound acyl-CoA. All procedures were performed at 26°C, and the flow rate of solutions through the cartridge was 0.2 ml/min. The bound acyl-CoAs in the cartridges were washed with 5.0 ml of 25 mM KH_2PO_4, dried with syringe air, and eluted with 0.4 ml of 75% 2-propanol containing 1 mM glacial acetic acid (GAC). The total time used for the solid-phase extraction was 3.5 h.

Sample preparation for HPLC

The two eluents were combined into one vial and stored for 1–5 days at 4°C with no observable breakdown of the acyl-CoAs. Before HPLC analysis, samples were concentrated by the reduction of the volume to ~0.1 ml under a stream of nitrogen. During this procedure (15 min), the sample was vortexed every 5 min. Acyl-CoA molecular species in this sample were separated by HPLC. To determine the recovery of the concentrating procedure and to determine the amount ejected into the HPLC system, 9.5 nmol of myristoyl-CoA was added to the sample before concentrating as an external standard.

HPLC separation

High-performance liquid chromatography of acyl-CoA molecular species was carried out using a Luna C-18(2) 5 μm column (250 × 4.6 mm; Phenomenex, Torrance, CA) with a stainless-steel frit filter (2 μm) and a security guard cartridge system (C-18; Phenomenex). The system was controlled by a Beckman 127 solvent module (Fullerton, CA). The eluent was monitored at 260 nm using a Beckman 166 ultraviolet/visible light detector.

The solvent program for elution was modified from a previous method (19). This modification was made to resolve the most common polyunsaturated acyl-CoAs (22:6-CoA, 20:4-CoA, and 18:2-CoA), which elute in a single peak containing shoulders in systems described previously. The gradient system was composed of 75 mM KH_2PO_4 (buffer A) and ACN containing 600 mM GAC (buffer B). The starting conditions were as follows: column temperature, 35°C; flow rate, 0.5 ml/min; 44% buffer B. During the first 80 min, the gradient of buffer B was increased to 50%. At 91 min, the percentage of buffer B was increased to 70% over 15 min and the flow rate was increased to 1 ml/min over 1 min to elute monounsaturated and saturated long-chain acyl-CoAs. At 120 min, the percentage of buffer B was increased to 80% to elute very hydrophobic compounds. At 140 min, the percentage of buffer B was returned to 44% over 5 min and the flow rate was reduced to 0.5 ml/min.

In liver, heart, muscle, and kidney extracts, there were substantially more peaks detected compared with brain. Therefore, we used a lower flow rate (0.25 ml/min) for these tissues, which showed higher resolution. For this flow rate, the gradient system and starting conditions were as follows. Over the first 140 min, the gradient of buffer B was increased to 50%. At 173 min, the percentage of buffer B was increased to 70% over 15 min and the flow rate was increased up to 1 ml/min over 0.75 min. At 210 min, the percentage of buffer B was increased to 80%, and at 230 min, the percentage of buffer B was returned to 44% over 5 min and the flow rate was reduced to 0.25 ml/min.

The long-chain acyl-CoAs were identified by comparing their retention times relative to the retention time of 17:0-CoA. The peak identity was confirmed by mixing tissue extracts and acyl-CoA standards before HPLC separation. For each acyl-CoA, the peak area was calculated by integrating the area under the curve (PeakSimple Data System; SRI Instruments, Torrance, CA) and the mass was calculated using a standard curve derived from 17:0-CoA.

Statistic analysis

All statistical comparisons were calculated using a two-way, unpaired Student's *t*-test or a one-way ANOVA combined with a Tukey-Kramer post hoc test using Instat II (Graphpad, San Diego, CA). Statistical significance was defined as $P < 0.05$. All values are expressed as means ± SD.

