What is the proper precursor-to-product labeling relationship for calculating the fractional synthetic rate of muscle triglyceride?  

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In this issue of the Journal of Lipid Research, Zhang et al. (1) report an investigation of the fractional synthetic rate of triglycerides in the muscle of anesthetized rabbits infused with $^{13}$C$_{16}$]palmitate tracer. The study was designed to solve a question that has been debated for a while among investigators in the field. The question is: “what is the proper precursor-to-product relationship used to calculate the fractional synthetic rate of triglyceride-bound palmitate from its isotopic enrichment?” Let us go over the parameters and the constraints of the problem illustrated in Fig. 1. In this figure, the numbers in red are the isotopic enrichments measured in muscle biopsies by Zhang et al. after 180 min of infusion of labeled palmitate [see Fig. 1 and Table 1 of (1)].

When a labeled fatty acid such as $^{13}$C$_{16}$]palmitate is infused intravenously, the enrichment of plasma free palmitate stabilizes within minutes. This is because of the small sizes and the short half-lives of the plasma pools of nonesterified fatty acids. In spite of the rapid stabilization of the enrichment of plasma palmitate, the enrichment of intramuscular palmitate is lower than that of plasma palmitate. This results from the turnover of intramuscular triglycerides. Unlabeled palmitate, released from triglycerides, dilutes the enrichment of the $^{13}$C$_{16}$]palmitate tracer entering the cell. This dilution is fairly constant because of the low fractional synthetic rate of muscle triglycerides [0.15–0.2%/hr; see Table 1 of (1)]. However, as the duration of the experiment increases, plasma triglycerides become increasingly labeled with a fairly long half-life of labeling [see Fig. 1 of (1)]. The hydrolysis of plasma triglycerides by lipoprotein lipase provides a second source of tracer $^{13}$C$_{16}$]palmitate to the muscle cell. Therefore, one should not expect the enrichment of intramuscular palmitate to stabilize rapidly. The progressive labeling of plasma triglycerides explains the acceleration with time of the labeling of intramuscular triglyceride-bound palmitate, presumably because of the progressive increase in enrichment of intramuscular palmitate [see Fig. 3 of (1)].

When tracing triglyceride synthesis with $^{13}$C$_{16}$]palmitate, the immediate precursor of triglyceride-bound palmitate is extra-mitochondrial palmitoyl-CoA. Because extra-mitochondrial palmitoyl-CoA cannot be isolated from frozen muscle, its labeling can be inferred from that of palmitoyl-carnitine, as previously hypothesized by the authors (2). They now present time profiles of enrichment of plasma palmitate and triglyceride-bound palmitate, as well as of intramuscular palmitate, palmitoyl-CoA and palmitoyl-carnitine. Two types of muscle sampling were conducted: i) freeze-cut, where the muscle is freeze-clamped in situ before being cut out around the clamp, and ii) cut-freeze, where a piece of muscle is cut, rapidly rinsed, and immersed in liquid nitrogen. In both types of biopsies, the enrichment of intramuscular palmitate was less than one-third that of palmitoyl-carnitine. The enrichment of palmitoyl-CoA was 10 and 20% lower than that of palmitoyl-carnitine in freeze-cut and cut-freeze biopsies, respectively. As a result, fractional synthetic rates of intramuscular triglycerides were 3.5 times greater when calculated using the enrichment of intramuscular palmitate compared with using the enrichment of palmitoyl-carnitine. It is thus clear that the fractional synthetic rate of intramuscular triglyceride-bound palmitate should not be calculated using the enrichment of intramuscular palmitate.

