Published in final edited form as:
Methods Enzymol. 2014 ; 542: 391–405. doi:10.1016/B978-0-12-416618-9.00020-0.

Measurement of Fatty Acid Oxidation Rates in Animal Tissues and Cell Lines

Frank K. Huynh[*], **Michelle F. Green**[*], **Timothy R. Koves**[*,†,‡], and **Matthew D. Hirschey**[*,†,‡,§,1]

[*]Duke Molecular Physiology Institute, Duke University Medical Center, Durham, North Carolina, USA

[†]Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, Durham, North Carolina, USA

[‡]Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA

[§]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina, USA

Abstract

While much oncological research has focused on metabolic shifts in glucose and amino acid oxidation, recent evidence suggests that fatty acid oxidation (FAO) may also play an important role in the metabolic reprogramming of cancer cells. Here, we present a simple method for measuring FAO rates using radiolabeled palmitate, common laboratory reagents, and standard supplies. This protocol is broadly applicable for measuring FAO rates in cultured cancer cells as well as in both malignant and nontransformed animal tissues.

1. Introduction

In order to sustain rapid cellular proliferation and growth, cancer cells undergo a complex metabolic rearrangement characterized by suppressing several catabolic pathways involved in energy production and activating anabolic processes including nucleotide and lipid biosynthesis (DeBerardinis et al., 2007; Frezza & Gottlieb, 2009; Lunt & Vander Heiden, 2011). Compared to studies on glucose or amino acid oxidation, remarkably few studies have addressed changes in lipid oxidation during oncogenesis or for cancer cell survival.

Lipids are oxidized primarily in the mitochondria in a multistep pathway called β-oxidation (or fatty acid oxidation, FAO) (McGarry & Foster, 1980). This process begins by importing long-chain fatty acyl-CoAs into mitochondria, followed by a four-step reaction. In the first step, acyl-CoA dehydrogenase oxidizes the long-chain acyl-CoA. In the second step, enoyl-CoA hydratase hydrates the acyl-chain forming hydroxy-acyl-CoA. In the third step, hydroxy-acyl-CoA dehydrogenase oxidizes the substrate forming a second keto-group. In the fourth step, thiolase cleaves acetyl-CoA and adds a free CoA to the new substrate, generating an acyl-CoA shortened by two carbons. This shortened acyl-CoA continues

[1]Corresponding author: matthew.hirschey@duke.edu.

through the four-step reaction until the entire chain is oxidized into acetyl-CoA (Fig. 20.1). A major result of metabolic shifts toward FAO is increased ATP production, and this is often associated with stress responses and survival.

Evidence is emerging for an important role for FAO in cancer (for a review, see Carracedo, Cantley, & Pandolfi, 2013). One recent study found that cells that undergo loss of attachment from solid tumors have less glucose uptake and oxidation, which results in decreased ATP and NADPH and increased reactive oxygen species (ROS) (Schafer et al., 2009). In this study, elevated ROS inhibited FAO, while antioxidant administration counteracted ROS and reactivated FAO, increased ATP levels, and prevented cell death by anoikis, demonstrating a crucial role for antioxidants and FAO in cancer cell survival. Another study identified a novel peroxisome proliferator-activated receptor-dependent mechanism by which the promyelocytic leukemia protein regulates FAO (Ito et al., 2012). In a final example, an atypical carnitine palmitoyltransferase 1 (CPT1) isoform C was identified as a potential oncogene, outside of its normal role in the brain (Zaugg et al., 2011). CPT1 converts long-chain fatty acyl-CoAs to fatty acylcarnitines to translocate lipids into the mitochondria, where acyl-CoAs are reformed to undergo FAO. In these examples, FAO is often associated with cancer cell survival, similar to postmitotic, metabolic cell types.

Thus, a picture is emerging for FAO to be an important metabolic pathway in cancer. Here, we describe detailed methods to measure mitochondrial FAO, which were adapted from previous studies (Kim, Hickner, Cortright, Dohm, & Houmard, 2000; Mannaerts, Debeer, Thomas, & De Schepper, 1979; Shindo, Osumi, & Hashimoto, 1978; Van Veldhoven et al., 1991). These methods use common supplies and reagents to measure FAO rates in either whole tissue homogenates (Fig. 20.2) or cultured cells (Fig. 20.3).

