Adaptation of Protein Metabolism to Endurance Training

INCREASED AMINO ACID OXIDATION IN RESPONSE TO TRAINING

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This study was conducted to investigate alterations in excretion of urea and total nitrogen after 6–8 weeks of daily exercise and to establish if the capacity for amino acid oxidation in muscle is influenced by endurance training. Urea nitrogen excretion was increased in trained compared with untrained rats and nitrogen balance was less positive in trained than in untrained rats. Increased [^{14}C]leucine oxidation with training was observed both *in vivo* and *in vitro*. The results of this study demonstrate that amino acid catabolism is increased during exercise training and that the muscle enzymes involved in leucine oxidation adapt to endurance training in a manner similar to the enzymes of carbohydrate and fat catabolism.

It is generally accepted that carbohydrate and fat are the primary sources of energy during exercise. However, there has been considerable controversy about whether protein catabolism is influenced by exercise. Although much of the older literature seems to discount protein as a source of energy when energy supply was adequate (for references, see Åstrand & Rodahl, 1970), the report by Mole & Johnson (1971) suggests that protein catabolism may indeed be increased by exercise. They found that urinary nitrogen excretion was significantly increased in exercising men who were on a diet providing more energy than was required for maintenance. The subsequent demonstration by Gontzea *et al.* (1974, 1975) that exercise caused a decrease in the nitrogen balance (increased nitrogen excretion) of men on a diet providing adequate energy supply provided further support for the hypothesis that exercise increases protein catabolism.

Until recently, the liver was generally believed to be the major site of amino acid oxidation in mammals. However, Odessy & Goldberg (1972) have demonstrated the ability of diaphragm and skeletal muscle to oxidize a number of amino acids. Dohm *et al.* (1976) investigated the maximum capacity for leucine oxidation in liver and muscle *in vitro*. They found that the rate of oxidation was considerably lower in muscle than in liver, but the total capacity for oxidation of branched-chain amino acids by muscle was appreciable, because of the large mass of muscle.

Since endurance training has been shown to alter a number of enzymes involved in the oxidation of carbohydrates (Huston *et al.*, 1975) and fats (Dohm *et al.*, 1972), we questioned whether amino acid oxidation in muscle might also be subject to exercise-induced adaptations. Thus the present study was conducted to investigate alterations in urea excretion and nitrogen balance after endurance training.

Experimental

Materials

In the first series of experiments (Table 2) L-[1- ^{14}C]leucine (59 mCi/mmol) and L-[U- ^{14}C]leucine (324 mCi/mmol) were obtained from Amersham/Searle Co., Arlington Heights, IL, U.S.A. In the second series of experiments (Figs. 1 and 2) L-[1- ^{14}C]leucine (50 mCi/mmol) and L-[U- ^{14}C]leucine (270 mCi/mmol) were obtained from New England Nuclear Corp., Boston, MA, U.S.A.

Methods

In the first series of experiments (see Tables 1 and 2), male Holtzman rats, weighing approx. 200 g initially, were trained by a progressive 6-week treadmill-running programme which culminated in a workload of 1 h/day, 6 days/week, at 35 m/min, up an 8° incline (Dohm *et al.*, 1975). Untrained and trained rats were allowed food (Wayne Lab Blox, Allied Mills, Chicago, IL, U.S.A.) and water *ad libitum*. Since trained rats consume approx. 10% less food than the untrained rats fed *ad libitum*, a second

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group of untrained rats (designated pair-fed) were pair-fed with the food intake of the trained rats.

Urine and faeces were collected during week 6 of training and were analysed for total nitrogen (Beecher & Whitten, 1970). Urea nitrogen was assayed by a modification (Technicon Auto Analyser Methodology Method File N-1C I/II) of the method of Marsh *et al.* (1965). For the assay of [^{14}C]leucine oxidation by muscle homogenates, rats were decapitated, the gastrocnemius muscles quickly removed, passed through a tissue press (Harvard Instrument Co., Millis, MA, U.S.A.) and homogenized in a glass/glass TenBroeck homogenizer in ice-cold homogenization medium (10 mM-potassium phosphate, pH 7.5, containing 1 mM-EDTA). The rate of leucine oxidation and the activity of leucine-2-oxoglutarate aminotransferase were then assayed as previously described (Dohm *et al.*, 1976).