The authors ascribe the low enrichment of intramuscular nonesterified palmitate to the large intramuscular concentration ratio (triglyceride-bound palmitate)/(nonesterified palmitate) estimated at about 1,000. I submit that a second cause of isotopic dilution of intramuscular nonesterified palmitate (isolated from an extract by thin-layer chromatography) is the ubiquitous contamination of the cleanest glassware, chromatography solvents, and methanol used for derivatization by traces of palmitate. The artifactual dilution of the isotopic enrichment of very small samples of palmitate in a biological matrix is unavoidable with current techniques. This artifact did not occur when the authors...
analyzed the enrichment of the large pool of triglyceride-bound palmitate in the muscle biopsies.

Note that stearate, but not oleate, is also a ubiquitous contaminant. This is why we have infused \( [^{13}\text{C}_{18}] \) oleate to trace lipolysis in rats (3). In another study (4), we tried to synthesize highly-labeled palmitate in vitro using \( [^{13}\text{C}_2] \) acetyl-CoA + \( [^{13}\text{C}_3] \) malonyl-CoA + fatty acid synthase purified from rat liver (5). In spite of all precautions, the amount of contaminating unlabeled palmitate in the product was much greater than the amount of \( [^{13}\text{C}_{16}] \) palmitate synthesized. In contrast, when we synthesized the odd-chain \( [^{13}\text{C}_{15}] \) pentadecanoate from \( [^{13}\text{C}_3] \) propionyl-CoA primer + \( [^{13}\text{C}_2] \) acetyl-CoA, the blank of unlabeled pentadecanoate was minuscule. In conclusion, because contamination of analytical procedures with traces of unlabeled palmitate is unavoidable, triglyceride synthesis may be more conveniently traced using \( [^{13}\text{C}_{18}] \) oleate.

Why was the enrichment of palmitoyl-carnitine slightly higher (10–20%) than that of palmitoyl-CoA in the authors’ study with \( [^{13}\text{C}_{16}] \) palmitate? Theoretically, the enrichment of mitochondrial palmitoyl-CoA, derived from infused \( [^{13}\text{C}_{16}] \) palmitate, could be diluted by unlabeled palmitoyl-CoA, an intermediate of the \( \beta \)-oxidation of endogenous unlabeled fatty acids with 18 or more carbons (Fig. 1). One would expect such dilution to be small because intermediates of \( \beta \)-oxidation do not accumulate in the metabolic tunnel (6) of the \( \beta \)-oxidation enzymes (7). If this mechanism is correct, the enrichment of palmitoyl-CoA extracted from the whole tissue would be slightly lower than the enrichment of cytosolic palmitoyl-CoA, a proxy of which is the enrichment of palmitoyl-carnitine. This argues for using the enrichment of palmitoyl-carnitine to calculate the fractional synthetic rate of triglyceride-bound palmitate labeled from \( [^{13}\text{C}_{16}] \) palmitate. However, computations of fractional synthetic rates from the enrichment of palmitoyl-CoA or from palmitoyl-carnitine yield similar data.

Zhang et al.’s study provides also a comparison of data from muscle biopsies obtained by freeze-cut and cut-freeze. The main differences are in the intramuscular concentrations of palmitate and total fatty acids which, in the cut-freeze, are about 1.5 those in the freeze-cut. This reflects the triggering of intramuscular triglyceride hydrolysis during the few seconds when the biopsy of the cut-freeze was anoxic, but not yet frozen.

This elegant study from Dr. Robert R. Wolfe’s group settles points of disagreement that have been hotly debated. First, neither the enrichment of plasma palmitate nor that of intramuscular palmitate should be used to calculate the fractional synthetic rate of triglyceride-bound palmitate. Second, the freeze-cut biopsy is preferable to the cut-freeze. Third, the labeling of tissue palmitoyl-CoA and palmitoyl-carnitine yield similar fractional synthetic rates of triglyceride-bound palmitate. Fourth, because the measurement of the labeling of tissue palmitoyl-CoA is somewhat difficult, the labeling of palmitoyl-carnitine is an acceptable surrogate of that of palmitoyl-CoA.
Finally, I recommend using labeled oleate instead of labeled palmitate or stearate to trace triglyceride synthesis in muscle.

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