RESULTS

Optimization of tissue extraction

Optimization of the tissue extraction procedure was focused on two parameters: *1*) method for tissue homogenization; and *2*) effectiveness of reextraction of the tissue residue. To this end, we tested two forms of tissue homogenization: sonication (22) and glass-on-glass homogenization using a Tenbroeck tissue grinder (Kontes Glass Co.). For brain tissue, total recovery of the internal standard, 17:0-CoA, after 20–60 s of sonication was 31.6 ± 3.1%, compared with 81.4 ± 2.4% for glass-on-glass homogenization. The recovery using glass-on-glass homogenization was stable over a wide range of tissue mass (~0.025–0.350 g). For heart tissue, glass-on-glass homogenization significantly

increased total recovery over sonication from 14.2 ± 5.2% to 68.8 ± 11.6%.

It is important to note that the second extraction increased the recovery of the internal standard by an additional 10.2 ± 2.9% after sonication and by 15.5 ± 3.2% after glass-on-glass homogenization for brain tissue. This effect was confirmed by extraction of other tissues. Furthermore, optimal recovery occurs when the tissue extraction procedure is done in 10–12 min (from tissue weighing until the start of centrifugation).

To compare endogenous long-chain acyl-CoA recovery after different methods of tissue homogenization, we calculated their concentration in brain tissue using the extraction recovery of the internal standard (17:0-CoA) to correct for the recovery of the endogenous acyl-CoAs. In this experiment, we used the previously described method for acyl-CoA separation (22). The concentration of the total long-chain acyl-CoA fraction [the sum of 22:6-CoA, 20:4-CoA, 18:2-CoA, palmitoyl-CoA (16:0-CoA), oleoyl-CoA (18:1-CoA), and stearoyl-CoA (18:0-CoA)] found after homogenization by sonication was 28.7 ± 1.2 nmol/g wet weight (n = 4) compared with 28.6 ± 0.5 nmol/g wet weight (n = 4) after glass-on-glass homogenization. These values are in the range of the reported levels for this method of separation (22). This indicates that the increase in the recovery of the internal standard found using glass-on-glass homogenization reflects a net increase in recovery of all of the endogenous acyl-CoAs as well. The intrasample variability determined by the comparison of the average relative standard deviation for individual acyl-CoA molecular species was also improved in our modification [21 ± 7% (n = 4) using sonication and 7 ± 5% (n = 4) using glass-on-glass homogenization].

Concentrating of acyl-CoA extracts

The combined volume of the eluent from the oligonucleotide purification cartridge was 0.8 ml and contained 75% 2-propanol. Because the 2-propanol in the sample decreased the resolution of the acyl-CoA (**Fig. 1**) and the large volume of the sample did not allow complete injection of the sample onto the HPLC column, we concentrated the eluent by the reduction of the volume to ~0.1 ml under a stream of nitrogen. The samples were vortexed every 5 min during volume reduction. The limited amount of 2-propanol left in the samples did not significantly influence acyl-CoA resolution, and the procedure allowed injection of 96.2 ± 3.0% (n = 12) of the sample onto the HPLC column.

Optimization of HPLC separation

The previously described method for the separation of long-chain acyl-CoAs (19) demonstrated poor resolution of 22:6-CoA, 20:4-CoA, and 18:2-CoA (Fig. 1). The mass of the standards used in this run was approximately the same as in 200 mg of brain tissue. Acyl-CoAs were dissolved in 80 μl of 75% 2-propanol containing 1 mM GAC. The two mobile phases for HPLC were 75 mM KH_2PO_4 (A) and 100% ACN (B). The starting conditions were flow rate of 1 ml/min and 44% solvent B. Solvent B was increased to

