Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting

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Summary

1. Measurements have been made of whole-body and skeletal muscle protein synthesis in fed and fasted adults with L-[1-13C]leucine.

2. The marked increase in whole-body synthesis on feeding largely reflects the changes in protein synthesis in muscle, which doubles on feeding, compared with a 40% increase in that of the rest of the body.

3. Skeletal muscle in fed man contributes more than half to total protein synthesis occurring in the whole body.

Key words: feeding and fasting, muscle, protein synthesis.

Introduction

In addition to its mechanical role skeletal muscle protein fulfils an important metabolic role as a store of energy and amino acids which may be called upon by the rest of the body during injury, starvation and disease [1, 2]. Skeletal muscle represents 70% of the lean body mass and is the major site of new protein deposition during growth [3]. Up to now, our understanding of the regulation of muscle growth and protein balance in health and disease has been based on animal experimentation because of the lack of a convenient method suitable for measurements in man. The metabolic role of muscle as a store of energy and amino acids depends on the dynamic state of its protein mass, which is well documented in animals [3]. In man the extent of protein turnover in muscle and its control are poorly documented because of a lack of suitable reliable methods.

We have developed such a method using stable isotope labelled leucine (i.e. [1-13C]leucine) to study muscle protein synthesis simultaneously with whole-body protein turnover. Here we report the first measurements of the response of muscle protein synthesis to feeding in normal man.

Methods

We studied seven normal healthy men (21–59 years, 80.8 ± 19.2 kg, mean ± SD). Our investigation was approved by the Ethics Committee of University College Hospital. The subjects, who gave their informed consent, were studied in the morning of the third day of a meat free diet (70–80 g of protein, 8400–13 500 kJ/day) either fasted (for at least 15 h) or fed hourly meals of a liquid food (Ensure, Abbott Laboratories Ltd) to provide 0–1 g of protein and 14 kJ/kg body wt. Feeding began 3 h before the collection of baseline samples. Urine collection (24 h) was made before the study and throughout the investigation. L-[13C]Leucine (90 atoms %; KOR Inc., Cambridge, MA, U.S.A.) was infused at 1 mg h⁻¹ kg⁻¹ body wt. via a cannula placed in a forearm vein, after a bolus injection of [13C]leucine (1.0 mg/kg) and NaH[13]CO₃ (KOR Inc.) (0.8 mg/kg). Blood samples were taken at
half-hourly intervals for 2 h and then at hourly intervals. Total CO₂ production was measured from the infrared absorbance of expired air sampled continuously from a metabolic tent. Expired ¹³CO₂ was also collected in a 5 litre Douglas bag and part immediately transferred to an evacuated glass vial for storage. The enrichment of CO₂ was measured in a VG micromass 602D isotope ratio mass spectrometer [4]. The isotopic enrichment of leucine and α-ketoisocaproate (oxoleucine; 4-methyl-2-oxovalerate) in blood and, in some cases, in acid extracts of muscle, was determined by gas chromatography–mass spectrometry [5, 6]. Muscle biopsy samples (60–300 mg) of both quadriceps muscles [7] were taken at 24 h and 74 h of the infusion when blood and intramuscular leucine labelling had achieved plateau values. Muscle RNA was analysed by the method of Munro & Fleck [8]. Alkali-soluble mixed muscle protein from fat-free, dried biopsy samples was hydrolysed for 24 h at 110°C in HCI (6 mol/l). Leucine in 12–20 mg of hydrolysed protein was separated preparatively (100% pure) on a Locarte amino acid analyser. Leucine was completely separated from other amino acids by using a 36 cm x 0.9 cm column eluted with sodium acetate solution (0.2 mol/l) at pH 4.25. The leucine fraction was collected and freeze-dried. Subsequently all reagents were CO₂-free. Leucine CO₂ was liberated by ninhydrin at pH 2-0, dried, and its enrichment measured in the isotope ratio-mass spectrometer [4]. Leucine flux was calculated according to the equations given in references [3] and [9] from the plateau labelling of blood α-ketoisocaproate. We believe that this is appropriate since the labelling of blood α-ketoisocaproate, which is produced intracellularly from leucine, is closer than the labelling of blood leucine to the labelling of the metabolic pool from which leucine is removed by protein synthesis and oxidation [10]. In four of our subjects the labelling of blood α-ketoisocaproate was 75–83% of blood leucine, but was 96–106% of leucine isolated from an acid extract of their muscle biopsy samples. For a bigger series, including nine patients undergoing elective surgery, the respective figures were 82 ± 3% and 108 ± 6%. This suggests that muscle protein synthesis estimated on the basis of blood oxo-leucine labelling will be slightly below the true value.