2. Protocol Overview

2.1. Biochemical principle of the assay

^{14}C-labeled palmitate is oxidized via β-oxidation to generate acetyl-CoA, which can then enter the tricarboxylic acid (TCA) cycle and be oxidized to CO_2 (Fig. 20.1). Figure 20.1 shows an example where the ^{14}C label is at position C-16 (IUPAC numbering), which allows for the measurement of complete oxidation of palmitate. Depending on the process of interest (i.e., complete oxidation or incomplete oxidation), palmitate with the ^{14}C label at different positions can be used. Maximal signal will likely be obtained with the radiolabel at position C-1, and this reagent is more readily available. This protocol has been optimized for use with radiolabeled palmitate, but other radiolabeled fatty acids can also be used.

The radiolabel can have several fates. ^{14}C-palmitate that does not get oxidized to fatty acyl-CoAs shorter than ∼6 carbons in length will precipitate out of solution upon addition of perchloric acid. Incompletely oxidized acid-soluble metabolites containing the ^{14}C radiolabel can include palmitoyl-carnitine, acetyl-carnitine, acetyl-CoA, ketone bodies (in the liver), gluconeogenic intermediates (depending on cell type), cataplerotic TCA cycle intermediates, and fatty acyl-CoAs that are shorter than 6 carbons in length (if using

palmitate labeled at the C-16 position). Radiolabeled acetyl-CoA can also enter the TCA cycle and be oxidized to $^{14}CO_2$.

2.2. Overview of the protocol

Figure 20.2 provides an overview of the protocol for measuring FAO in animal tissues. Similarly, Fig. 20.3 shows the protocol adapted for cultured cells.

3. Equipment

Mouse dissection tools (forceps and scissors)

Bell jar or other anesthesia apparatus

Isoflurane or other mouse anaesthetic

2-mL Dounce homogenizer (Kimble Kontes, 8853000002)

Water bath or heating block

1.5-mL Eppendorf tubes

Whatman filter paper

Scissors

Scintillation counter, fluid, and vials

24-Well culture plates

4. Materials

1 *M* sucrose

1 *M* Tris–HCl, pH 7.4

50 m*M* EDTA, pH 8.0

Radiolabeled fatty acid (e.g., 1-^{14}C-palmitate)

Nonradiolabeled fatty acid (same fatty acid as above)

30% fatty acid-free bovine serum albumin (BSA) (Sigma, A9205)

1 *M* KH_2PO_4

1 *M* KCl

1 *M* $MgCl_2$

50 m*M* L-carnitine

20 m*M* malate

10 m*M* coenzyme A

100 m*M* ATP

1 *M* dithiothreitol (DTT)

1 *M* perchloric acid or other strong acid

1 *M* sodium hydroxide or other strong base

Milli-Q or other purified water (ddH_2O)

Dulbecco's modified Eagle's medium (DMEM)

4.1. STE buffer

0.25 *M* sucrose

10 m*M* Tris–HCl

1 m*M* EDTA

pH to 7.4

4.2. Preparation of 7% BSA/5 m*M* cold palmitate mixture

BSA is required to act as a carrier for palmitate to ensure lipid solubility in an aqueous solution. Cold palmitate is included in the FAO reaction in order to increase the total rate of oxidation and signal measured in the assay. In order to prepare a solution of 7% BSA/5 m*M* palmitate (∼5:1 molar ratio), do the following:

1. Prepare a 7.5% BSA solution by diluting a 30% stock (e.g., fatty acid-free BSA; Sigma, A9205) in ddH_2O. Warm to 42 °C in a water bath. Prepare 20 mL for the 7% BSA/5 m*M* palmitate solution and 20 mL for a 7% BSA only control solution.
2. Weigh 27.8 mg sodium palmitate (Sigma, P9767) and put in a 50-mL conical tube. Add 1.3 mL of ddH_2O and keep cap closed but loosened.
3. Place the tube in a boiling water bath until the fatty acids are dissolved (a few minutes).
4. Cool the palmitate until it can be held in a bare hand, but the fatty acids are still dissolved (∼70 °C). If the palmitate precipitates, then warm again.
5. Immediately add 18.7 mL of the 7.5% BSA. Place in a 42 °C water bath for 30 min. For the 7% BSA only control, add 18.7 mL of the 7.5% BSA to 1.3 mL of ddH_2O.
6. If particles form and are visible to the naked eye, sonicate for 5 min. Increase the temperature to 47 °C if particles are still present, but do not go above 50 °C to avoid denaturing the BSA.
7. Store unused aliquots at −80 °C for no more than 6 months. Avoid freeze–thaw cycles.

