Measuring muscle protein turnover in vivo: what can 3-methylhistidine production tell us?

Changes in the amount of protein in a cell, tissue or organism are brought about by differences in the rates of protein synthesis and/or protein breakdown [1]. Hence understanding how protein abundance is controlled requires the ability to make reliable, quantitative measurements of both these two processes in vivo. Protein synthesis rates can be determined by measuring the rate of incorporation of a labelled amino acid into tissue protein, so long as the isotopic enrichment of the labelled amino acid in the precursor pool for protein synthesis (i.e., the aminoacyl tRNA) is known [1,2]. Several methods have been developed to measure the rate of protein synthesis at the level of the organ or tissue (for a fuller discussion, see [3,4]). For small laboratory animals, the most widely adopted approach is the so-called ‘flooding dose’ method, which has been applied to a wide range of tissues in numerous species, including man [5]. This method has yielded extremely valuable information about the regulation of tissue protein, but has not yet been routinely applied to measuring the synthesis rates of individual proteins other than in a handful of special cases such as albumin [6].

In contrast, measurement of the rate of protein breakdown in vivo has proved much more problematical. This is largely because a proportion of the amino acids released by protein breakdown is immediately subject to re-incorporation into protein. Hence any method based on measuring the release of amino acids or the decline in labelling of a labelled protein has conceptual and practical limitations [1]. In addition, the release of an amino acid represents only the final stage of a multifaceted pathway. In the case of muscle, for example, this involves the cleavage and disassembly of the complex myofibrillar structure [7]. As a consequence, there was considerable interest when it was proposed that the measurement of urinary excretion of 3-methylhistidine (3MH) could be used to assess the rate of breakdown of skeletal muscle protein [8]. 3MH is formed by post-translational modification of certain histidine residues in all forms of actin and the myosin heavy chains found in type-1 muscle fibres. When these proteins are broken down, the 3MH is not re-utilized for protein synthesis, so the net rate at which 3MH is produced is proportional to the rate at which these proteins are broken down. The method was taken up enthusiastically by Vernon Young, Hamish Munro and colleagues who published an extensive series of studies validating various aspects of the method (summarized in [9]).

Although 3MH-containing proteins are present in most cells in the body, their concentration is greatest in muscle. Skeletal muscle is the most abundant tissue in the body, and it has been shown to contain at least 75% of the protein-bound 3MH in young rats [10]. Much of the rest appears to be in tissues that contain significant amounts of smooth muscle, such as gut, skin and lung. However, the rate of protein breakdown in many of these tissues is considerably higher than that in skeletal muscle, so that skeletal muscle may contribute no more than 50% of the body’s 3MH production [11,12]. Moreover, there are circumstances where 3MH production from skeletal muscle appears to decrease at the same time as the urinary excretion of 3MH increases [13]. These doubts about the interpretation of 3MH excretion as an index of the rate of skeletal muscle breakdown have prevented more widespread application of the method during the last 20 years.

Some authors have side-stepped these doubts by interpreting 3MH excretion as an index of myofibrillar protein breakdown, or more specifically the breakdown of actin and myosin. Unfortunately, such an interpretation means little in physiological terms, since it does not relate to any specific organ or tissue. For example, when rats are fasted, the rate of protein breakdown in the smooth muscle of the gut rises rapidly, whereas skeletal muscle protein breakdown rate remains low and only rises after the second day of fasting [14]. Urinary excretion of 3MH shows that myofibrillar protein breakdown rate is elevated throughout this period, but gives no information on the tissue in which this is taking place. The situation is analogous to measuring whole-body protein synthesis, which gives no information on changes at the tissue level. Thus, for example, whole-body protein synthesis rate in cancer patients was shown to be not significantly different from that in healthy controls. However, muscle protein synthesis was decreased by 80%, but offset by an increase in protein synthesis in non-muscle tissues [15].

The paper by Vissers et al. [16] in this issue of Clinical Science describes a refinement of the 3MH technique for use in mice. It is known that 3MH excretion cannot be measured satisfactorily in mice, because some of the 3MH is excreted as conjugated metabolites (although these can be released by hydrolysis) and some is temporarily sequestered in unidentified pools. Hence Vissers et al. [16] have developed a method for measuring 3MH production directly. This has the
additional advantage of being sensitive to changes over a shorter timescale than the 24 h over which urine collections are conventionally made. On the other hand, the method is invasive and technically demanding and it is known that both anaesthesia and surgery can perturb tissue protein turnover [17,18]. Nevertheless, the method provides the opportunity to make measurements on protein breakdown in a species which is becoming central to our understanding of molecular biology, because of the relative ease with which transgenic strains and other molecular manipulations can be produced.

It will be interesting to see what use can be made of this technique [16]. Recent advances in proteomics are showing that huge numbers of different proteins exist in different tissues [19], so that data on the average rate of protein turnover even at the level of the whole tissue are likely to mask important differences between individual proteins. It will certainly be technically challenging to develop methods for routinely measuring the turnover rates of individual proteins in vivo, but ultimately that is the level at which information will be needed if we are really going to understand how protein abundance is controlled, especially in the year of DNA50.

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