Measurement of muscle proteolysis and the impact on muscle wasting

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In general terms, muscle mass begins to decrease after the age of 30 years and continues to decrease at varying rates throughout the rest of life (see Fig. 1; Forbes & Reina, 1970). As human subjects approach 75–80 years of age they may have lost as much as 40 % of the peak muscle mass which places them very near the minimum point of critical muscle mass necessary to maintain both ambulatory and respiratory functions (Windsor & Hill, 1988). The impact of any process that accelerates this gradual loss of muscle will, therefore, depend on the current muscle mass relative to the minimal critical muscle mass. Thus, the impact of a 2–3 kg loss of muscle mass, which can occur during a 14 d period after trauma (Hill, 1992), is much more serious in a 75-year-old subject than in a young subject. The elderly subject can easily fall into the danger range, while the younger subject has a great deal of reserve capacity.

The decision to therapeutically intervene to slow or prevent muscle loss or wasting is very much dependent on first knowing what muscle reserves are present and second the rate at which muscle loss is occurring. Knowing if the muscle loss is due to either an accelerated protein breakdown or an impairment of protein synthesis are both critical in the design of a rational approach to therapeutic intervention.

At present there are several methodological gaps and uncertainties in all aspects of muscle metabolism, including difficulties in measuring muscle mass and poor methods for the measurement of protein synthesis and proteolysis *in vivo*. In addition, the current methods are generally only applicable in research settings designed to monitor the effects of metabolism and/or endocrine interventions. The present paper will not review in detail all the issues in the regulation of muscle mass but will concentrate mainly on protein breakdown in muscle.
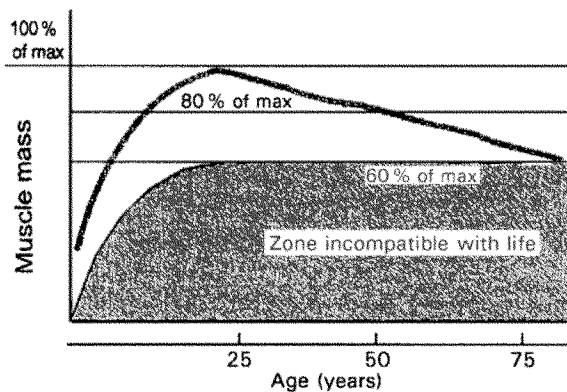

Fig. 1. Theoretical representation of the changes in muscle mass with age. (▩), 'Zone incompatible with life', which is a zone in which the risks of muscle failure for both locomotion and respiration are greatest. This increase in risk is thought to occur at about a loss of 40 % of peak muscle mass, although definitive studies have not been carried out because of the difficulty in evaluating muscle mass *in vivo*. max, Maximum muscle mass, usually reached at about 30 years of age.

MUSCLE WASTING AND PROTEIN BREAKDOWN

To effectively study the problems of muscle wasting, it is important to be able to measure over time changes in muscle mass, proteolysis and protein synthesis. By knowing how fast muscle is being lost and why it is being lost, effective therapies can be implemented to maintain muscle mass and maximize muscle function, and, hence, minimize the health risks associated with depleted muscle mass.

Measurement of muscle mass in vivo

Central to assessing and treating the problem of muscle wasting is measuring the mass of muscle present in a subject. Despite great progress in methodology for assessing body composition, there is still no practical method of measuring muscle mass in vivo. The advances in methodology and instrumentation allow partitioning of lean, fat and bone, but the lean compartment is largely indivisible using current techniques. Because muscle tissue represents only about half the components of the lean mass, changes in lean mass do not always reflect changes in muscle mass.

Methods for the measurement of muscle mass have ranged from anthropometry to neutron activation (Cohn et al. 1969, 1980). The latter is probably the best indicator of muscle mass, but is only available in a very few sites in the world and is not easily adapted to clinical situations. A sophisticated variant of anthropometric measurement is the direct computerized tomography (CT) scanning of specific muscles. The CT scanning techniques (either NMR or X-ray) are useful in measuring specific muscle groups but do not indicate what may be occurring in the whole body.