   Tip: If performing this assay for the first time, it may be useful to vary the amount of cold palmitate per reaction to find the ideal concentration of total palmitate per reaction for your sample, tissue of choice, and experimental conditions. To do this, use the 7% BSA control solution to adjust the concentration of cold palmitate. Typical concentrations of cold palmitate for tissue homogenates and cells are 0.1–0.5 m*M* and 0.3–1.0 m*M*, respectively.

4.3. Solubilization of radiolabeled palmitate into the aqueous phase

The radiolabeled palmitate is typically stored as a liquid in ethanol, which is not compatible with the FAO assay. In order to remove the ethanol and resolubilize the palmitate in BSA, perform the following:

1. Calculate the amount of radiolabeled palmitate required. Each sample should be assayed in triplicate with 0.4 μCi per reaction for animal tissues and 0.2 μCi per well for cell lines.

   Tip: When optimizing this assay, up to 1–2 μCi labeled palmitate can be used to increase the signal.

   Tip: Be sure to include enough for a triplicate blank sample as well as a triplicate measurement of the total radioactivity input per reaction.

2. Dry down the radiolabeled palmitate in an Eppendorf tube under nitrogen gas. The ethanol will evaporate and leave a film of radiolabeled palmitate at the bottom of the tube.

3. For tissue samples, resolubilize the radiolabeled palmitate in the appropriate amount of 7% BSA/5 m*M* palmitate warmed to 37 °C to get a solution of 7% BSA/5 m*M* palmitate/0.01 μCi/μL ^{14}C-palmitate. For cultured cells, use the 7% BSA control solution to adjust the 7% BSA/5 m*M* palmitate solution to a concentration of 7% BSA/2.5 m*M* palmitate. Resolubilize the radiolabeled palmitate in the appropriate amount of 7% BSA/2.5 m*M* palmitate that when added to the required amount of DMEM will give a final media concentration of 0.3% BSA/100 μ*M* palmitate/0.4 μCi/mL ^{14}C-palmitate. 500 μL of media will be required for each well of a 24-well plate.

4. Heat the radioactive BSA/palmitate solution at 37 °C for at least 30 min with frequent vortexing to ensure complete solubilization. Alternatively, the solution can be heated at 37 °C overnight in a thermomixer to aid solubilization.

   Tip: The radioactive BSA/palmitate solution can be prepared the day before and stored at 37 °C overnight.

5. Protocol

5.1. FAO in animal tissues

1. Prepare the oxidation reaction mixture according to Table 20.1 and keep at 37 °C for 1–2 h.

2. Prechill the required amount of sucrose–Tris–EDTA (STE) buffer at 4 °C.

3. Prechill the 2 mL Dounce homogenizer on ice.

4. Anesthetize the mouse (e.g., isoflurane) according to institutional animal safety protocols.

5. Quickly remove the tissue(s) of interest and euthanize the mouse.

Tip: For liver, about 200 mg of liver should be enough. For heart tissue, use the whole heart. A tumor xenograft could also be used. The appropriate amount to collect for other tissues should be determined empirically. More tissue homogenate is always better to increase the signal in this assay.

6. Rinse the tissue several times in ice-cold STE buffer to remove any blood.

   Tip: For more collagenous tissues, such as heart tissue, use a razor blade and mince the tissue in ice-cold STE buffer which will facilitate homogenization by the Dounce homogenizer.

7. Place the tissue directly into the 2-mL Dounce homogenizer containing approximately 5 volumes of chilled STE buffer.

   Tip: For 200 mg of liver, use 1 mL of STE buffer. For one regularsized mouse heart, use 0.8 mL of STE buffer.

8. Homogenize liver tissue using 5 strokes of the loose-fitting A pestle of the Dounce homogenizer (5 down and 5 up-strokes).

   Tip: For tough, firm tissues such as the heart, use the A pestle and perform 1 firm down stroke right to the bottom of the homogenizer. For the up-strokes, twist the A pestle while gently pulling up in order to avoid forming a strong vacuum pressure that will damage the mitochondria. The first complete up-stroke can take 1–2 min to perform, but each successive up-stroke will become easier. Repeat this process up to 25 times for heart tissue to ensure thorough homogenization.