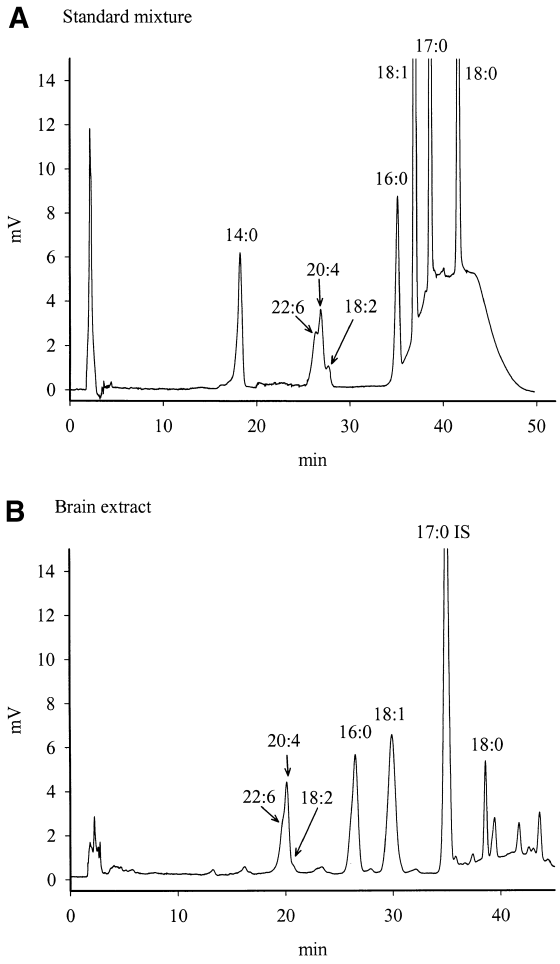

Fig. 1. The effect of 2-propanol on the HPLC separation of acyl-CoAs. Acyl-CoAs were dissolved in 80 μl of 75% 2-propanol containing 1 mM glacial acetic acid (GAC). The initial conditions were as follows: flow rate, 1 ml/min; 56% solvent A (75 mM KH_2PO_4, pH 4.9) and 44% solvent B [acetonitrile (ACN)]. The solvent program for elution was as described by Rabin et al. (19). IS, internal standard.

49% over 25 min and then to 70% over the next 5 min, held at this level for 9 min, and then decreased to 44% during 4 min (19). Other HPLC separations, based on different gradients of ACN/phosphate buffer or methanol/ACN/phosphate buffer, had similar or even lower resolution for long-chain acyl-CoAs (11, 17, 20), as was estimated after the comparison of the retention times of standard acyl-CoAs.

Several approaches were used in an attempt to increase the resolution of 22:6-CoA, 20:4-CoA, and 18:2-CoA. The ability of the column temperature to resolve these acyl-CoAs was tested using temperatures of 20, 35, 45, and 55°C. Temperature did not have an impact on the resolution of the acyl-CoA but did increase the retention time for standards (data are not shown). Therefore, we performed all HPLC separations at a stable column temperature of 35°C. However, removal of the 2-propanol from the sample significantly increased the resolution of these acyl-CoAs. Thus, we recommend the use of the procedure

described in Materials and Methods for concentrating acyl-CoA extracts by evaporating 2-propanol to minimal amounts to achieve good resolution of acyl-CoA.

Because a decrease in the flow rate can improve the separation of poorly resolved components (11), we examined the resolution of these acyl-CoAs at three different flow rates. The best resolution was found at a flow rate 0.25 ml/min, although the total time for the separation was 3.5 h. The key to the separation was to balance resolution with run time. To shorten the running time, we added GAC to solvent B (ACN). We tried different concentrations of GAC ranging from 10 to 600 mM and found the optimal concentration to be 600 mM.

Application of the method to tissue acyl-CoA analysis

Typical chromatograms of the acyl-CoA separation from rat brain, heart, and liver tissue are shown in **Fig. 2**. For brain tissue, the flow rate was 0.5 ml/min, and for liver and heart tissue, the flow rate was 0.25 ml/min. The concentrations of different acyl-CoAs in brain, liver, heart, kidney, and skeletal muscle are presented in **Table 1**. In summary, liver and brain tissues contained the highest concentration of acyl-CoAs. The highest content of the most common polyunsaturated acyl-CoAs was found in liver tissue. In contrast, the amounts of saturated and monounsaturated acyl-CoAs (16:0-CoA, 18:0-CoA, and 18:1-CoA) were higher in brain tissue. The concentration of 22:6-CoA was about the same in all tissues with the exception of liver, which had 4-fold higher mass of 22:6-CoA. The magnitudes for 18:2-CoA were significantly higher for liver and heart compared with brain, kidney, and skeletal muscle. Skeletal muscle contained the lowest amount of 20:4-CoA, whereas in liver and kidney the concentration of 20:4-CoA was the highest of the tissues analyzed.