The components of the flux not directly measured, protein synthesis (S) and degradation (D), are calculated from the flux (Q), oxidation rate (Ô) and dietary input rate (D) according to a two-pool stochastic model whereby \( \dot{Q} = \dot{S} + \dot{O} \) (i.e. the sum of processes removing leucine) and also \( \dot{Q} = \dot{D} + \dot{B} \) (i.e. the sum of processes supplying leucine [3, 9]).

Muscle protein synthesis rates were measured by the increase in incorporation of ¹³C into leucine in quadriceps proteins in biopsies at times \( t_1 \) and \( t_2 \) h. It was assumed that the labelling of the precursor pool for protein synthesis was similar to that of α-ketoisocaproate in blood. Then, synthesis rate

\[
k_s = \frac{\Delta E_{\text{muscle}}}{\sum_{i=1}^{n} E_{\text{blood}}^{(i)}} \times \frac{(1/t_2 - 1/t_1)}{100} \text{ h}^{-1}
\]

where \( \Delta E_{\text{muscle}} \) is the change in enrichment of muscle and \( \sum_{i=1}^{n} E_{\text{blood}}^{(i)} \) is mean enrichment over the time in hours. Creatinine was measured in urine by the alkaline picrate method [11] and 3-methylhistidine by ion-exchange chromatography with fluorescent detection [12].

Results

In every infusion isotopic enrichment of blood leucine and α-ketoisocaproate, and blood CO₂, achieved plateau within 2 h. In all cases the variability of individual sample enrichments of leucine and α-ketoisocaproate was less than 10% about the mean plateau value, and for ¹³CO₂ the variability was less than 5%. Intramuscular free leucine labelling (determined from acid extracts of 14 muscle biopsies in both fed and fasted states) was 82 ± 4% (mean ± SD) of blood leucine enrichment. An erroneously higher value than this previously reported by us was due to D-leucine contamination of the infused [13]. Reproducibility of labelling in both free and protein-bound muscle leucine was excellent (ratio of left to right legs for fed and fasted states) was 91 ± 11%; protein-bound 99 ± 8%; means ± coefficient of variation, \( n = 17 \).

Markedly increased rates of whole-body protein synthesis, amino acid catabolism and skeletal muscle protein synthesis were observed in the fed compared with the fasted state with no significant differences in whole-body protein degradation (Table 1). Muscle RNA activity (g of protein synthesized/g of RNA) in the fed state was double the value in the fasted state, the difference being entirely due to alterations in the protein synthesis rate, since RNA protein remained constant (2.17 ± 0.41, 2.23 ± 0.38 mg of RNA/g of protein).

The excretion of creatinine and 3-methylhistidine was not different in the fed and fasted state and so values have been combined. Mean 24 h excretion of creatinine was 13-85 mmol. Muscle mass calculated from this, assuming that...
TABLE 1. Whole-body leucine turnover and muscle protein synthesis during feeding and fasting in normal adult man

Mean values ± sd are shown for seven subjects. N.S., Not significant.

<table>
<thead>
<tr>
<th></th>
<th>Normal men (fed) n = 7</th>
<th>Normal men (fasted 18 h) n = 7</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-body leucine turnover (µmol of leucine h⁻¹ kg⁻¹ body wt.)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Leucine flux (Q)</td>
<td>194 ± 20</td>
<td>106 ± 19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Leucine oxidation (O)</td>
<td>40 ± 8</td>
<td>14 ± 4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Q/O (%)</td>
<td>20 ± 8</td>
<td>13 ± 4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Leucine removed by synthesis (S) (Q – O)</td>
<td>154 ± 30</td>
<td>92 ± 16</td>
<td>&lt;0.01</td>
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<tr>
<td>Leucine supplied by diet D</td>
<td>55 ± 4</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>D/O (%)</td>
<td>73 ± 4</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Leucine supplied by protein breakdown (b) (Q – D)</td>
<td>139 ± 20</td>
<td>106 ± 19</td>
<td>N.S.</td>
</tr>
<tr>
<td>Protein balance (S – b)</td>
<td>+15 ± 2</td>
<td>–14 ± 2</td>
<td>&lt;0.01</td>
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<td>Skeletal muscle turnover</td>
<td></td>
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<tr>
<td>Mixed protein synthesis rate k₆ (%/h)</td>
<td>0.198 ± 0.055</td>
<td>0.098 ± 0.043</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Muscle RNA activity (g of protein synthesized h⁻¹ g⁻¹ of RNA)</td>
<td>0.91 ± 0.30</td>
<td>0.44 ± 0.016</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Calculated protein synthesis (g of protein 12 h⁻¹ 70 kg⁻¹ body wt.)</td>
<td></td>
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<tr>
<td>Whole body</td>
<td>212 ± 41</td>
<td>127 ± 22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>113 ± 31</td>
<td>55 ± 20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Non-skeletal muscle</td>
<td>99 ± 34</td>
<td>71 ± 24</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

8.85 mmol excreted per 24 h is equivalent to 20 kg of muscle [14], was 31.3 ± 7.0 kg (mean ± sd); 24 h excretion of 3-methylhistidine was 253 ± 32 µmol (mean ± sd).

