The Effect of Surgical Trauma on Muscle Protein Turnover in Rats

A SERIOUS METHODOLOGICAL MISUNDERSTANDING

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(Received 25 January 1979)

The reported rates of protein degradation in a recent paper on the effect of surgical trauma on muscle protein turnover [Hoover-Plow & Clifford (1978) *Biochem. J.* 176, 137-142] have no real meaning because of a serious methodological misunderstanding by the authors. In addition, there are problems involved in the determination of synthesis rates, so that the reported effects of trauma on muscle protein turnover can be discounted.

Hoover-Plow & Clifford (1978) recently reported experiments in which they examined the effect of surgical trauma on muscle protein turnover in rats. They concluded that protein synthesis and catabolism were increased in the sarcoplasmic-muscle fraction in operated rats compared with food-restricted rats, that the synthesis rate of myofibrillar protein decreased in operated rats, but that the catabolic rate was not altered in the operated rats. In addition, the net change of muscle protein was calculated from the measured protein synthesis and degradation rates, and this calculation indicated a loss of muscle protein in the operated animals. Their methods involved the use of $\text{Na}_2^{14}\text{CO}_3$ to label muscle protein, and they reported that their analysis of the experimental results and the calculation of turnover rate and half-lives for the muscle proteins were as described by Millward (1970a). In fact Hoover-Plow & Clifford (1978) have seriously misunderstood the basis of the method as described by Millward (1970a,b) to the extent that their reported rates of protein degradation have little meaning. Indeed, it is not possible to calculate rates of protein degradation from their results. In addition, there are serious problems relating to their measurements of protein synthesis. This present paper describes these problems.

Measurement of Protein Synthesis and Degradation

The basis of the determination of rates of protein synthesis and degradation by measurement of the decay rates of labelled proteins is that the rate of fall of total radioactivity (C , which might be d.p.m./muscle) indicates the rate of degradation, whereas the rate of fall of specific radioactivity (S , which might be d.p.m./mg of protein) indicates the rate of protein synthesis.

Assuming only that degradation is a random first-order (constant fraction) process, then:

$$\frac{C_t}{C_0} = e^{-k_d t} \quad (1)$$

where k_d is the fractional degradation rate. That is:

$$k_d = \frac{1}{t} \cdot \ln \frac{C_0}{C_t} \quad (2)$$

In the steady state synthesis and degradation are equal, so that the rate of decay of specific radioactivity is the same as that of total radioactivity.

$$\frac{S_t}{S_0} = e^{-k_s t} \quad (3)$$

where k_s is the fractional rate of synthesis. That is:

$$k_s = \frac{1}{t} \cdot \ln \frac{S_0}{S_t} \quad (4)$$

In the non-steady state, when $k_s \neq k_d$ decay of specific and total radioactivities will be different, but, provided that measurements are made over short time periods, then eqns. (2) and (4) still hold (Koch, 1962; Millward, 1970a; Waterlow *et al.*, 1978).

Determination of Total Radioactivity

The important practical consideration is that total radioactivity (C) is the radioactivity in the system under study, e.g. in an individual muscle, group of muscles or total muscle mass. Hoover-Plow & Clifford (1978) report measurement of total radioactivity that appears to be the radioactivity in a 2g portion of hind-limb muscle. This is not the appropriate measurement of total radioactivity for the determination of k_d . It is closer to a measurement of specific radioactivity (S), differing from the actual

measurement of S only to the extent that the protein content of the muscle varies. Thus the reported values for synthesis and degradation of sarcoplasmic proteins in Table 2 of Hoover-Plow & Clifford (1978) are very similar, since the protein contents of 2g of muscle are not very different between day 2 and day 4. In contrast, the reported values for synthesis and degradation of myofibrillar proteins are different, because of the change in protein content of 2g of muscle between day 6 and day 8, when the turnover rates were calculated. In fact, no meaningful information can be obtained from an analysis of such measurements of total radioactivity. The appropriate experimental design would have been to measure the protein mass in a particular muscle or anatomically defined group of muscles.