There are several indirect measurements of metabolites of muscle metabolism such as creatinine (Heymsfield et al. 1983) and 3-methylhistidine (3MH; Lukaski et al. 1981) that can be related to muscle mass. These 'metabolic' estimates of muscle mass have been used, but are generally only applicable in young normal human subjects because any disease or metabolic disturbance can markedly affect these indicators of muscle mass. For example, in the case of urinary 3MH, changes in muscle protein turnover can alter estimates of muscle mass. Urinary creatinine can be influenced by the energy state of the muscle and can affect the estimate of muscle mass. Muscle strength is also related to muscle mass, but the effects of disuse and joint flexibility make it difficult to directly relate strength to muscle mass. It is clear that the lack of accurate and easy-to-use methods for the measurement of muscle mass results in a great deal of uncertainty in assessing current muscle protein status and even more uncertainty in predicting what muscle mass might be in the long term.

Measurement of muscle proteolysis in vivo

The most-commonly-used method of assessing muscle protein turnover involves quantitative collection of urine for 24 h and measurement of the amount of 3MH produced daily. Assuming a 3MH content of 4·2 nmol/g muscle (Johnson et al. 1967), the daily rate of muscle breakdown (g) can be calculated; this value can range from 40 to 80 g/d in a normal man. Based on 3MH production rate and muscle mass, the daily muscle turnover (%) can be calculated. Of the two values, the percentage muscle turnover daily may be more useful in evaluating treatment strategies since it relates to the current rate of muscle breakdown.

The usefulness of 3MH as an indicator of muscle proteolysis is based on the presence of 3MH in actin and myosin of muscle, and the fact that once released from protein by

proteolysis it cannot be re-used for protein synthesis. In many mammals (including man) 3MH is quantitatively lost in the urine, making daily losses relatively easy to measure. Thus, it is a relatively easy task to calculate the rate of muscle breakdown (g/d).

Like most biological techniques, the 3MH model of muscle proteolysis depends on a number of assumptions which are the subject of criticism. The first assumption is that 3MH is derived only from actin and myosin in skeletal muscle. This is not a valid assumption in that 3MH is found in all forms of actin in the body and there are considerable amounts of smooth muscle in the body, especially in the intestine, skin and the uterus (Wassner & Li, 1982). The difficulty arises in determining the impact of the non-skeletal muscle contribution to total 3MH production. Second, it is important to know the effect that disturbances in intestinal metabolism have on the rate of 3MH release from smooth muscle. It is thought that smooth-muscle proteins turn over at a higher rate than skeletal-muscle proteins. Although there is evidence in rats that this could be a major problem, several catheter studies in human subjects and animals indicate that only a small proportion of 3MH is derived from the intestine, even under conditions of stress, disease or trauma (Sjolin et al. 1989a,b). There are, however, a few reports that seem to indicate that other tissues may be contributing disproportionally more than skeletal muscle to the production of urinary 3MH. Studies in which 3MH production was measured across the leg of subjects after surgery indicate that 3MH output decreases while urinary 3MH increases, suggesting that problems exist with the method (Rennie & Millward, 1983). However, recent data from human subjects with varying degrees of infection indicate that the release of 3MH from the leg is highly correlated with the urinary output of 3MH, and the splanchnic region contributes less than 10 % to total urinary 3MH production (Sjolin et al. 1989a,b).

The second assumption of the model is that all 3MH produced from the muscle is quantitatively lost in the urine. Quantitative recovery of 3MH has been shown in human subjects (Long et al. 1988), rabbits (Harris et al. 1977) and cattle (Harris & Milne, 1981a). However, the tracer is not quantitatively recovered in urine of sheep (Harris & Milne, 1980) and pigs (Harris & Milne, 1981b). Despite the previous evidence, it has not been proved that intracellular 3MH is quantitatively exported from the cell and in turn lost in the urine. In pigs, sheep and whales there is conversion of 3MH to balanine in muscle (3MH linked to β-alanine). This dipeptide accumulates in pigs, sheep and whales, with very little free 3MH lost in urine. Although it appears that human subjects make very little balanine, there has been no systematic study to verify this.

