Reliability of results and interpretation of measures of 3-methylhistidine in muscle interstitium as marker of muscle proteolysis

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To the Editor: We were interested in the contribution by Tesch and colleagues to the evidence base concerning the events subsequent to acute immobilization in human muscle. Tesch and colleagues (14) used a microdialysis technique applied to human muscle in an attempt to measure an index of myofibrillar proteolysis 72 h after immobilization using the unilateral leg suspension technique. The authors discuss their results as though they had good evidence that the technique they used produces results that are sufficiently firm to be the basis of conjecture about mechanisms of protein balance in muscle after immobilization.

We demur and propose that they are on very shaky ground. First, we wonder about the size of the effect reported. The authors claim it to be 44%, but calculation of the change shows it to be actually 29% with the SEs sufficiently large that they almost span the difference; SDs must overlap. Significance at the 5% level can only barely have been reached. Are the authors convinced that they have not ignored a type I error?

The authors (14) begin their explanation of the advantages of the dialysis technique using 3-methylhistidine (3MeHis) by describing the gold standard method, i.e., the stable isotope-labeled amino acid tracer dilution method, as cumbersome and possibly unjustified in study of otherwise healthy subjects. We find this notion strange since Ferrando and colleagues (6, 12) have used the technique in many studies of how immobilization affects muscle protein turnover subsequent to disuse. We have also used the technique in a wide variety of studies of proteolysis in healthy and infirm subjects (1, 2, 10, 11, 15) with no problems and, we would propose, producing results with a greater degree of certainty and biological appropriateness than those obtained by the 3MeHis dialysis technique.

The authors (14) cite studies validating their technique from their own previous work. Thus “In addition, the measurements obtained with microdialysis have been confirmed in parallel experiments using the arterial-venous balance technique (16). With the aid of microdialysis, altered skeletal myofibrillar proteolysis has been noted with aging (16) and following strenuous artificial (i.e., electrically stimulated) contractile activity (8)”.

In fact, the first study they quote did not validate the 3MeHis by use of the arterial-venous balance technique because only blood concentrations but not blood flow were used and thus no dynamic net balances were presented; furthermore it is the only paper we know of that claims to find a higher rate of muscle protein breakdown in elderly than young persons. Many publications have now demonstrated that muscle proteolysis in healthy older people is not elevated and that muscle synthesis is not depressed (e.g., Refs. 5, 7, 17). Accepting these data, we have to contend that if muscle proteolysis were elevated, given the anabolic resistance of old people to feeding (5, 7), their muscles would palpably vanish before our eyes. They clearly do not.

The study the authors then quote, on electrical stimulation, cannot, in fact, be used to justify the technique because it contains no objective measure of muscle proteolysis at all (but simply extrapolates from the dialysis finding), and there is no nonexercised control data. Also it (like the study quoted previously) contains the finding that according to the 3MeHis dialysis technique muscle proteolysis is not significantly increased after strenuous voluntary exercise despite the fact that the tracer dilution method (3) does indeed show just this!

Let us try to explain why the authors (and others possibly wishing to use the method) need to be careful. First, the authors, we propose, have not used a suitable marker for 3MeHis recovery. The amino acid in question, 3MeHis, is basic and thus positively charged at physiological pH, whereas glucose, the molecule used by the authors to assess recovery of the amino acid, is an electrically neutral molecule. Compared with 3MeHis, which probably uses the very rapid ion- and hormone-independent system L for transport (9), glucose has completely different muscle transport systems (GLUTs 1 and 4 predominantly, the latter being insulin sensitive and probably affected by the insulin resistance of immobilized muscle). A suitable recovery marker would be stable isotope-labeled 3MeHis (which is available) or a nonproteic amino acid (such as ε-aminoproprionic acid), which would not interfere with the chromatographic analysis of 3MeHis and yet is similarly charged and probably uses the same transporter.

Second, although in the paper initially describing the technique (16) the authors made use of the “ethanol in/out” ratio as an index of local blood flow around the dialysis probe, no such measures appear in their present publication; indeed there is no direct measure of local or bulk leg blood flow at all. Even if the values of 3MeHis in interstitial fluid are correct, as leg blood flow after immobilization is likely to be depressed (4), the observations of elevated 3MeHis may be an artefact of its decreased washout from the leg.

The authors are to be congratulated on attempting to bring new tools to the workbench, but they need to be sure that they are reliable and that the answers they provide make biological sense and are in accord with the large amount of data present within the literature. Whether there is elevation of muscle protein breakdown early after immobilization is unproven [see our letter on this topic elsewhere (13)] and is too important a question for the answer to be regarded as unreliable.

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