Recently, more attention has been focused on DAG because of their purported role to induce muscle and liver insulin resistance when present in excess (1–4). Because of the biological functions of DAG, especially DAG species containing saturated fatty acids, we endeavored to develop a method to precisely and rapidly measure muscle DAG.

Most methods for DAG measurement quantify total DAG content. One of the first and common quantitative analysis of DAG was performed by enzymatic analysis using diacylglycerol kinase assay (5) using labeled ATP\(^{32}\). A non-radioactive technique for total DAG quantification uses HPLC analysis following derivatization (6). The composition of DAG fatty acids can be determined by hydrolysis of DAG and analysis of the liberated and derivatized fatty acid by the means of gas-liquid chromatography with flame ionization detector (GLC-FID). Typically, DAG are separated from an extract of total lipids using thin-layer chromatography (TLC) or HPLC. TLC separation requires long processing times, leads to loss of analyte, and is susceptible to sample contamination. Development of mass spectrometry methods has facilitated analysis of DAG by reducing the analysis time, the amounts of consumables, and the likelihood of sample contamination. Diacylglycerols and triacylglycerols are classes of neutral lipids without any permanent charge; therefore, they are usually analyzed by ESI/MS as a sodium [M + Na]\(^{+}\), lithium [M + Li]\(^{+}\), or ammonium adduct that is created spontaneously by adding the appropriate salt to the solvent system (7, 8) or after the derivatization procedure (9, 10).

Because the potential contribution of circulating FFA to DAG synthesis is unknown, we developed a new method for measuring both concentration and isotopic enrichment of DAG in tissue from animals infused intravenously with uniformly labeled [U-\(^{13}\)C]palmitate. Our method displays excellent inter- and intraassay reproducibility, low detection limits, and satisfactory precision. Applying this protocol to biological muscle samples proves that the method is sensitive, accurate, and efficient. —Blachnio-Zabielska, A. U., P. Zabielski, and M. D. Jensen. Intramyocellular diacylglycerol concentrations and [U-\(^{13}\)C]palmitate isotopic enrichment measured by LC/MS/MS. \(J.\) Lipid Res. 2013. 54: 1705–1711.

**Supplementary key words**  Diacylglycerols measurement • mass spectrometry • skeletal muscle • liquid chromatography

Diacylglycerols (DAG) are an important lipid class because of their function as second messengers in many cellular processes, modulating vital biochemical mechanisms.

**Method**

**Abstract**  Diacylglycerols (DAG) are important lipid metabolites thought to induce muscle insulin resistance when present in excess; they can be synthesized de novo from plasma free fatty acids (FFA) or generated by hydrolysis of preexisting intracellular lipids. We present a new method to simultaneously measure intramyocellular concentrations of and the incorporation of [U-\(^{13}\)C]palmitate from an intravenous infusion into individual DAG species. DAG were extracted from pulverized muscle samples using isopropanol: water:ethyl acetate (35:5:60; v:v:v). Chromatographic separation was conducted on reverse-phase column in binary gradient using 1.5 mM ammonium formate, 0.1% formic acid in water as solvent A, and 2 mM ammonium formate, 0.15% formic acid in methanol as solvent B. We used UPLC-ESI\(^{-}\)-MS/MS in the multiple reaction monitoring (MRM) mode to separate the ions of interest from sample. Because DAG are a neutral lipid class, they were monitored as an ammonium adduct [M+NH\(_4\)]\(^{+}\). To measure isotopic enrichment (for \(^{13}\)C\(_16:0/16:0\)-DAG and \(^{13}\)C\(_{16:0}/C_{18:1}\)-DAG), we monitored the basic ions as [M+2+NH\(_4\)]\(^{+}\) and the enriched compounds as [M+16+NH\(_4\)]\(^{+}\). We were able to measure concentration and enrichment using 20 mg of skeletal muscle samples obtained from rats receiving a continuous infusion of [U-\(^{13}\)C]palmitate. Applying this protocol to biological muscle samples proves that the method is sensitive, accurate, and efficient. —Blachnio-Zabielska, A. U., P. Zabielski, and M. D. Jensen. Intramyocellular diacylglycerol concentrations and [U-\(^{13}\)C]palmitate isotopic enrichment measured by LC/MS/MS. \(J.\) Lipid Res. 2013. 54: 1705–1711.