In the second series of experiments (Figs. 1 and 2) male Carworth CFN rats (Carworth, Portage, MI, U.S.A.), 4–5 weeks old and weighing approx. 100 g, were trained according to a standardized treadmill-running programme described by Huston *et al.* (1975). Rats were randomly allotted to groups designated as trained or untrained. The untrained rats remained sedentary in their cages for the duration of the study. Both groups received food (Ralston Purina, St. Louis, MO, U.S.A.) and water *ad libitum* throughout the experiment. At the end of the 12 weeks of training the rats were running for 2 h/day, 5 days/week, at 29.5 m/min, 8° incline, with 30 s sprints at 42.9 m/min interspersed every 10 min. The rats were housed in individual cages in a room with temperature regulated to 22°C, 12 h of light and 12 h of dark.

Oxidation rates were determined *in vivo* by monitoring the expired $^{14}\text{CO}_2$ from rats that had received L-[^{14}C]leucine intraperitoneally (15 μCi /100 g body wt.) as described by Meikle & Klain (1972). To assay leucine oxidation *in vitro* by muscle slices, a 100–150 mg muscle slice was incubated for 1 h in 3.0 ml of Krebs–Ringer bicarbonate buffer (Krebs, 1950) containing 15 μmol of L-[^{14}C]leucine (0.2 μCi). The reaction was terminated with 1 ml of 1 M- H_2SO_4 , the $^{14}\text{CO}_2$ was trapped in 0.2 ml of Hyamine hydroxide

and the radioactivity measured as described by Klain & Burlington (1967).

For statistical analysis of the data, a one-way analysis of variance was performed and, when significance was indicated, a Neuman–Keuls analysis was performed as described by Weiner (1971).

In conducting the research described in this report the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care', as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institutes of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

Results

Urea nitrogen excretion was increased as a result of training (Table 1). Nitrogen balance (Table 1) was positive for all three groups, which was expected, since all rats were increasing in body weight as a result of growth. Trained rats had a less-positive nitrogen balance than either the untrained group fed *ad libitum* or the untrained pair-fed group.

Since the increased urea excretion suggested increased amino acid catabolism in endurance-trained rats, it was decided to confirm this conclusion by determining amino acid oxidation in trained and untrained animals. Fig. 1 demonstrates that training increased the rate of $^{14}\text{CO}_2$ production from [1- ^{14}C]leucine *in vivo*. In another experiment we observed that trained animals oxidized [1- ^{14}C]leucine at a faster rate than did the pair-fed controls, indicating that the difference observed in Fig. 1 was the result of training and not lower food consumption (A. L. Hecker, G. J. Klain & E. W. Askew, unpublished work).

Although the absolute rate of leucine oxidation cannot be calculated from the data in Fig. 1, the comparison of trained and untrained animals should be valid if the specific radioactivity of leucine in the tissues of oxidation is not different in the trained and untrained groups. In experiments where [^{14}C]leucine was administered, the trichloroacetic

Table 1. *Changes in urea excretion and nitrogen balance in response to endurance training*

Values in the Table are means $\pm$ S.E.M., with the numbers of observations in parentheses. To take account of body-weight differences, data are presented on a basis of metabolic body size, i.e. (body wt. in $\text{kg}^{0.734}$). * indicates statistically different from untrained rats ($P < 0.05$), and † indicates significant difference ($P < 0.05$) between trained and pair-fed rats. Nitrogen balance was calculated as the difference between nitrogen intake in food and nitrogen excretion in faeces and urine.