Discussion
The values we report here for whole-body leucine flux calculated from blood α-ketoisocaprate enrichment are about 22% higher than those previously reported by us and others [15, 16] from calculations based on blood leucine enrichment. Part of the reason for this is because the present calculations are based on the enrichment of plasma α-ketoisocaprate, which is similar to the enrichment of muscle free leucine but has a lower value than that of leucine in plasma. We believe that our values for flux are therefore near to the ideal value, being based upon estimates of the enrichment of a pool of leucine close to the precursor pool for protein synthesis and oxidation [10].

Our results show that feeding in normal man results in increases in whole-body protein synthesis and amino acid catabolism as previously observed in studies of young men [16] and obese women [15]. However, we found that whole-body protein breakdown (b) was not significantly different in the fed and in the fasted states. Other studies have shown that in young adult men [15], feeding apparently depressed breakdown and the results of studies on obese adult women have been interpreted to show either no change [16] or a decrease [17] during feeding. However, the direction of the change in breakdown depends crucially on the accuracy of D, and if D is underestimated then b (as Q – D) is more likely to apparently decrease on feeding. Our estimates of D are likely to be accurate since food was given as a liquid diet, the contents of which was known precisely. Furthermore, our findings are consistent with our measurements of protein synthesis in muscle.

Our present values for muscle protein synthesis in fed man are double our previously reported measurements [18], made using [¹⁵N]lysine. The precursor for protein synthesis was assumed to be enriched to the same extent as plasma lysine, which probably resulted in erroneously low values. Infusions of both lysine and leucine in pigs show that the ratio of labelling of intracellular free to plasma lysine was only half that of leucine, and similar synthesis rates were obtained only when the intracellular leucine and lysine labelling were used in the calculation [19].

Our confidence in the values we obtained for
muscle protein synthesis in fed and fasted man is increased by the finding that the muscle RNA activities were comparable with those obtained in fed and starved rats [20].

During feeding the rate of muscle protein synthesis is 0.198%/h. From creatinine excretion as a measure of muscle mass and from our measurements of muscle protein content (17%) and leucine content of protein (8%) we estimate that in a 70 kg man 113 g of protein is synthesized per 12 h in the fed state, i.e. 53% of the whole-body synthesis rate. This is a higher proportion than that observed in smaller animals [3, 21]. During fasting muscle protein synthesis rate halves to 0.098%/h, equivalent to 55 g of protein/12 h, i.e. 44% of whole-body protein synthesis. Since this fall on fasting (51%) is greater than that in the whole body (41%), it is clear that protein synthesis in muscle is more sensitive than that in the rest of the body. These marked changes in protein synthesis in muscle on fasting are consistent with the conclusion based on animal experiments that protein synthesis rather than degradation is the regulated process in muscle [22]. Given the large contribution of muscle protein turnover to the whole-body rate the lack of sensitivity of whole-body protein degradation is also consistent with this idea, especially since we found no differences in 3-methylhistidine excretion on short-term fasting, confirming the findings of others [23]. Indeed, the present results suggest that, for physiological purposes in healthy adults of normal body composition, 3-methylhistidine excretion may be an adequate index of myofibrillar degradation, despite the problems associated with its use in muscular dystrophy [24].

The high proportion of whole-body protein turnover accounted for by skeletal muscle not only emphasizes its potential for participation in protein and energy homoeostasis but also means that this role is more important in man than in laboratory animals in which models of human disease states are studied. This results, in part, from the increase in muscle as a proportion of total lean tissue mass with increase in body size (which can be inferred from the increasing muscle/liver ratio pointed out by Munro [21]). Another fact points to muscle's important role: the value of the non-muscle tissue protein synthesis rate. This can be calculated from our data if muscle mass is known. We calculate that a 70 kg adult man with 10.5 kg of lean, non-muscle active cell tissue would synthesize 99 g of protein/12 h in the fed state and 71 g/12 h in the fasted state. These values represent fractional synthesis rates of 0.46%/h in the fed state and 0.33%/h in the fasted state. Thus these estimates give non-skeletal muscle protein a synthesis rate of only 2–3.5-fold that in skeletal muscle, much less than expected from relative measurements of individual tissue rates in, for example, the rat [25, 26]. Nevertheless, recent measurements made by us in samples taken during surgery from patients infused with [1-13C]leucine before operation show only a fourfold range in incorporation of labeling of leucine isolated from mixed protein of gut, skin and skeletal muscle [27]. Together these results indicate that although human tissues synthesize protein at a generally lower rate than in animals, the difference is more pronounced in non-muscle tissue.

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References
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