**Supplementary key words**  Diacylglycerols measurement • mass spectrometry • skeletal muscle • liquid chromatography

Diacylglycerols (DAG) are an important lipid class because of their function as second messengers in many cellular processes, modulating vital biochemical mechanisms.
chemotaxonomy, and can detect concentration and enrichment in as little as 20 mg of muscle. Measuring both concentration and isotopic enrichment of DAG and other lipid groups (11, 12) can help to elucidate the contribution of plasma FFA to intramyocellular fatty acid-containing compounds. We believe that this method can be used to elucidate the dynamics of the intramyocellular DAG pool and its relationship to plasma FFA in physiological and pathological states.

MATERIALS AND METHODS

Chemicals

HPLC grade solvents. Methanol, water, ammonium formate, and formic acid was purchased from Sigma-Aldrich (St. Louis, MO).

Standards. Enriched standards 1-palmitoyl[13C16]3-palmitoyl-glycerol (1.3 [13C16]16:0/16:0-DAG), 1-palmitoyl[13C15]3-oleyl-glycerol (1.5 [13C16]16:0/18:1-DAG) as well as standards 1-palmitoyl-2-linoleoyl-glycerol (1.2 16:0/18:2-DAG), 1-oleoyl-2-linoleoyl-glycerol (1.2 18:1/18:2-DAG), and 1-stearoyl-2-oleoyl-glycerol (1.2 18:0/18:1-DAG) were synthesized by the Lipidomics Core, Medical University of South Carolina. Standards 1,2-di-palmitoyl-glycerol (1.2 16:0/16:0-DAG), 1,3-dilinoleoyl-glycerol (1.3 18:2/18:2-DAG), and internal standard (ISTD) 1,3-dipenta decanoyl-glycerol (1.3 15/15-DAG) were purchased from Sigma-Aldrich. Standards 1,2-dioleoyl-glycerol (1.2 18:1/18:1-DAG), 1-stearoyl-2-linolenoyl-glycerol (1.2 18:0/18:2-DAG), and 1-palmitoyl-2-oleoyl-glycerol (1.2 16:0/18:1-DAG) were obtained from Avanti Polar Lipids (Alabaster, AL).

Extraction of diacylglycerols

Muscle samples were pulverized and weighed, and 20 mg of each was taken for analysis. To these samples we added 50 mg of the internal standard (ISTD) (1,3 dipentadecanoyl-sn-glycerol) as well as 200 µl of homogenization buffer consisting of 0.25 M sucrose, 20 mM KC1, 50 mM Tris, and 0.5 mM EDTA, pH 7.4. After homogenization, 1.5 ml of the extraction mixture isopropanol:water:ethyl acetate, (55:50:5; v:v:v) was added to each sample. The samples were then vortexed, sonicated, and centrifuged for 10 min at 4000 rpm (Sorvall Legend RT). The supernatant was transferred to a new tube, and the pellet was reextracted using the same extraction mixture. After centrifugation, supernatants were combined and evaporated under nitrogen. The dried sample was reconstituted in 100 µl of LC solvent B for LC/MS/MS analysis. To prevent sample contamination, all the glassware used for extraction of lipids, including glass pipette tips and vials, was disposable. All solvents, including water, were of LC/MS grade.

Animals and isotope infusion

Male Wistar rats weighing approximately 350 g were acclimated for one week prior to the experiment by housing them in controlled environment under ambient temperature and humidity in 12 h light/dark cycle and feeding them standard laboratory rat chow. Experiments were approved by Institutional Animal Care and Use Committee of Medical University of Bialystok.

Tracer preparation

The palmitate tracer was prepared as previously described (13). Briefly, 13C16-potassium-palmitate (uniformly labeled, 88.66% M+16 isotopic purity, Sigma-Aldrich, St. Louis, MO) was mixed with concentrated albumin solution (essentially fatty acid-free, Sigma-Aldrich) with a ratio of 1 g of albumin per 10 mg tracer. The solution was heated to 60°C, stirred until clear, and sonicated for 1 min with probe sonicator. Despite the use of fatty acid-free albumin, residual unlabeled palmitate decreased final isotopic enrichment of the infusates to 86.65% (± 0.18% SD, n = 6 independent preparations). The solution was diluted with sterile phosphate buffered saline to a final concentration of 1 mM [13C16-palmitate, taking into account both the tracer isotopic purity and the presence of preexisting albumin-bound palmitate. This concentration was used to calculate infusion rates. The total palmitate concentration in infusion (M+16 palmitate and all other sources) was 1.134 mM. The infusate was passed through 0.2 µm sterile syringe filter, divided into aliquots, and stored at −20°C until use. Plasma palmitate concentration and isotopic enrichment in both the infusate and plasma samples was measured according to Persson et al. (14).