The third assumption is that all the 3MH derived from muscle is derived from endogenous muscle proteolysis and not from exogenous sources. For this to be true a meat-free diet must be fed for at least 3 d before urine collections (half-life of 3MH is from 12 to 24 h; Rathmacher et al. 1995). This is necessary because all meat products contain 3MH as part of the muscle. Dietary 3MH is digested and absorbed just like any amino acid and will in turn be lost in urine. Thus, urinary 3MH from dietary sources cannot be distinguished from endogenous 3MH. A meat-free diet is, therefore, a necessary component of the experimental protocol. At best this creates an inconvenience, and at worst it makes use of this technique impractical.

The fourth assumption is that the content of 3MH in muscle is constant and does not change appreciably throughout life or with metabolic state. This clearly is not the case (Mussini et al. 1984) and a systematic examination has not been conducted; thus, to be accurate in the measurement of muscle proteolysis a biopsy sample of muscle must be taken to correct for differences in 3MH content. Furthermore, the biopsy assumes that all muscles have the same content of 3MH, which may not be true.

Despite the many difficulties with the use of the urinary 3MH model of measuring protein breakdown in muscle, the method can be useful in understanding the physiology of the regulation of muscle protein breakdown. In these situations, pretreatment and post-treatment measurements make many of the difficult assumptions less of a problem. Thus, short-term insight into nutritional and hormonal effects on muscle proteolysis can be achieved. However, the use of 3MH to clinically assess the muscle protein breakdown state is not possible without the additional measurement of both muscle mass and 3MH content of the muscle.

Use of a compartmental model to measure 3-methylhistidine metabolism in vivo

A variation of the 3MH technique is the use of 3MH labelled with a stable isotope to measure 3MH production without collection of urine (Rathmacher et al. 1992, 1993, 1995). This technique involves injection of a small amount of 3MH tracer into a vein and collection of four to eight blood samples over the following 36 h. Analysis of the decay of this isotope in the blood combined with compartmental analysis results in an estimate of 3MH production which is similar to that obtained by urine collection methods.

This method has both theoretical and practical advantages over the urinary 3MH model methods. First, this method is not subject to quantitative urine collections. In addition, there is some evidence that the mass of one of the compartments relates well to the total muscle mass in the body (Rathmacher et al. 1995). Thus, it may be possible, with further refinement, to predict total turnover, fractional turnover and total muscle mass using this method. Further studies will be necessary to fully understand the usefulness of this model.

A further variation of the 3MH technique is the measurement of the uptake and output of 3MH across muscle (Rennie & Millward, 1983; Sjolin et al. 1989a). Theoretically this could be used to assess changes in muscle proteolysis over short periods of time. However, because the half-life of 3MH is 12–24 h (Rathmacher et al. 1995), there are very small concentration gradients across the muscle beds which necessitates extremely accurate measurement of 3MH concentrations. These measurements are beyond the capabilities of most amino acid analysers.

Other techniques used to measure muscle proteolysis in vivo

Table 1 lists the major methods used to measure muscle proteolysis in vivo. Beyond the 3MH technique and its variations, the other methods are mainly research tools not applicable to clinical situations. The arterial–venous method measures the uptake or output of amino acids across the leg or forearm. It involves the catheterization of the artery and the vein and measurement of total blood flow. By using isotopes the absolute rates of muscle protein breakdown can be measured. The other variable necessary is an estimate of the muscle mass supplied and drained by the artery and vein. It is also assumed that the metabolism occurring across the limb represents only the muscle tissue.

