Application of deuterium oxide (D$_2$O) to metabolic research: just D$_2$O it? Depends just how you D$_2$O it!

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Letter to the Editor: the recent article by Lambert et al. (4) reports the effects of aquatic vs. land-based aerobic treadmill exercise combined with resistance exercise in the context of muscle anabolism. These authors quantified muscle protein synthesis (MPS) over 24 h using a method that has garnered a great deal of interest over the past few years, i.e., deuterium oxide (D$_2$O), or “heavy water.” We are ardent advocates of the application of stable isotope tracers to the study of physiology and metabolism; nonetheless, we feel it is imperative that such specialist techniques are scrutinized carefully to ensure that they have been applied and analyzed with rigor. If not, then erroneous conclusions could and no doubt have been drawn. In Fig. 3 of the article by Lambert et al. (4), rates of MPS (myofibrillar) were reported as ~8–15%/day. This is approximately five to 10 times greater than what is normally observed in healthy human skeletal muscles when conventional amino acid tracers are employed (0.07–0.15%/h; 1.6–3%/day) (3, 6). Moreover, utilizing the same D$_2$O tracer techniques (albeit more sensitive, accurate mass spectrometric techniques, i.e., gas chromatography-pyrolysis-isotope ratio mass spectrometry), we recently demonstrated rates of MPS, even after resistance exercise in the fed state, of ~2%/day (7). Lambert et al. (4) provide limited details of their analytical methods but reference previously coauthored papers detailing past work using this approach. In one such paper (1), they describe similarly high MPS (~0.7–0.9%/h) again eight to 10 times that typically observed; this time, however, they acknowledge the discrepancy in the discussion without providing a convincing explanation (1). Moreover, earlier work by the same authors reports MPS of ~0.2–0.9%/h in 6- to 8-mo-old (i.e., growing) exercising rats (2). Although these MPS rates are more consistent with those reported for rat muscles (5), how do these authors explain humans and rats having similar rates of MPS? Based on the information provided in Lambert et al. (4) and previous (1, 2) papers, we believe that any analytical related shortfalls leading to such incongruent rates of MPS should be reconciled. For example, Gasier et al. (1) reported that, due to low levels of enrichment (<0.5% molar percent excess), they enhanced detection of this low enrichment (e.g., using gas chromatography-mass spectrometry, which has a typical accurately quantifiable limit of detection of ~0.1%) by integrating the first 20% of the chromatographic peak. Since deuterium-labeled compounds elute faster in chromatographic runs (as is the case with all isotopically enriched compounds), integration of only the first ~20% of the chromatographic peak will significantly overestimate the true enrichment. Given the similarity of the approaches, have Lambert et al. (4) perhaps employed similarly imprecise integration methods in their paper? Finally, it should always be remembered that rates of MPS are absolute and should never be considered as arbitrary values; MPS in humans, over several hours to day(s), has been reported for many years and consistently within a defined range. Methods should rightly be critiqued when apples are made out to be equal to pears. The reported data (4) are far outside of normal ranges; it may be conjectured that this could translate into relative changes, thus questioning the veracity of the scientific findings.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


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