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Examination of a protein-sparing effect of exogenous leucine during physical activity

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Abstract. Muscle leucine oxidation increases due to physical activity and thus contributes to enhanced energy expenditure. Since oxidized amino acids are withdrawn from protein metabolism, physical activity causes a negative nitrogen balance. Therefore a possible protein-sparing effect was studied of exogenous leucine injected prior to nearly exhaustive swimming exercise of the rat. Physiological saline and glucose served as controls for specific effects on energy and protein metabolism.

Immediately after exercise or a non-exersice period the leucine utilization was examined by tracing the metabolic fate of intraperitoneally injected L-[^{14}C] leucine. In both controls the $^{14}CO_2$ production was similarly increased by swimming exercise. This increase was potentiated and not suppressed by exogenous leucine. After exercise the incorporation of radiolabelled leucine in muscle protein tended to decrease.

This study suggests increased leucine catabolism due to physical activity to be the secondary effect of a decreased leucine utilization for protein synthesis. Therefore application of exogenous leucine does not have a protein-sparing effect during exercise but mainly stimulates its own oxidation. Nevertheless it might have a positive effect during recovery.

Key words: amino acid oxidation, amino acid utilization; exercise, leucine, muscle protein, physical activity

Introduction. Several workers (Rennie et al., 1981; Dohm et al., 1980; Viru, 1987) have reported increased muscle leucine oxidation during exercise. This means that the energy required during exercise is partly supplied by protein, although normal energy stores (fat and glycogen) can easily cover increased energy expenditure. Therefore the question rises whether leucine is oxidized because it is available by altered protein metabolism or because leucine is specifically required for muscle energy supply during activity. Anyhow, muscle protein degradation in order to maintain muscle performance does not seem to be teleological.

Amino acids in the blood can be utilized for either protein synthesis or energy ex-

penditure. In the latter case they are deaminated and decarboxylated, whereafter they can be completely oxidized or be transformed and stored into fat or glycogen.

Amino acid utilization can be examined after application of a ^{14}C-labelled amino acid. The expired $^{14}CO_2$ produced by decarboxylation then indicates which fraction of the total flux of the amino acid concerned is not used for protein synthesis. The difference with $^{14}CO_2$ produced by complete oxidation indicates that part of the label is retained within the body by energy stores.

Most amino acids are decarboxylated and oxidized in the liver. However, branched-chain amino acids (leucine, isoleucine and valine) are mainly catabolized in muscle. So these amino acids can directly supply energy for muscle activity.

Furthermore, leucine has been suggested to have a controlling role in protein turnover (Goldberg & Chang, 1978; Rannels et al., 1974) by stimulating protein synthesis and depressing protein degradation.

In this study we have investigated the effect of exogenous leucine on protein and energy metabolism of the rat immediately after a single bout of nearly exhaustive swimming exercise.

Materials and methods. Measurements were made on 48 male Wistar rats (6 months, ca. 430 g) divided in a non-exercising and an exercising group (nearly exhaustive exercise: 45 minutes swimming with a tail load of 25 g; water temperature 35 °C). Animals of both groups were intraperitoneally injected 5 min prior to the exercise/non-exercise period with either physiological saline (1 ml) alone or in combination with 182 μmol glucose or 157 μmol leucine. The amounts of leucine and glucose were equicaloric (ca. 0.5 kJ ME) and were supposed to cover about 10 % of the energy expenditure expected for the swimming exercise.

On the day of the experiment the rats were kept in an air-conditioned room (26–29 °C), either swimming or staying in their cage. Immediately thereafter the rats were injected with either 1 μCi L-[Carboxyl-^{14}C] leucine (1-^{14}C leu; to determine decarboxylation (n = 24)) or respectively 1 or 10 μCi L-[Universal-^{14}C] leucine (U-^{14}C leu; to determine complete oxidation (n = 24) and incorporation in muscle protein (n = 12)). Expired $^{14}CO_2$ was trapped in a solution of potassium hydroxide. The cumulated amount expired during four hours was expressed as a percentage of the injected dose. In the animals who received the high-dose U-^{14}C leu the protein specific activity (SA in dpm/g, normalized for dose per body weight) was determined after protein isolation by SDS gel filtration (modified from Schreurs et al., 1983) for several muscles (M. soleus, the sural muscle and upper and lower muscle complex of the foreleg).