The other major technique for the estimation of muscle proteolysis in vivo involves the use of isotope injection combined with biopsy so that the rate of protein synthesis can be measured (Garlick et al. 1989; Smith et al. 1992; Rennie et al. 1994). Knowing the rate of synthesis and the rate of muscle mass lost or gained, estimates of muscle breakdown can be determined indirectly. The difficulty, however, lies in the measurement of the change in muscle mass over time. In animals this can be determined by dissection of muscle from the animals. In human subjects, the only way to accurately estimate the change in muscle mass is with sequential CT scans. Even this is somewhat difficult to assess as only one or two

Table 1. *Comparison of methods used to measure muscle protein breakdown* in vivo

Method or model	Blood collected	Urine collection (complete)	Special diet	Independent estimate of muscle mass	Isotopes	Invasive or biopsy
Urinary 3MH	No	Yes	Meat-free for at least 3 d	No; yes, if exact estimate	No	No; yes, if exact estimate
Compartmental 3MH	Yes	No	Meat-free for at least 3 d	No, if model proves to predict mass	Stable isotope	No; with further model refinement
Artery–vein difference	Yes	No	No; yes, if using 3MH difference	Yes; estimate local mass drained	No; yes, for exact estimate	Artery and vein catheter
Indirect calculation by measuring protein synthesis	Yes	No	No	Yes; must estimate change in mass over time	Yes	No, if CT scans used

3MH, 3-methylhistidine; CT, computerized tomography.

sections are usually evaluated. The recent advances in three-dimensional NMR may allow more accurate quantifying of changes in muscle mass over short periods of time. These data combined with isotope–biopsy techniques may allow great progress to be made in understanding both protein synthesis and proteolysis.

Theoretical implications of muscle protein breakdown and intervention

The contribution of increased muscle protein breakdown to well-being has been widely measured in human subjects. Fig. 2 depicts some of the physiological events that cause increases in muscle protein breakdown (as measured by urine 3MH). As can be seen, the stress of disease and trauma as well as the stress of resistance exercise can all result in muscle proteolytic rates approximately twice that of normal. Thus, a normal rate of muscle protein breakdown of 45–60 g/d can easily double to over 90 g/d, which can be equal to or up to double the daily dietary intake of protein. Thus, the large quantities of amino acids being released into the blood are in effect 'feeding' the protein synthetic machinery at saturation levels, which makes supplementary dietary protein of little or no use in increasing protein synthesis. Table 2 depicts the various theoretical scenarios that could result from changes in muscle proteolysis and the interaction with dietary protein intake. As can be seen, the most critical situation arises when a subject with an already low muscle mass and low protein breakdown, such as occurs in the elderly, undergoes trauma or disease. In this situation, low inputs of amino acids from muscle make dietary supplies of amino acids critical to maintaining the ability to mount acute-phase responses. Increased protein intake would be necessary, therefore, to offset the decrease in amino acids from muscle breakdown.
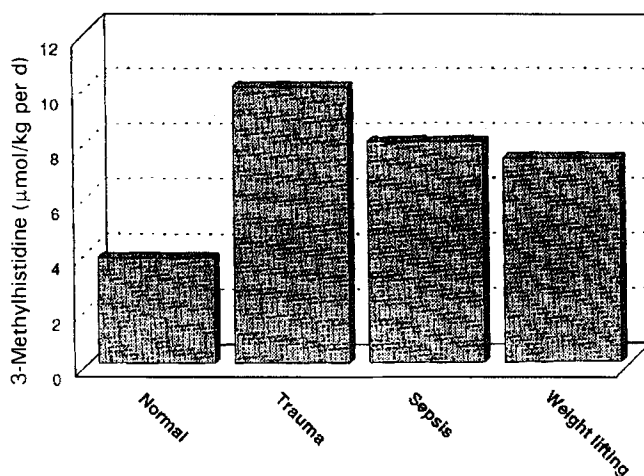
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Fig. 2. Changes in muscle protein breakdown as measured by urinary 3-methylhistidine production in subjects with trauma and sepsis (Long *et al*. 1981) and with exercise (Nissen *et al*. 1996).