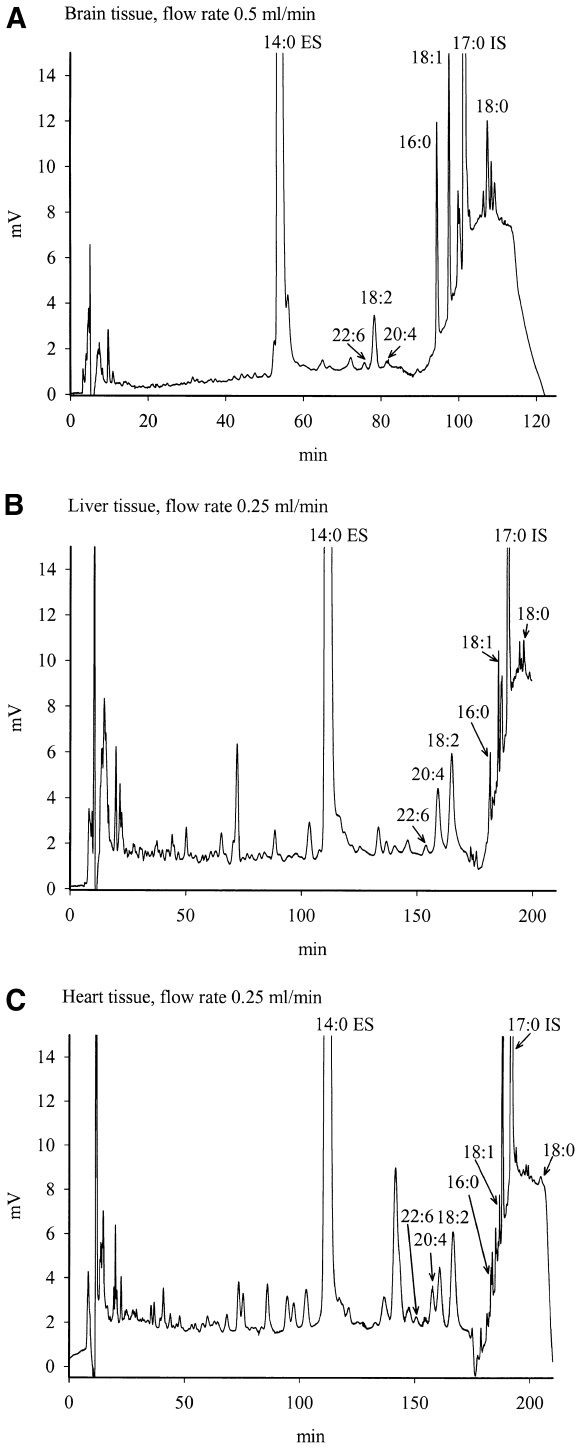

Fig. 2. Chromatograms of the acyl-CoAs extracted from tissues at initial conditions of 56% solvent A (75 mM KH_2PO_4) and 44% solvent B (ACN in 600 mM GAC). The acyl-CoAs were extracted, concentrated under the nitrogen stream, and separated as described in Materials and Methods. ES, external standard; IS, internal standard.

DISCUSSION

Acyl-CoA recovery from most reported extraction procedures ranges from 10% to 45% (7, 12, 16, 21). A recovery of 80% has been reported using a chloroform/methanol extraction procedure (8); however, when used by others, the recovery with this method was only 13% for heart and 34% for liver tissues (12), and in an unrelated study it did not exceed 10% (16). Furthermore, this method is extremely laborious and time consuming. In the present study, we modified the solid-phase extraction protocol (17, 19) by the substitution of homogenization by sonication with glass-on-glass homogenization, which increased acyl-CoA recovery by 2.6- and 4.8-fold for brain and heart tissues, respectively.