Isotope infusion and tissue collection

The food was withdrawn from animals 6 h prior to infusion. Animals received pentobarbital anesthesia (40 mg/kg of body weight) and were placed on a heating blanket. The tracer was delivered into proximal dorsal tail vein using a Model NE-1000 syringe pump (New Era Syringe Pumps, Farmingdale, NY) via 0.5 mm id tubing (Rotirolab, Dreieich, Germany) and 25 gauge needle at a constant rate of 50 nmol·min−1·kg−1 body weight for 2 h. A priming dose of 500 nmol/kg was given in the first 10 s of infusion to prime the palmitate pool and accelerate isotopic equilibration. Every 15 min, a 50 µl blood sample was taken from saphenous vein into a heparinized Microrvette capillary tube (Stardstedt, Numbrecht, Germany). Plasma was obtained by centrifugation for analysis of palmitate concentration and enrichment. To prevent vascular volume overload, the infused volume did not exceed 17% of total plasma volume [calculated according to Lee et al. (15)] and was less than estimated 2 h urine output according to the values for Wistar rats from Rat Phenome Database (16). The tracer infusion had no effect on the level of plasma FFA. The pre- and postinfusion concentration of total plasma FFA and FFA palmitate did not differ significantly (274 µmol/l versus 271 µmol/l for total FFA; 64 µmol/l versus 64 µmol/l for palmitate). The mean plasma palmitate tracer enrichment at plateau was 0.107 ± 0.005 molar percentage excess (MPE, n = 6). At the end of 2 h infusion, the last blood sample was collected from inferior vena cava, and rats were sacrificed by heart incision. Samples of longissimus thoracis muscle were collected and immediately frozen in liquid nitrogen for analysis of DAG concentration and enrichment.

Liquid chromatography condition

The chromatographic separation was performed using an Agilent 1290 Infinity Ultra Performance Liquid Chromatography (UPLC). The analytical column was a reverse-phase Zorbax SB-C8

| Molecular Species | Precursor Ion [M+NH4]+ | Product Ion |
|-------------------|------------------------|------------|
| 18:0/18:1-DAG     | 640.5                  | 339.4      |
| 18:0/18:2-DAG     | 638.5                  | 341.4      |
| 18:1/18:1-DAG     | 638.6                  | 339.8      |
| 18:1/18:2-DAG     | 636.5                  | 339.4      |
| 18:2/18:2-DAG     | 634.5                  | 337.4      |
| 16:0/18:1-DAG     | 612.6                  | 313.4      |
| 16:0/18:2-DAG     | 610.5                  | 313.4      |
| 16:0/16:0-DAG     | 586.5                  | 313.4      |
| 15/15-DAG         | 558.5                  | 299.4      |
To measure concentration, all DAG were monitored as [M+NH₄⁺]⁺ in positive mode. To measure the low levels of isotopic enrichment from the incorporation of plasma [U-¹³C]palmitate into DAG, we used the strategy of increasing the relative abundance of the enriched ions [M+16+NH₄⁺]⁺ ions by monitoring the [M+2+NH₄⁺]⁺ species as a base peaks. This approach uses the lower-abundance natural [M+2] isotopomer to increase precision of enrichment measurement in samples with extremely low enrichment as described by Patterson et al. (17). The entire analysis was performed in MRM mode with collision energy of 10 eV. Mass transitions and collision are shown in Table 1 (for concentration) and Table 2 (for enrichment).

### Preparation of standard solutions

The ISTD solution and stock solution of DAG standards for standard curve preparation were prepared in ethanol. Each 50 µl of the ISTD contained 50 ng of 1,3 ¹⁵/¹⁵-DAG. Each 10 µl of the standard curve stock solution contained 50 ng of the particular DAG. After preparation of the six-point standard curve (representative plot is presented in Fig. 1), each aliquot was spiked with 50 µl of ISTD solution, and all aliquots underwent the extraction procedure. For enrichment analysis, a ten-point standard curve was prepared by mixing constant amounts of unlabeled standards and different amounts of labeled standards for each point (Fig. 2).