Table 2. *Theoretical relationship between protein intake and muscle proteolysis relative to the ability of the liver and immune system to respond acutely to a stimulus for increased protein synthesis such as that which can occur in sepsis and trauma*

State of muscle proteolysis*... Dietary protein intake†	Elevated	Normal	Low
Low	+	−	−
Normal	+	+	−
High	+	+	+

+, Should be able to increase liver and immune protein synthesis; −, inadequate amino acid supply to increase liver or immune protein synthesis.
* Muscle proteolysis is described relative to a young, non-stressed normal subject.
† Protein intakes are described in terms relative to recommended dietary allowance.

CONCLUSION

Technological advances in measuring protein metabolism in muscle *in vivo* are not yet adequate to develop rational strategies to treat muscle wasting. 3MH is a good technique for estimating absolute rates of muscle proteolysis in longitudinal studies. However, without better techniques for the estimation of muscle mass and rates of muscle loss, muscle protein breakdown data are of little use in 'fine tuning' therapeutic regimens.

Based on current knowledge of muscle wasting and muscle protein breakdown, therapeutic intervention should be implemented in cases where over 30 % of peak muscle mass is lost. This intervention group would include many, if not most, middle-aged and elderly subjects. Intervention strategies, either nutritional or drug, would be aimed at minimizing muscle breakdown and maximizing protein synthesis. In this target group, it appears that adequate protein intake is essential to support maximal protein synthesis in liver and immune tissues and replace the amino acids now unavailable due to successful suppression of muscle proteolysis.

REFERENCES

Cohn, S. H., Dombrowski, C. S., Pate, H. R. & Robertson, J. S. (1969). A whole-body counter with an invariant response to radionuclide distribution and body size. *Physics in Medicine and Biology* **14**, 645–658.

Cohn, S. H., Vartsky, D. & Yasumura, S. (1980). Compartmental body composition based on total-body nitrogen, potassium and calcium. *American Journal of Physiology* **239**, 524–530.

Forbes, G. B. & Reina, J. C. (1970). Adult lean body mass declines with age: some longitudinal observations. *Metabolism* **19**, 653–663.

Garlick, P. J., Wernerman, J., McNurlan, M. A., Essen, P., Lobley, G. E., Milne, E., Calder, G. A. & Vinnars, E. (1989). Measurement of the rate of protein synthesis in muscle of postabsorptive young men by injection of 'flooding dose' of [1-^{13}C]leucine. *Clinical Science* **77**, 329–336.

Harris, C. I. & Milne, G. (1980). The urinary excretion of Nt-methyl histidine in sheep: an invalid index of muscle protein breakdown. *British Journal of Nutrition* **44**, 129–140.

Harris, C. I. & Milne, G. (1981a). The urinary excretion of N-tau-methyl histidine by cattle: validation as an index of muscle protein breakdown. *British Journal of Nutrition* **45**, 411–422.

Harris, C. I. & Milne, G. (1981b). The inadequacy of urinary (N-tau)-methyl histidine excretion in the pig as a measure of muscle protein breakdown. *British Journal of Nutrition* **45**, 423–429.

Harris, C. I., Milne, G., Lobley, G. E. & Nicholas, G. A. (1977). 3-Methylhistidine as a measure of skeletal-muscle protein catabolism in the adult New Zealand white rabbit. *Biochemical Society Transactions* **5**, 706–708.

Heymsfield, S. B., Arteaga, C., McManus, C., Smith, J. & Moffitt, S. (1983). Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *American Journal of Clinical Nutrition* **37**, 478–494.

Hill, G. L. (1992). *Metabolic Management of Patients Undergoing Major Surgery*, pp. 19–32. London: Churchill Livingstone.