There are a number of possibilities that may account for the low extraction recovery of tissue acyl-CoAs using sonication to homogenize tissue. One possibility is the retention of acyl-CoAs in denatured binding proteins found in various tissues (24–27). However, it is unlikely that trapping of tissue acyl-CoAs in these proteins would result in such a large decrease in internal standard recovery in the tissue homogenized via sonication. It is also unlikely that the decreased recovery is the result of direct acyl-CoA destruction, because 120 s of sonication destroyed only 12% (n = 1) of the 17:0-CoA in extraction solutions. The most likely possibility is that sonication increases the quantity of oligonucleotides as a result of the destruction of intracel-

TABLE 1. Tissue acyl-CoA mass

Acyl-CoA	Brain	Liver	Heart	Kidney	Skeletal Muscle
22:6-CoA	0.39 ± 0.13	2.82 ± 0.71	0.36 ± 0.06	0.51 ± 0.10	0.26 ± 0.03
20:4-CoA	2.93 ± 0.21	4.20 ± 0.36	0.66 ± 0.17	4.14 ± 0.20	0.22 ± 0.04
18:2-CoA	0.37 ± 0.07	8.59 ± 0.59	4.48 ± 0.26	1.27 ± 0.10	1.63 ± 0.24
16:0-CoA	6.05 ± 0.64	3.66 ± 0.43	1.21 ± 0.15	2.42 ± 0.20	3.31 ± 0.25
18:1-CoA	7.30 ± 0.29	6.23 ± 0.35	1.84 ± 0.08	1.32 ± 0.14	2.94 ± 0.19
18:0-CoA	3.37 ± 0.43	1.41 ± 0.47	1.15 ± 0.08	0.93 ± 0.12	0.31 ± 0.03
Total	20.41 ± 1.43	26.90 ± 0.92	9.70 ± 0.50	10.58 ± 0.41	8.68 ± 0.41
Percent recovery	81.2 ± 1.6	78.0 ± 3.2	69.4 ± 8.0	77.7 ± 6.4	73.8 ± 9.7

16:0-CoA, palmitoyl-CoA; 18:0-CoA, stearoyl-CoA; 18:1-CoA, oleoyl-CoA; 18:2-CoA, linoleoyl-CoA; 20:4-CoA, arachidonoyl-CoA; 22:6-CoA, docosahexaenoyl-CoA. Individual long-chain acyl-CoA masses in rat tissues were separated using the improved HPLC method. Acyl-CoAs from tissue samples (0.138–0.205 g) were extracted after using the glass-on-glass homogenization procedure. Values are expressed as nanomoles per gram wet weight and are means ± SD (n = 4 except skeletal muscle, where n = 3). The percent recovery of the acyl-CoA extraction procedure was calculated for heptadecanoyl-CoA as the internal standard added before homogenization.

lular structures and the breakdown of nucleic acids, thus competing with acyl-CoAs for binding sites in the oligonucleotide purification cartridge.

Our new HPLC separation uses a high concentration of acetic acid (600 mM) to increase the resolution of polyunsaturated acyl-CoAs. GAC may decrease the lipophilic properties of ACN, therefore prolonging separation time and improving interaction between the reversed-phase column and long-chain acyl-CoAs. Higher concentrations of GAC, exceeding 600 mM, may cause a significant shift of the baseline and may damage the column because of low pH (<2).

We used this new extraction procedure and HPLC separation to quantify acyl-CoA species in a number of tissues. For liver, heart, and kidney tissues, we report lower values for some acyl-CoA mass as well as a lower total mass of the acyl-CoAs compared with other reports (**Table 2**). Very likely, this is the result of the better separation of the polyunsaturated acyl-CoAs 16:0-CoA and 18:1-CoA, which can be coeluted with other substances in other HPLC separations (Fig. 2B, C, compared with Fig. 1B). To check this point, we used the previously described method for acyl-CoA separation (19, 22) (Fig. 1B), but with our modified extraction procedure. In this experiment, we found the concentration of the total long-chain acyl-CoA fraction in brain tissue to be similar to the reported levels (22). Another explanation for these differences may be different dietary rations for the rats, which may significantly influence liver acyl-CoA composition and mass (13, 14). The application of the gas-liquid chromatography method demonstrates a significantly different distribution of acyl-CoAs in the tissue compared with HPLC analysis (7, 14, 21). However, in our hands, we could not demonstrate an effective transesterification of the acyl-CoAs using either acid-catalyzed (28) or base-catalyzed (29) methods. Regardless, we assume that our values are more accurate because of the enhanced resolution and recovery of our modified procedure.