Interpretation of Decay Curves of Protein Mixtures

Although the above is the most serious error in the analysis of the data, there are several others. The decay curves of specific radioactivities of sarcoplasmic and myofibrillar protein and of aspartate and glutamate isolated from these proteins are shown in Fig. 1 (data from Hoover-Plow & Clifford, 1978).

The authors calculate synthesis rates from the decay of S between 2 and 4 days for sarcoplasmic protein and for aspartate and glutamate isolated from these proteins, and from the decay of S between 6 and 8 days for the myofibrillar proteins. In fact, the decay curves of mixtures of proteins with heterogeneous turnover rates are complex even in the absence of isotope reutilization, so that the time

Fig. 1. Decay of specific radioactivity in rat muscle proteins labelled by injection of $\text{Na}_2^{14}\text{CO}_3$

Measurements were made on muscle proteins isolated from rats after the removal of the uterine horns, i.e. operated rats (□), sham-operated rats (Δ) or rats fed with the same intake as that of the operated rats, i.e. food-restricted rats (○), at the stated times after the operations. The $\text{Na}_2^{14}\text{CO}_3$ was injected at day 1 and measurements were made of the specific radioactivities of sarcoplasmic protein (a), myofibrillar protein (b), aspartate and glutamate isolated from sarcoplasmic protein (c) and aspartate and glutamate isolated from myofibrillar proteins (d). Results for (a) and (b) are shown as means $\pm$ 1 S.E.M. of groups of four to six rats, whereas results for (c) and (d) are for pooled muscle samples. The data are taken from the paper by Hoover-Plow & Clifford (1978).

over which the decay is measured crucially influences the determined rate constant (Garlick & Millward, 1972; Garlick *et al.*, 1976; Waterlow *et al.*, 1978). Presumably the authors chose to measure myofibrillar protein turnover between 6 and 8 days after the operation because only after this time was S falling (and even then the 6-day and 8-day values of S were not significantly different; Fig. 1b).

Isotope Reutilization

The most serious difficulty in the determination of protein turnover from decay rates results from the continued incorporation of labelled amino acids for long periods after their administration and from the reutilization of labelled amino acids. Although the $\text{Na}_2^{14}\text{CO}_3$ method was designed to minimize this, it does not eliminate it completely, and it is apparent that reutilization was occurring in the experiments conducted by Hoover-Plow & Clifford (1978). Thus S rose in myofibrillar proteins for up to 6 days after the injection (Fig. 1b), and even in one case when the measurements were made on aspartate and glutamate isolated from the protein (Fig. 1d).

Finally, in the analysis of decay curves, in order that some confidence can be placed in the results, it is advisable to inspect the overall decay curve to check that it is sensible. The results for the decay of S (aspartate + glutamate) in the sarcoplasmic proteins from the restricted group (Fig. 1c) are not sensible, since after the initial rapid fall (from which k_s is calculated) S increases. This rise in S is highly unlikely to be a real phenomenon and must raise doubts over the significance of the rapid fall in S .

Calculation of Net Change in Protein Content

The rate of change of muscle protein mass in these experiments was calculated as the difference between

the calculated values of protein synthesis and degradation. Given the problems discussed above in accepting the validity of the dynamic measurements, it is highly unlikely that the values for net change in muscle protein are reliable. In any case the effect of surgical trauma on muscle protein mass can be measured directly in a relatively easy way.

Conclusions

There are several serious flaws in the analysis of the experimental results reported by Hoover-Plow & Clifford (1978), the major one involving the misinterpretation of the meaning of total radioactivity from which degradative rates are calculated. As a result the rates of degradation described in that paper have no meaning. Other problems raise doubts about the accuracy of the measurements of synthesis rates. The net changes in muscle protein have little meaning therefore, since they have been calculated from rates of protein synthesis and degradation. All of the individual problems raised in this present paper have been discussed in detail in a recent review (Waterlow *et al.*, 1978).

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