	Body wt. (g)	Nitrogen (g/24 h per $\text{kg}^{0.734}$)	
		Urea nitrogen excretion	Nitrogen balance
Untrained	358 $\pm$ 7 (12)	0.55 $\pm$ 0.04 (8)	+0.72 $\pm$ 0.07 (10)
Pair-fed	353 $\pm$ 8 (12)	0.57 $\pm$ 0.04 (4)	+0.54 $\pm$ 0.08 (11)*
Trained	328 $\pm$ 7 (10)*†	0.80 $\pm$ 0.03 (7)*†	+0.28 $\pm$ 0.05 (8)*†

acid-soluble radioactivity and leucine concentration in muscle, liver and plasma were not different in trained and untrained rats (G. L. Dohm, unpublished work). These observations suggest that the specific radioactivity of leucine was the same in the tissues of trained and untrained rats and that the difference in $^{14}\text{CO}_2$ evolution in Fig. 1 reflects a higher rate of leucine oxidation in trained than in untrained rats.

A considerable portion of the increase observed in leucine oxidation *in vivo* may be occurring in the muscle tissue. This was confirmed by the muscle-slice incubations *in vitro* (Fig. 2) in which, beginning with week 8 of training, there was a significant increase ($P<0.05$) in the oxidation of $[\text{U-}^{14}\text{C}]$ leucine

in tissue slices. However, the oxidation of leucine by muscle tissue slices can be influenced by the rate of uptake into the cell as well as the rate of oxidation inside the cell. This question was partially resolved by the data obtained with muscle homogenates shown in Table 2.

The data in Table 2 seem to confirm that the capacity for leucine oxidation is increased in muscle of trained rats. Because of the great degree of variability in the individual values, a statistically significant

Fig. 1. L-[1- ^{14}C]Leucine oxidation by trained and untrained rats

Rats were injected with L-[1- ^{14}C]leucine and $^{14}\text{CO}_2$ was collected over a 60 min period. $\circ$, Average for four untrained rats; $\bullet$, average for four trained rats (12-week training regimen). The mean body weights ($\pm$ S.E.M.) were $374\pm15\text{g}$ for untrained and $308\pm6\text{g}$ for trained rats. There was a statistically significant difference ($P<0.05$) between the trained and untrained groups at 30, 45 and 60 min.

Fig. 2. Effect of physical training on L-[U- ^{14}C]leucine oxidation in gastrocnemius-muscle slices

Rats were trained on the 12-week training schedule previously described (Huston *et al.*, 1975). Six animals from each group were killed at 4, 6, 8, 10 and 12 weeks after the start of training. $\bullet$, Average for six trained rats; $\circ$, average for six untrained rats. The mean body weights ($\pm$ S.E.M.) were $225\pm7\text{g}$ for untrained rats and $238\pm5\text{g}$ for trained rats at 4 weeks. At 12 weeks the body weights were $382\pm16\text{g}$ and $326\pm15\text{g}$ for untrained and trained rats respectively. There was a statistically significant difference ($P<0.05$) between trained and untrained groups at 8, 10 and 12 weeks of training.

Table 2. Effect of endurance training on the oxidation of L-[U- ^{14}C]- and L-[1- ^{14}C]-leucine and on leucine-2-oxoglutarate transaminase activity in gastrocnemius-muscle homogenates in response to exercise training

Values in the Table are means $\pm$ S.E.M., with the numbers of observations in parentheses. An untrained pair-fed group was also included in the experiment, but results for this group were not different from those of the untrained control group, so data from the two groups were combined. * indicates statistical significance at $P<0.1$.

	Body wt. (g)	$^{14}\text{CO}_2$ produced (nmol/min per g)		Leucine-2-oxoglutarate transaminase (nmol/min per g)
		From [U- ^{14}C]leucine	From [1- ^{14}C]leucine	
Untrained	378 ± 7 (18)	0.21 ± 0.02 (15)	0.51 ± 0.09 (16)	94 ± 20 (8)
Trained	312 ± 5 (8)	0.31 ± 0.05 (8)*	0.64 ± 0.16 (8)	92 ± 7 (8)

($P < 0.05$) difference between trained and untrained groups could not be established. Nevertheless, the trend in leucine oxidation by muscle homogenates agrees with data obtained with tissue slices and $^{14}\text{CO}_2$ production from intact rats oxidizing $[^{14}\text{C}]$ leucine.

In contrast with the alteration in leucine oxidation, there does not appear to be any change in the activity of the leucine transaminase as a result of training (Table 2). However, since the transaminase activity is several orders of magnitude greater than the activity of 4-methyl-2-oxopentanoate dehydrogenase (oxidation of $[1-^{14}\text{C}]$ leucine), little or no effect on the overall oxidation rate would be expected.