9. Pour the homogenate from the Dounce homogenizer into a prechilled Eppendorf tube and place on ice.

10. Continue until all tissues of interest are homogenized.

    Tip: No more than 30 min should pass between the time the tissue is collected and when the FAO reaction is started. Waiting longer than 30 min will result in a poor assay signal. Working quickly will ensure the integrity of the mitochondria and strengthen the signal in the assay. It may be useful to have more than one person and more than one Dounce homogenizer to expedite tissue processing.

    Tip: If harvesting tissues from several animals, the whole tissue is more stable than the homogenate. Leave tissues intact in ice-cold STE buffer until all tissues are harvested and then process homogenates as quickly as possible.

11. Centrifuge the crude homogenates at 420 × *g* for 10 min at 4 °C.

12. Decant the supernatant containing crude mitochondria and place in fresh, chilled Eppendorf tubes. Keep on ice.

13. Resuspend the pellet in 400 μL STE buffer and centrifuge again at 420 ×*g* for 10 min at 4 °C. Decant the supernatant and pool with the previous supernatant. (This step is optional but may release additional mitochondria trapped in the low-speed pellet.)

14. Discard the pellet.

15. Take a small aliquot of homogenate and set aside for a BCA assay to measure protein concentration.

    Tip: A 1:10–1:50 dilution of each tissue homogenate should be sufficient for the BCA assay.

16. Add 30 μL of tissue homogenate to fresh, chilled Eppendorf tubes and keep on ice.

    Tip: Each tissue sample should be assayed in triplicate. Be sure to include a triplicate blank sample containing 30 μL of STE buffer *in lieu* of tissue homogenate.

17. Immediately add 370 μL of the oxidation reaction mixture.

18. Incubate at 37 °C for 30 min.

19. While the reaction is incubating, prepare acidification vials with a $^{14}CO_2$ trap. First, cut out small round discs of Whatman filter paper that are slightly larger than the inside of a cap of an Eppendorf tube. Use a cap to make an imprint on the filter paper, which acts as a cutting guide. Stuff the filter paper into the inside of the cap of a fresh Eppendorf tube, ensuring that it does not easily fall out.

20. Add 200 μL of 1 *M* perchloric acid into the tube.

    Tip: Perform the acid and base steps in a fume hood.

21. To the same tube containing acid, add 20 μL of 1 *M* NaOH or other strong base to the paper disc in the cap.

    Tip: Make sure that the paper disc will not fall out when the cap is closed. Use the large bore of a pipette tip to ensure the filter paper is firmly placed. The caps should be open when the strong base is applied to the filter paper resting in the tube top (Fig. 20.2).

22. When the reaction is done incubating, transfer the entire reaction mixture into the tubes containing perchloric acid and the paper disc.

23. Quickly close the cap. Work quickly but gently to prevent the paper disc from falling out.

24. Incubate for 1 h at room temperature.

    Tip: Gently shaking on an orbital shaker can increase the signal, but also risks the paper disc from dislodging from the cap.

    Tip: Incubating for more than 1 h does not result in greater signal and often causes the paper disc to fall into the acid solution. If this happens, the sample is not salvageable.

25. Carefully open the reaction vial in a fume hood and transfer the paper disc to a scintillation vial.

    Tip: A pipette tip or small disposable tweezers can be used to transfer the paper disc to the scintillation vial.

26. Centrifuge the remaining acid solution at maximum speed (≥ 14,000 × *g*) for 10 min at 4 °C.

27. Transfer 400 μL of the supernatant to a scintillation vial.

28. Additionally, add 3 × 370 μL of nonincubated reaction mixture to three scintillation vials to get a triplicate measurement of the amount of radioactivity input into each reaction.

    Tip: This will be used to calculate the specific activity, which will allow for converting counts per minute into moles of palmitate.

29. Add 4 mL of scintillation fluid to all scintillation vials and measure the average counts per minute over 3 min with a standard scintillation counter.

5.2. FAO in cell lines

1. Plate cells in 24-well plates and grow until confluent.

   Tip: This step needs to be optimized depending on the cell type. Growing cells have very different metabolic needs than confluent cells. For example, MEFs become senescent when they reach confluency and therefore should be used at a stage immediately prior to confluence (e.g., approximately 85%; equal to 50,000 cells/well plated 48 h before the assay).

