The following is the abstract of the article discussed in the subsequent letter:

Watt, Matthew, George J. F. Heigenhauser, and Lawrence L. Sprriet. Intramuscular triacylglycerol utilization in human skeletal muscle during exercise: is there a controversy? J Appl Physiol 93: 1185–1195, 2002; 10.1152/japplphysiol.00197.2002.—Intramuscular triacylglycerols (IMTGs) represent a potentially important energy source for contracting human skeletal muscle. Although the majority of evidence from isotope tracer and 1H-magnetic resonance spectroscopy (MRS) studies demonstrate IMTG utilization during exercise, controversy regarding the importance of IMTG as a metabolic substrate persists. The controversy stems from studies that measure IMTG in skeletal muscle biopsy samples and report no significant net IMTG degradation during prolonged moderate-intensity (55–70% maximal O2 consumption) exercise lasting 90–120 min. Although postexercise decrements in IMTG levels are often reported from direct muscle measurements, the marked between-biopsy variability (~23%) that has been reported with this technique in untrained subjects is larger than the expected decrease in IMTG content, effectively precluding significant findings. In contrast, recent data obtained in endurance-trained subjects demonstrated reduced variability between duplicate biopsies (~12%), and significant changes in IMTG were detected after 120 min of moderate-intensity exercise. Therefore, it is our contention that the muscle biopsy, isotope tracer, and 1H-MRS techniques report significant and energetically important oxidation of free fatty acids derived from IMTGs during prolonged moderate exercise.

Muscle fat utilization during exercise: controversial only methodologically

To the Editor: Watt and colleagues (8) recently reviewed the issue of intramyocellular triacylglycerol (IMTG) utilization during exercise. The authors concluded that the simple method of directly measuring IMTG content changes in muscle samples obtained before and after prolonged, intermediate-intensity exercise produces results that are too variable (~23%) to be useful for detecting small to modest utilization (reduction) of IMTG. They recommended that this direct method be used only in endurance-trained subjects and not in other populations such as untrained or obese individuals. The authors also praised the muscle fiber-dissecting method for IMTG analysis as more effective and reliable. Their extensive review will undoubtedly benefit research in this area. However, two points may be worth further discussion.

First, the many reported negative results on IMTG utilization during prolonged exercise, as reviewed, stem from the fact that direct measurement of IMTG changes is not a valid method of determining IMTG utilization simply because of the existence of simultaneous esterification of IMTG (5). Second, the well-known high variability of IMTG measurements, attributable either to extracellular lipid contamination (4) (only a few groups use detailed microdissection techniques to remove fat cells) or to inherent muscle heterogeneity (2), further points to the invalidity of the direct method. Therefore, even in endurance-trained individuals, in whom simultaneous esterification is not expected to decrease even though extracellular lipid contamination may be less of a concern, the results obtained with the direct method are most likely confounded and, thus, questionable. The simple direct method was originated decades ago when better tracer methods were not available and some key IMTG metabolic pathways, such as simultaneous esterification, were not recognized. The method has been repeatedly negated by many positive results obtained with more sophisticated techniques (3, 7, 8). For these reasons, this simple “weighing” method should be discontinued. The review seems to have implied this, but it would be scientifically beneficial to recommend it explicitly and advocate for it. Tracer techniques, especially when combined with breath CO2 analysis and muscle biopsy, and nuclear magnetic resonance should be the primary techniques to study IMTG utilization, although quantitation reliability appears to be a challenge of the latter technique.

Regarding muscle specimen lipid decontamination, the two studies that used the perceptually more effective method of dissecting freeze-dried muscle samples into individual fibers to remove lipid contaminants both reported paradoxically higher IMTG than other methods (1, 6). Therefore, contrary to the perception, this technique does not appear to be a better or even a valid method for removing lipid contaminants, although the exact reason is not apparent. Until the reason is clarified, this time-consuming method should not be used in IMTG studies.

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REPLY

To the Editor: In the letter by Guo, concerns were raised regarding the legitimacy and usefulness of the muscle biopsy and chemical extraction technique for the measurement of IMTG content. In our initial review (3), our intention was to discuss whether net exercise-induced changes in IMTG were physiologically real and not a function of methodological limitations. We have previously acknowledged that the muscle biopsy and chemical extraction technique measures net changes in IMTG following an intervention (e.g., exercise) and, thus, questionable. The simple direct method should be discontinued. The review seems to have implied this, but it would be scientifically beneficial to recommend it explicitly and advocate for it. Tracer techniques, especially when combined with breath CO2 analysis and muscle biopsy, and nuclear magnetic resonance should be the primary techniques to study IMTG utilization, although quantitation reliability appears to be a challenge of the latter technique.

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extramyocellular contamination must be low because biopsy and chemical extraction methods produce results that are quantitatively similar to results given in studies that used the 1H-magnetic resonance spectroscopy technique, not withstanding the concern of translating these signals into contents. In addition, Dr. Guo’s own results (1) suggest that reesterification during exercise is not a major concern, as it amounted to only \( \sim 7\% \) of the lipolytic rate. Although we generally agree with many of the assertions of Dr. Guo, we also raise some concerns with his recommendations.

It is well known that the pulse-chase technique can be utilized for the simultaneous measurement of IMTG hydrolysis and esterification in vitro (4) and perhaps in vivo (1); however, there are numerous limitations inherent to this method. These include incorporation of radiolabeled substrate into pools other than triacylglycerol, equilibrium of the pulse label into the IMTG pool, equilibrium of CO2 into the bicarbonate pool, and the prelabeling of only a small fraction of the IMTG pool that may not represent homogenous labeling across the entire pool. Although Dr. Guo recommends the simultaneous use of breath CO2 measures and muscle biopsy, it is clear that these methods do not always match, both quantitatively and qualitatively, and may further confuse this issue (1). Clearly, all techniques have inherent limitations that result in variability of the final measurement, and investigators should carefully consider these when designing experiments.

It was also stated that the variability of the IMTG measure was \( \sim 23\% \) (5). This often-quoted figure was derived from repeated biopsies in untrained men and women. We have since demonstrated reduced variability (12%) in endurance-trained men (3) and recreationally active men (15%; Watt, unpublished observations) and believe that careful dissection can provide investigators with reliable and reproducible measures of IMTG in these populations.

It seems from the careful work conducted by Dr. Guo and colleagues that the use of a stereomicroscope may aid in the dissection of extramyocellular lipid from aging and/or obese rat muscle (2). In practical terms, however, skeletal muscle often needs to be freeze-dried to prevent the activation of enzymes and changes in metabolite contents that are “trapped” when a muscle sample is snap-frozen. Thus we maintain that the muscle biopsy and chemical extraction technique, when performed with careful dissection, is a practical, valid, and reliable measure of IMTG content.

REFERENCES

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