Results and discussion. The data for the expiration of $^{14}CO_2$ presented in Table 1 show the values for 1-^{14}C leu always to be higher than the corresponding values of U-^{14}C leu. This had to be expected since oxidation of leucine starts with decarboxylation (after deamination) but is not always completed. After decarboxylation the remainder of the U-^{14}C leu molecule looses its amino acid-specific properties and becomes part of general catabolic pathways. Ultimately the acetyl-CoA pool will be labelled. Therefore production of $^{14}CO_2$ with U-^{14}C leu as tracer not solely depends

Table 1. Cumulated amount of $^{14}CO_2$ expired after injection of L-[^{14}C] leucine (expressed as a percentage of the injected dose).

	Non-exercising			Exercising		
	saline	glucose	leucine	saline	glucose	leucine
U-14C	12.1 ± 1.9	11.5 ± 2.0	16.8 ± 1.7	18.3 ± 3.3	18.1 ± 2.6	20.6 ± 2.9
1-14C	16.3 ± 1.8	15.9 ± 3.8	25.7 ± 1.9	21.6 ± 1.9	20.7 ± 4.6	29.3 ± 5.0

Table 2. Muscle protein specific activity (dpm/g protein) ×100.

	Non-exercising			Exercising		
	saline	glucose	leucine	saline	glucose	leucine
Muscle 1	416 ± 52	461 ± 19	319 ± 29	376 ± 54	377 ± 30	431 ± 2
Muscle 2	237 ± 11	256 ± 8	200 ± 10	220 ± 57	226 ± 2	218 ± 19
Muscle 3	242 ± 2	251 ± 2	225 ± 47	203 ± 36	226 ± 18	229 ± 2
Muscle 4	211 ± 21	222 ± 2	172 ± 6	190 ± 36	218 ± 2	224 ± 3

Muscle: 1 = M. soleus; 2 = upper muscle complex of foreleg; 3 = lower muscle complex of foreleg; 4 = sural muscle.

on amino acid supply but also on aspects of general energy metabolism such as total energy supply and the ratio of acetyl-CoA utilization for direct oxidation and the conversion to fat.

The increased $^{14}CO_2$ production ($P < 0.01$) at rest after application of exogenous leucine indicated that these amino acids became available as energy substrate. The supply of glucose had no effect on leucine utilization.

$^{14}CO_2$ production increased ($0.001 < P < 0.05$) after exercise for both tracers at all three administrations. The increase for the glucose group was not significantly different but tended to be slightly less than for the saline group. In the third group the effects of exogenous leucine and exercise seemed to be complementary. The relative larger increase of leucine decarboxylation compared to complete oxidation suggests that exogenous leucine after decarboxylation is not primarily used to cover energy expenditure but is rather retained in the body and probably channelled into fat metabolism.

The incorporation of U-^{14}C leu expressed as the specific activity (SA; dpm/g protein) was used for the comparison of the amino acid utilization for muscle protein synthesis (Table 2). The specific activities of the muscle proteins in the exercising groups seemed to be lowered with minor counteraction of the administrations.

The increased leucine decarboxylation in all exercising groups indicated a rise of leucine catabolism regardless of the availability of exogenous energy substrates. The influence of exercise on leucine metabolism seemed to be similar to application of exogenous leucine. Our data on amino acid utilization for muscle protein synthesis suggest that protein synthesis might be suppressed due to exercise. This is in ac-

cordance with several data from the literature (Viru, 1987). As a result the amino acid utilization shifts from protein to energy metabolism (Harper & Benjamin, 1984). The elevated leucine oxidation in both leucine groups (exercising and non-exercising) indicate that in both cases amino acid catabolism is mainly a matter of supply and not of a demand for amino acids for energy generation in muscle. In similar experiments tyrosine oxidation was also found to increase, which confirms this conclusion (Schreurs et al., 1987). However it cannot be excluded that, in addition, leucine plays a specific role in muscle energy metabolism during exercise. This could cause a deficiency of muscle catabolized amino acids. In that case application of exogenous branched-chain amino acids would have a positive effect on the re-instatement of the nitrogen balance during the recovery phase.

In similar experiments with 1-^{13}C leu in man, Rennie et al. (1981) described a relative larger dose of glucose to decrease protein breakdown. However they failed to present data on leucine decarboxylation and protein synthesis. They suggested the natural ^{13}C-labelling of their glucose to interfere with a proper interpretation of the measured $^{13}CO_2$ production. So they have no conclusive evidence for the effect of exogenous glucose on leucine decarboxylation.

Finally, it has to be stressed that in this type of experiments the ultimate results strongly depend on relative measures such as intensity and duration of the activity. Significant results on protein metabolism can be expected with a more strenuous exercise load.

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