2. Add the BSA/palmitate mixture prepared in Section 4.3 to the required amount of prewarmed DMEM to obtain DMEM containing 0.3% BSA/100 μ*M* palmitate/0.4 μCi/mL ^{14}C-palmitate.

3. Add 1 m*M* carnitine to the media.

   Tip: Depending on the radiolabeled substrate used and the cell type, serum-free media may help to increase the signal in the assay.

4. Rinse cells with PBS two times to remove residual media.

5. Add 500 μL of media containing radiolabeled palmitate to each well.

6. Seal the plate with parafilm and incubate at 37 °C for 3 h.

   Tip: Because the plate is sealed with the addition of HEPES, incubation can be done in either a CO_2 or standard incubator.

7. During the incubation, set up the acidification vials and $^{14}CO_2$ trap as described in Steps 19–21 of Section 5.1.

8. At the end of the incubation, add 400 μL of the media into the acidification vial to stop the reaction and quickly close the tube.

   Tip: Cells could be scraped from the plate and placed in acidification media to determine the amount of acid-soluble metabolites remaining in the cells. Alternatively, cells can be used for protein determination or other metabolite measurements.

9. Measure the radioactivity in the captured $^{14}CO_2$ and acid-soluble metabolites as outlined in Steps 23–29 of Section 5.1.

10. In order to normalize to the amount of protein in each well, collect the cells and perform a BCA assay.

    Tip: Make sure to take special care when measuring protein concentration in cells containing radiolabeled palmitate. Alternatively, a separate replicate plate of cells that does not get ^{14}C-palmitate added could be used to estimate protein concentrations per well.

5.3. Alternative method of $^{14}CO_2$ capture

If the above carbon capture method is not successful, this alternative method of $^{14}CO_2$ capture has also been used with success.

1. Cut ∼5 mm off of the bottom tip of a 200-μL pipette tip.
2. Cut the lid off of a 0.25-mL microdialysis tube (Fisher, 02-681-230) and press firmly into the top of the 200 μL tip.
3. Add 200 μL of 1 *M* NaOH to the microdialysis tube.
4. At the end of the reaction incubation, transfer 400 μL of either the tissue reaction mixture or the cell culture media into a 5-mL disposable culture tube (Simport, T400-3).
5. Gently place the tip/tube assembly into the culture tube and cover with a rubber septum (Sigma, Z124567).
6. Using a 23-gauge 1.5-in. needle and syringe, inject 100 μL of 1 *M* perchloric acid through the rubber gasket into the reaction mixture while avoiding introducing acid into the NaOH in the trap tube.
7. Place the culture tubes upright in a rack and shake on an orbital shaker for 1 h.
8. Transfer the 200 μL of NaOH containing trapped $^{14}CO_2$ to a scintillation vial.
9. Add 4 mL of scintillation fluid to the scintillation vials and measure the average counts per minute over 3 min with a scintillation counter.
10. Transfer the reaction mixture containing acid-soluble metabolites to an Eppendorf tube and allow to settle at 4 °C overnight. Centrifuge at 10,000 × *g* for 10 min at room temperature.
11. Transfer 150 μL of the reaction mixture to a scintillation vial.
12. Add 4 mL of scintillation fluid to the scintillation vials and measure the average counts per minute over 3 min with a scintillation counter.

    Tip: To further increase the signal obtained from either animal tissues or cell culture, perform the assay on isolated mitochondria instead of whole tissue lysates or cultured cells. A detailed method for isolating intact mitochondria has been previously published (Frezza, Cipolat, & Scorrano, 2007).

6. Data Handling and Calculations

Determine the specific activity of the reaction mixture by dividing the disintegrations per minute (DPM) of the input by the number of nanomoles of palmitate (cold + hot) per reaction. The specific activity can be used to convert DPM to nanomoles for the other samples.

$$\text{Specific activity} = \frac{\text{DPM of input}}{\text{total nmol of palmitate}}$$

To determine the rate of conversion of ^{14}C-palmitate to $^{14}CO_2$, divide the nanomoles of $^{14}CO_2$ by the amount of time the reaction was allowed to run before addition of perchloric acid. The rate of conversion can also be normalized to the amount of protein or milligrams of tissue per reaction. A similar calculation can be done to determine the rate of conversion of ^{14}C-palmitate to acid-soluble metabolites.