It is also important to note that in rats ischemic conditions can influence brain acyl-CoA mass. Three minutes of a global ischemia causes a significant increase (4-fold) in brain 20:4-CoA mass and a decrease in 22:6-CoA mass (22). Hence, all procedures with brain tissue must be done rapidly and with the same postmortem time. In our analysis, the brain tissue was removed from the cranium within 40 s and placed immediately in liquid nitrogen.

Previously described methods require 0.8–1 g of brain tissue to obtain reproducible results (16, 17). Our modification allows the analyst to reliably use tissue masses of <0.1 g, because of the higher recoveries of the extraction procedure and the concentrating procedure. Furthermore, our method permits the injection of 96% of ex-

TABLE 2. Acyl-CoA mass of fed rat tissues

Acyl-CoA	Brain[a]	Brain[b]	Liver[a]	Liver[c]	Heart[a]	Heart[d]	Kidney[a]	Skeletal Muscle[a]	Skeletal Muscle[d]
22:6-CoA	N/A	1.71 ± 0.25	N/A	N/A	N/A	NA	N/A	N/A	N/A
20:4-CoA	N/A	1.66 ± 0.65	N/A	6.6	N/A	0.32 ± 0.27	N/A	N/A	0.1 ± 0.14
18:2-CoA	4.0 ± 0.3	0.98 ± 0.14	16.5 ± 1.4	10.0	7.7 ± 1.3	1.2 ± 0.99	9.1 ± 0.8	3.7 ± 0.4	0.73 ± 0.11
16:0-CoA	6.6 ± 0.6	8.45 ± 0.37	8.6 ± 0.5	16.6	5.1 ± 1.1	1.96 ± 0.81	5.7 ± 0.4	1.7 ± 0.2	0.87 ± 0.29
18:1-CoA	8.5 ± 0.8	10.85 ± 0.91	11.2 ± 0.4	11.6	4.8 ± 0.8	2.1 ± 0.44	4.3 ± 0.5	3.5 ± 0.3	1.33 ± 0.41
18:0-CoA	4.6 ± 0.3	5.08 ± 0.27	1.2 ± 0.4	27.4	0.7 ± 0.2	4.73 ± 1.54	0.7 ± 0.2	0.1 ± 0.0	0.71 ± 0.46
Total	23.7	28.73 ± 1.1	37.5	72.2	18.3	10.31	19.8	9.0	3.74

Values are expressed as nanomoles per gram wet weight and are means ± SD. Total acyl-CoA mass was calculated as the sum of the individual acyl-CoAs presented. N/A, not assayed.

[a] Values are from ref. (16).
[b] Values are from ref. (22).
[c] Values are from ref. (7).
[d] Values are from ref. (10).

tracted acyl-CoAs onto the HPLC column, thereby introducing the maximum amount of acyl-CoAs from the sample onto the column.

In summary, the method described for the analysis of tissue acyl-CoA mass shows significantly higher recovery for the extraction procedure than previously published methods. Furthermore, the HPLC procedure described demonstrates much higher resolution of the polyunsaturated as well as saturated acyl-CoA species. Collectively, the increased resolution and recovery make this analytical method suited for the analysis of small tissue amounts (<100 mg) and an accurate means to assess the individual acyl-CoA content of any given tissue. Recently, we have used this method to accurately assess acyl-CoA mass in as little as 15 mg of brain tissue. Overall, our new method provides comparable masses compared with previously reported values, and we report for the first time the mass of 20:4-CoA and 22:6-CoA in liver, heart, muscle, and kidney.

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