Johnson, P., Harris, C. I. & Perry, S. V. (1967). 3-Methylhistidine in actin and other muscle proteins. *Biochemical Journal* **105**, 361–370.

Long, C. L., Birkhahn, R. H., Geiger, J. W., Betts, J. E., Schiller, W. R. & Blakemore, W. S. (1981). Urinary excretion of 3-methylhistidine: An assessment of muscle protein catabolism in adult normal subjects and during malnutrition, sepsis and skeletal trauma. *Metabolism* **30**, 765–776.

Long, C. L., Dillard, D. R., Bodzin, J. H., Geiger, J. W. & Blakemore, W. S. (1988). Validity of 3-methylhistidine excretion as an indicator of skeletal muscle protein breakdown in humans. *Metabolism* **37**, 844–849.

Lukaski, H. C., Mendez, J., Buskirk, E. R. & Cohn, S. H. (1981). Relationship between endogenous 3-methylhistidine excretion and body composition. *American Journal of Physiology* **240**, E302–E307.

Mussini, E., Colombo, L., De Ponte, G. & Marcucci, F. (1984). Low 3-methylhistidine levels in skeletal muscles of genetically dystrophic mice. *Research Communications in Chemical Pathology and Pharmacology* **43**, 477–486.

Nissen, S., Sharp, R., Rathmacher, J. A., Rice, J., Fuller, J. C. Jr, Connelly, A. S. & Abumrad, N. N. (1996). The effect of the leucine metabolite β-hydroxy β-methylbutyrate on muscle metabolism during resistance-exercise training. *Journal of Applied Physiology* **81**, 2095–2104.

Rathmacher, J. A., Flakoll, P. J. & Nissen, S. L. (1995). A compartmental model of 3-methylhistidine metabolism in humans. *American Journal of Physiology* **296**, E193–E198.

Rathmacher, J. A., Link, G. A., Flakoll, P. J. & Nissen, S. L. (1992). Gas chromatographic/mass spectrometric analysis of stable isotopes of 3-methylhistidine in biological fluids: application to plasma kinetics in vivo. *Biological Mass Spectrometry* **21**, 560–566.

Rathmacher, J. A., Link, G. & Nissen, S. (1993). Measurement of 3-methylhistidine production in lambs by using compartmental-kinetic analysis. *British Journal of Nutrition* **69**, 743–755.

Rennie, M. J. & Millward, D. J. (1983). 3-Methylhistidine excretion and the urinary 3-methylhistidine/creatinine ratio are poor indicators of skeletal muscle protein breakdown. *Clinical Science* **65**, 217–225.

Rennie, M. J., Smith, K. & Watt, P. W. (1994). Measurement of human tissue protein synthesis: an optimal approach. *American Journal of Physiology* **266**, 298–307.

Sjolin, J., Stjernstrom, H., Arturson, G., Andersson, E., Friman, G. & Larsson, J. (1989a). Exchange of 3-methyl-histidine in the splanchnic region in human infection. *American Journal of Clinical Nutrition* **50**, 1407–1414.

Sjolin, J., Stjernstrom, H., Henneberg, S., Andersson, E., Martensson, J., Friman, G. & Larsson, J. (1989b). Splanchnic and peripheral release of 3-methylhistidine in relation to its urinary excretion in human infection. *Metabolism* **38**, 23–29.

Smith, K., Barua, J. M., Watt, P. W., Scrimgeour, C. M. & Rennie, M. J. (1992). Flooding with L-[1-^{13}C]leucine stimulates human muscle protein incorporation of continuously infused L-[1-^{13}C]valine. *American Journal of Physiology* **262**, E372–E376.

Wassner, S. J. & Li, J. B. (1982). Nt-methylhistidine release: contributions of rat skeletal muscle, GI tract, and skin. *American Journal of Physiology* **243**, E293–E297.

Windsor, J. A. & Hill, G. L. (1988). Risk factors for postoperative pneumonia: the importance of protein depletion. *Annals of Surgery* **208**, 290–296.