### Method validation

**Recovery and efficiency.** The recovery was examined by comparing the LC/MS/MS peak areas of a standard mixture with and without extraction procedure. A mixed standard solution

![Fig. 1](image1.png)  
**Fig. 1.** Representative concentration standard curves for dipalmitoyl-DAG (A) and palmitoyl/oleoyl-DAG (B). Equations of linear regression curves fitting and coefficients of determination are shown above respective linear regression curves.

![Fig. 2](image2.png)  
**Fig. 2.** Enrichment standard curves for dipalmitoyl-DAG (A) and palmitoyl/oleoyl-DAG (B) molecular species. The values are expressed as MPE. Enriched species were analyzed as [M+16+NH₄⁺]⁺ ions versus the base peak of the [M+2+NH₄⁺]⁺ ions.

### Mass spectrometry

The DAG species were analyzed as the ammonium adducts, without derivatization, using electrospray ionization conditions in the positive ion mode. To generate this charged adduct species, a 2 mM ammonium formate in solvent A and 1.5 mM ammonium formate in solvent B were used. The following conditions were employed: the drying gas temperature was 300°C, the drying gas flow was 5 l/min, the spray voltage was 3,500 V, sheath gas was 11 L/min, and transfer capillary was 250°C.

### Chromatographic separation

Chromatographic separation was conducted in binary gradient using 1.5 mM ammonium formate, 0.1% formic acid in water as solvent A; and 2 mM ammonium formate, 0.1% formic acid in methanol as solvent B at the flow rate of 0.4 ml/min. The gradient conditions were as follows: 0 min at 90% B, 0–1.5 min 90–96% B, 1.5–3.0 min 96–97% B, 3.0–5.0 min 97–95% B, 5.0–7.0 min 95–97.5% B, 7.0–7.5 min 97.5–90% B, 7.5–8.0 min isocratic at 10% B. Standards and samples were resuspended in 100 µl buffer B prior to injecting 3 µl onto the UPLC/MS/MS.

### Masses monitored for enrichment measurement

| Molecular Species                  | Precursor Ion | Product Ion |
|-----------------------------------|---------------|-------------|
| 16:0/16:0-DAG [M+2+NH₄⁺]⁺         | 588.5         | 315.4       |
| ¹³C16:0/16:0-DAG [M+16+NH₄⁺]⁺     | 602.5         | 329.4       |
| 16:0/18:1-DAG [M+2+NH₄⁺]⁺         | 614.5         | 315.4       |
| ¹³C16:0/18:1-DAG [M+16+NH₄⁺]⁺     | 628.5         | 329.4       |
Fig. 3. Total ion chromatogram (A) and MRM chromatograms of individual DAG molecular species extracted from skeletal muscle (B) after chromatographic resolution.
Measurement of diacylglycerols by LC/MS/MS

After chromatographic separation, the eluate was directed to the ionization source without derivatization. Because neutral lipids, such as DAG and triacylglycerols, lack a permanent charge, we analyzed DAG as the ammonium adduct \([M+NH_4]^+\) (the masses for precursor and product ions are provided in Table 1). Using the sample preparation and analytical conditions described above, we obtained clean total ion chromatogram in MRM mode (Fig. 3). Enriched peaks from skeletal muscle and corresponding base peaks \([M+2+NH_4]^+\) are presented in Fig. 4. Our approach contrasts to published mass spectrometry methods for DAG measurement by applying triple quadrupole mass spectrometry equipment designed specifically for quantitative analysis. Most LC/MS methods for DAG quantification use ion trap mass spectrometers, more suitable for identification then for quantification, with a derivatization step before analysis (9, 10). A method for DAG quantification without derivatization prior to analysis has been described using a direct infusion ionization source operating in full-scan mode (18), but without chromatographic separation. The principle advantages of our method are simple sample preparation without derivatization, short run time (10 min), and accurate and precise analysis of both concentration and enrichment based on mass transition (MRM mode) and retention time. The method requires as little as 20 mg of muscle.