Acknowledgments

We would like to acknowledge the members of the Hirschey and Muoio Labs for thoughtful feedback. We would also like to acknowledge the funding support to the Hirschey Lab from the American Heart Association grants 12SDG8840004 and 12IRG9010008, The Edward Mallinckrodt, Jr. Foundation, The Ellison Medical Foundation, and the National Institutes of Health grants AA022146 and AG045351. M. F. G. is supported by a Postdoctoral Fellowship from the NIH/NCI (CA059365-19). F. K. H. is supported by an American Diabetes Association/ Canadian Diabetes Association Joint Post-doctoral Fellowship (PF-3-13-4342-FH).

References

Carracedo A, Cantley LC, Pandolfi PP. Cancer metabolism: Fatty acid oxidation in the limelight. Nature Reviews Cancer. 2013; 13:227–232.

DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, et al. Beyond aerobic glycolysis: Transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:19345–19350. [PubMed: 18032601]

Frezza C, Cipolat S, Scorrano L. Organelle isolation: Functional mitochondria from mouse liver, muscle and cultured fibroblasts. Nature Protocols. 2007; 2:287–295.

Frezza C, Gottlieb E. Mitochondria in cancer: Not just innocent bystanders. Seminars in Cancer Biology. 2009; 19:4–11. [PubMed: 19101633]

Ito K, Carracedo A, Weiss D, Arai F, Ala U, Avigan DE, et al. A PMLPPAR-delta pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. Nature Medicine. 2012; 18:1350–1358.

Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. American Journal of Physiology Endocrinology and Metabolism. 2000; 279:E1039–E1044. [PubMed: 11052958]

Lunt SY, Vander Heiden MG. Aerobic glycolysis: Meeting the metabolic requirements of cell proliferation. Annual Review of Cell and Developmental Biology. 2011; 27:441–464.

Mannaerts GP, Debeer LJ, Thomas J, De Schepper PJ. Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. The Journal of Biological Chemistry. 1979; 254:4585–4595. [PubMed: 438207]

McGarry JD, Foster DW. Regulation of hepatic fatty acid oxidation and ketone body production. Annual Review of Biochemistry. 1980; 49:395–420.

Schafer ZT, Grassian AR, Song L, Jiang Z, Gerhart-Hines Z, Irie HY, et al. Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment. Nature. 2009; 461:109–113. [PubMed: 19693011]

Shindo Y, Osumi T, Hashimoto T. Effects of administration of di-(2-ethylhexyl)phthalate on rat liver mitochondria. Biochemical Pharmacology. 1978; 27:2683–2688. [PubMed: 728223]

Van Veldhoven PP, Vanhove G, Vanhoutte F, Dacremont G, Parmentier G, Eyssen HJ, et al. Identification and purification of a peroxisomal branched chain fatty acyl-CoA oxidase. The Journal of Biological Chemistry. 1991; 266:24676–24683. [PubMed: 1761563]

Zaugg K, Yao Y, Reilly PT, Kannan K, Kiarash R, Mason J, et al. Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. Genes & Development. 2011; 25:1041–1051. [PubMed: 21576264]

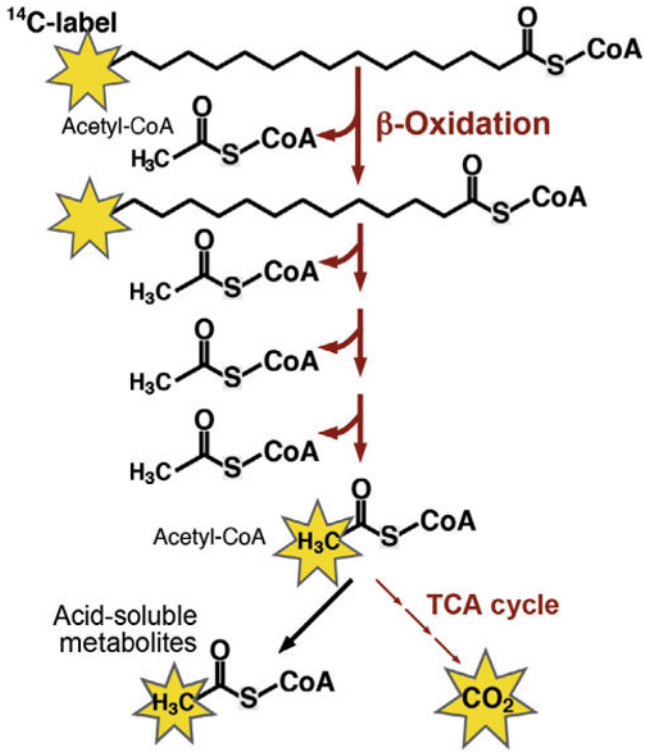

Figure 20.1. Biochemical overview of the assay

^{14}C-palmitate is oxidized via β-oxidation to generate acetyl-CoA, which can then enter the tricarboxylic acid (TCA) cycle or be incorporated into acid-soluble metabolites.

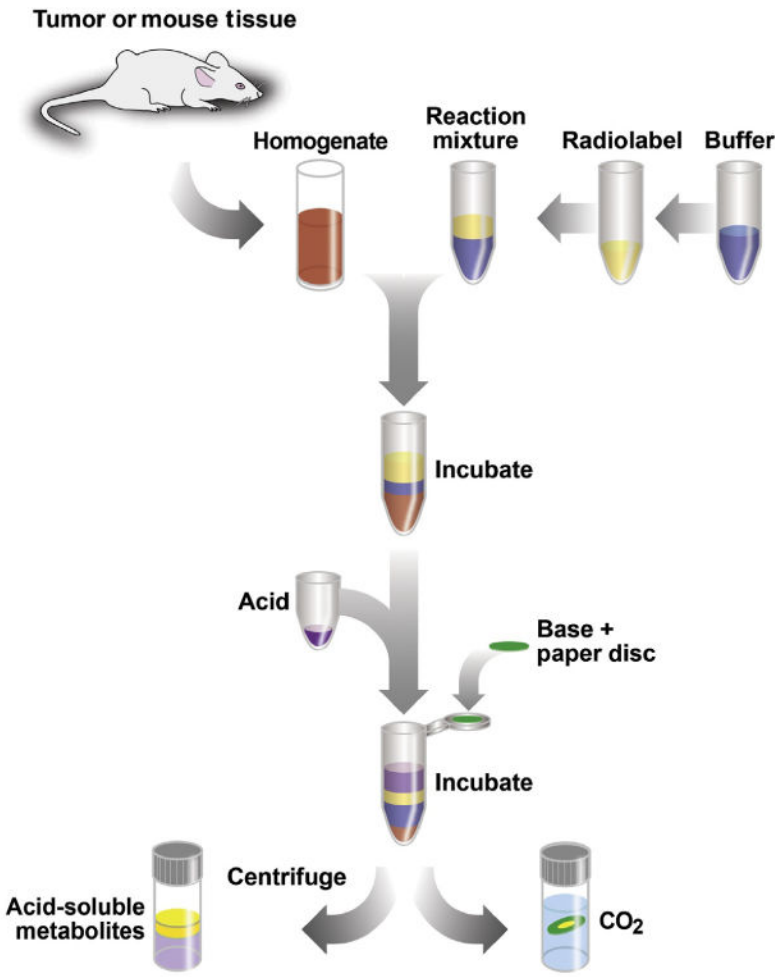

Figure 20.2. Schematic of the protocol for tissue homogenates

The tissue or tumor of interest is homogenized with a Dounce homogenizer to lyse cells, while keeping the mitochondria intact. The tissue homogenate is then incubated with a reaction mixture containing radiolabeled palmitate. A strong acid is added to stop the reaction. Unoxidized bound palmitate will precipitate out of solution upon addition of acid. Acetyl-CoA, acetylcarnitines, ketone bodies, and tricarboxylic acid (TCA) cycle intermediates (acid-soluble metabolites) will remain soluble after the addition of the strong acid. Acetyl-CoA that enters the TCA cycle can be oxidized to CO_2 and this will be captured by the strong base in the Whatman paper disc. The amount of ^{14}C in the acid-soluble metabolite fraction and the paper disc CO_2 fraction can each be measured by scintillation counting.