### Standard curves for enrichment estimation

The standard curve samples contained \([U-^{13}C]_{16:0}/16:0\) DAG or \([U-^{13}C]_{16:0}/18:1\) DAG in an MPE range of 0.01% to 0.5%. The calculated \([U-^{13}C]_{16:0}/16:0\) DAG/\([U-^{13}C]_{16:0}/18:1\) DAG peak area ratios and theoretical MPEs of \([U-^{13}C]_{16:0}/16:0\) DAG and \([U-^{13}C]_{16:0}/18:1\) DAG were taken to construct a ten-point enrichment standard curve. We found excellent linear relationships with \(R^2\) of 0.9997 and 0.9995 for \([U^{13}C]_{16:0}/16:0\) DAG and \([U^{13}C]_{16:0}/18:1\) DAG, respectively (Fig. 2).

### Method validation

**Recovery and efficiency.** The average calculated recovery of extracted standards ranged from 86 to 93%. In the biological matrix, the extraction recovery ranged from 82 to 89%.

**Reproducibility.** To test the sample-to-sample reproducibility, three rat longissimus thoracis muscle samples were taken after \([U-^{13}C_{16}]_{palmitate}\) infusion. Samples were analyzed five times by LC/MS/MS, using 20 mg of tissue for each analysis. To study day-to-day reproducibility, the samples were analyzed on three different days.

**Sensitivity.** Using the sample preparation and extraction procedure described above, we could reliably quantify DAG concentration and isotopic enrichment using 20 mg of tissue. This amount of sample was sufficient to precisely establish both values. The tissue mass equivalent used for each injection was comparable to only 0.6 mg of the original muscle sample. The measurement of DAG was prepared by dissolving DAG standards in 100 µl of solvent B and directly analyzing the standard solution by LC/MS/MS, while another 100 µl solution was dried under gentle N\(_2\) flow, extracted as described for the samples, and redissolved in 100 µl of solvent B for LC/MS/MS analysis. The peak areas for DAGs \([M + NH_4]^+\) ions were integrated and compared. This experiment was performed three times, and the average recovery was calculated.

To estimate recovery from the biological matrix, we prepared homogenate from muscle samples that was kept at room temperature for 24 h and then incubated at 45°C for 1 h. A portion of the homogenate was extracted to ensure there was no detectable DAG. The remaining homogenate was divided into three aliquots to which 100 µl of the above DAG standard mixture was added, followed by the extraction and LC/MS/MS analysis procedures. To measure the extraction recovery from sample containing the biological matrix and standard mixture, we compared the DAG peak areas of both sample types.

**RESULTS AND DISCUSSION**

We used UPLC (1290 Infinity, Agilent) connected to an Agilent 6460 triple quadrupole mass spectrometer.
TABLE 3. Reproducibility of the measurement of individual DAG concentrations

| Muscle sample I | 18:2/18:2 | 16:0/18:2 | 18:1/18:2 | 16:0/16:0 | 16:0/18:1 | 18:1/18:1 | 18:0/18:2 | 18:0/18:1 |
|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Day1            | 35.9 ± 0.85 | 21.9 ± 1.23 | 23.1 ± 1.19 | 32.4 ± 1.64 | 113.2 ± 2.11 | 25.5 ± 1.16 | 193.3 ± 3.36 | 38.5 ± 1.69 |
| Day2            | 35.3 ± 2.15 | 22.9 ± 1.67 | 23.5 ± 2.08 | 32.4 ± 2.15 | 113.4 ± 2.96 | 25.9 ± 2.06 | 194.5 ± 5.30 | 39.5 ± 1.45 |
| Day3            | 36.0 ± 1.51 | 23.5 ± 1.44 | 24.1 ± 1.52 | 32.2 ± 1.84 | 114.6 ± 2.20 | 26.1 ± 0.78 | 195.7 ± 3.71 | 40.3 ± 2.87 |
| Day to day (CV)| 35.7 ± 1.51 (4.2%) | 22.8 ± 1.52 (6.7%) | 23.6 ± 1.58 (6.7%) | 32.3 ± 1.77 (5.3%) | 113.7 ± 2.36 (2.1%) | 25.8 ± 1.35 (5.3%) | 194.5 ± 4.03 (2.1%) | 39.5 ± 2.09 (3.3%) |

| Muscle sample II| 18:2/18:2 | 16:0/18:2 | 18:1/18:2 | 16:0/16:0 | 16:0/18:1 | 18:1/18:1 | 18:0/18:2 | 18:0/18:1 |
|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Day1            | 37.3 ± 1.18 | 24.7 ± 1.61 | 36.4 ± 1.88 | 44.0 ± 1.58 | 163.4 ± 2.43 | 31.6 ± 1.43 | 206.4 ± 8.12 | 42.8 ± 1.69 |
| Day2            | 36.4 ± 0.99 | 25.5 ± 1.92 | 35.5 ± 2.64 | 44.6 ± 1.95 | 164.2 ± 7.41 | 32.2 ± 2.34 | 203.6 ± 9.56 | 43.0 ± 1.98 |
| Day3            | 38.4 ± 1.18 | 26.1 ± 1.92 | 37.3 ± 1.47 | 45.4 ± 2.36 | 166.6 ± 5.70 | 33.2 ± 2.52 | 207.8 ± 9.50 | 44.2 ± 1.40 |
| Day to day (CV)| 37.3 ± 1.90 (5.1%) | 25.4 ± 1.79 (7.9%) | 36.4 ± 2.05 (5.6%) | 44.7 ± 1.93 (4.3%) | 164.7 ± 5.34 (3.2%) | 33.4 ± 2.11 (6.5%) | 205.9 ± 8.6 (4.2%) | 43.3 ± 1.70 (3.9%) |

| Muscle sample III| 18:2/18:2 | 16:0/18:2 | 18:1/18:2 | 16:0/16:0 | 16:0/18:1 | 18:1/18:1 | 18:0/18:2 | 18:0/18:1 |
|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Day1            | 43.3 ± 1.92 | 25.2 ± 1.73 | 32.5 ± 1.46 | 42.9 ± 1.88 | 131.6 ± 1.86 | 23.5 ± 1.73 | 190.0 ± 4.38 | 34.0 ± 1.84 |
| Day2            | 44.1 ± 1.81 | 26.3 ± 1.62 | 33.5 ± 1.28 | 43.9 ± 1.76 | 133.6 ± 2.87 | 24.5 ± 2.27 | 191.0 ± 4.80 | 33.2 ± 2.52 |
| Day3            | 44.3 ± 1.50 | 25.8 ± 1.45 | 33.7 ± 1.85 | 44.7 ± 1.85 | 130.8 ± 2.08 | 25.1 ± 1.92 | 192.0 ± 4.28 | 34.4 ± 1.67 |
| Day to day (CV)| 43.9 ± 1.68 (3.8%) | 25.7 ± 1.56 (6.1%) | 33.2 ± 1.53 (4.6%) | 43.9 ± 1.86 (4.3%) | 132.0 ± 2.46 (1.9%) | 24.4 ± 1.97 (8.1%) | 191.0 ± 4.24 (2.2%) | 33.9 ± 1.96 (5.8%) |

Data are expressed as pmol/mg tissue ± SD. Each sample was analyzed five times daily for three days. Values in parentheses represent day-to-day coefficient of variation (CV%) for the respective DAG molecular species.
Biological application

Animals with greater plasma palmitate enrichment had greater tissue DAG enrichment (Fig. 5A). The muscle DAG enrichments were compared with the corresponding plasma palmitate enrichments. There was a close correlation between the enrichment of plasma FFA and tissue DAG in rat muscle samples (Fig. 5B). Enrichment of both molecular species of DAG displayed a linear relationship with plasma tracer enrichment. We also noticed differences between labeling efficiency of dipalmitoyl-DAG and palmitoyl/oleoyl-DAG (curve slope and mean enrichment value under the same tracer pool), which suggests differences in synthesis or degradation of intramyocellular DAG containing saturated and monounsaturated fatty acids. These data suggest to us that plasma FFA are an important source of fatty acids for intramyocellular, de novo DAG synthesis. It also suggests that the individual intramyocellular DAG species differ in respective intracellular turnover.

CONCLUSION

We found that this UPLC-ESI/MS/MS in the MRM mode is a rapid, simple, and reliable method to simultaneously detect low levels of stable isotopic tracer enrichments (13C16:0/16:0 DAG and 13C16:0/18:1DAG) and DAG concentrations in small samples of muscle tissue. Remarkably, as little as 3 μl of lipid extract, equivalent to 0.6 mg of original sample weight, can be reliably analyzed for DAG concentration and enrichment with excellent quantification accuracy and sample-to-sample and day-to-day reproducibility. One limitation to this method is the limited availability of DAG species standards and enriched standards, which can be quite costly to purchase.

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