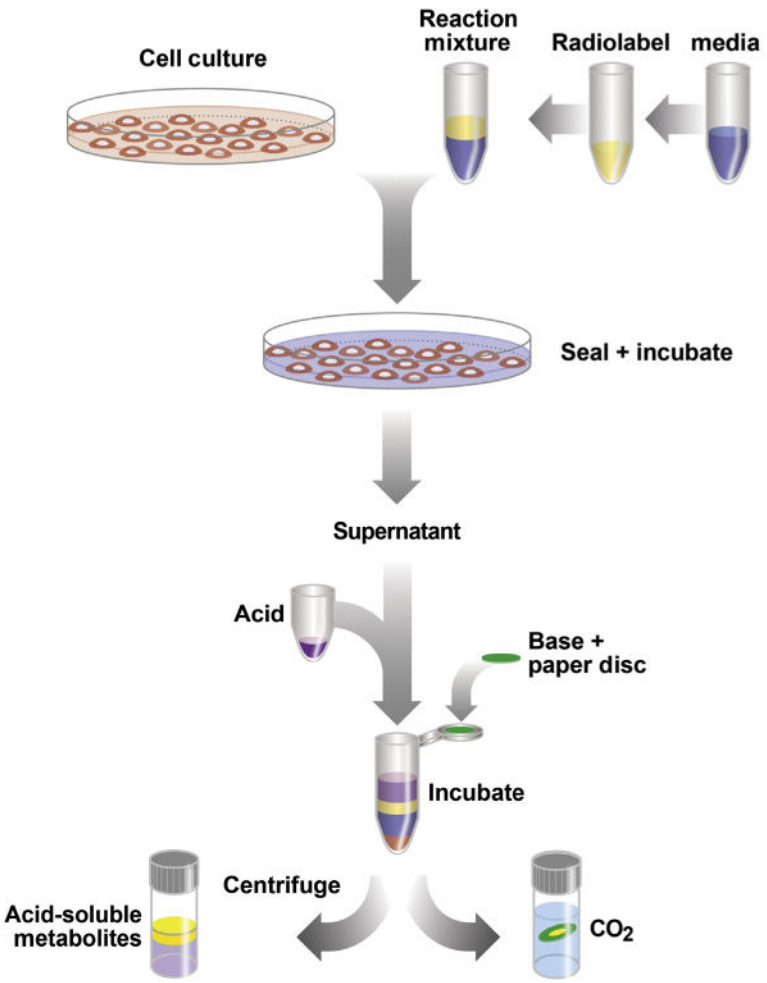

Figure 20.3. Schematic of the protocol for cultured cells

In a similar manner to tissue oxidation, the cells of interest are incubated in media containing ^{14}C-palmitate. The supernatant/cells are then added to a strong acid to stop the reaction and the amount of ^{14}C in the acid-soluble metabolite fraction and the paper disc CO_2 fraction can each be measured by scintillation counting.

Table 20.1
Oxidation reaction mixture for animal tissues

Component	Concentration of stock	Volume of stock per 400 μL reaction (μL)	Final concentration per reaction mixture
Sucrose[a]	1 *M*	40	100 m*M*
Tris–HCl, pH 7.4	1 *M*	4	10 m*M*
KH_2PO_4	1 *M*	2	5 m*M*
EDTA, pH 8.0	50 m*M*	1.6	0.2 m*M*
KCl	1 *M*	32	80 m*M*
$MgCl_2$	1 *M*	0.4	1 m*M*
L-Carnitine	50 m*M*	16	2 m*M*
Malate	20 m*M*	2	0.1 m*M*
Coenzyme A	10 m*M*	2	0.05 m*M*
ddH_2O	–	221.6	–
pH to 8.0			
ATP[b]	100 m*M*	8	2 m*M*
DTT	1 *M*	0.4	1 m*M*
BSA:palmitate solution	7% BSA/5 m*M* palmitate/0.01 μCi/μL ^{14}C-palmitate	40	0.7% BSA/500 μ*M* palmitate/0.4 μCi ^{14}C-palmitate
Tissue homogenate	–	30	30 μL/rxn

[a] Components highlighted in dark grey (blue in online) can be combined ahead of time and stored at 4 °C for up to 6 months. Heat the solution to 37 °C before adding the components highlighted in light grey (green in online).

[b] Components highlighted in light grey (green in online) should be added fresh. After the addition of these reagents, incubate the reaction mixture at 37 °C for 1–2 h to ensure complete solubilization of the palmitate.