Discussion

The results of the present study demonstrate that protein catabolism is increased during exercise and that muscle enzymes involved in amino acid oxidation adapt to endurance training similarly to the enzymes of carbohydrate and fat catabolism. Since most investigators (Åstrand & Rodahl, 1970) discount the contribution of protein as an energy source, the question arises as to the purpose served by an increased utilization of amino acids in the trained animals.

To assess the possible contribution of protein as an energy source in the trained rat, we have calculated that approx. 8.4 kJ of energy could have been derived from protein catabolism that resulted in the excess urea nitrogen excretion (0.08 g more urea nitrogen excreted/24 h in trained than in untrained rats, divided by 0.16 g of nitrogen/g of protein, multiplied by 16.7 kJ/g of protein). This amounted to approx. 37% of the calculated energy expenditure during an exercise bout (22.8 kJ expended in excess of resting metabolism; calculated from the equations of Shepherd & Gollnick, 1976). Although these calculations do not prove conclusively that protein catabolism is a significant energy source, they suggest that it may be unwise to dismiss protein as an energy source during exercise in trained animals. More detailed studies are needed to assess the extent of amino acid utilization during exercise and to investigate the mechanisms that control amino acid oxidation.

There are other possible explanations of the function of increased amino acid catabolism during exercise and in endurance-trained animals. Felig & Wahren (1971) observed that alanine is released by exercising muscle and they proposed the 'glucose-alanine cycle'. Odessey *et al.* (1974) found that alanine release was increased when leucine was provided in the medium bathing the muscle and suggested that branched-chain amino acids contribute a large portion of the amino groups for transamination of pyruvate. Thus amino acids in muscle may be catabolized at an increased rate during exercise to

contribute amino groups so that the glucose-alanine cycle can function. Also the increased capacity of muscle to oxidize branched-chain amino acids may be an adaptation to utilize the oxo acids formed by transamination as immediate energy sources.

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References

- Åstrand, P. O. & Rodahl, K. (1970) *Textbook of Work Physiology*, p. 456, McGraw-Hill, New York
- Beecher, G. R. & Whitten, B. K. (1970) *Anal. Biochem.* **36**, 243-246
- Dohm, G. L., Huston, R. L., Askew, E. W. & Weiser, P. C. (1972) *Am. J. Physiol.* **223**, 783-787
- Dohm, G. L., Barakat, H., Stephenson, T. P., Pennington, S. N. & Tapscott, E. B. (1975) *Life Sci.* **17**, 1075-1080
- Dohm, G. L., Brown, W. E. & Barakat, H. A. (1976) *Biochem. Med.* **15**, 306-310
- Felig, P. & Wahren, J. (1971) in *Muscle Metabolism during Exercise* (Pernow, B. & Saltin, B., eds.), pp. 205-214, Plenum Press, New York
- Gontzea, I., Sutzescu, R. & Dumitrache, S. (1974) *Nutr. Rep. Int.* **10**, 35-43
- Gontzea, I., Sutzescu, R. & Dumitrache, S. (1975) *Nutr. Rep. Int.* **11**, 231-236
- Huston, R. L., Weiser, P. C., Dohm, G. L., Askew, E. W. & Boyd, J. B. (1975) *Life Sci.* **17**, 369-376
- Klain, G. J. & Burlington, R. F. (1967) *Am. J. Physiol.* **213**, 209-214
- Krebs, H. A. (1950) *Biochim. Biophys. Acta* **4**, 249-269
- Marsh, W. H., Fingerhut, B. & Miller, H. (1965) *Clin. Chem.* **11**, 624-627
- Meikle, A. W. & Klain, G. J. (1972) *Am. J. Physiol.* **222**, 1246-1250
- Mole, P. A. & Johnson, R. E. (1971) *J. Appl. Physiol.* **31**, 185-190
- Odessey, R. & Goldberg, A. L. (1972) *Am. J. Physiol.* **223**, 1376-1383
- Odessey, R., Khairallah, E. A. & Goldberg, A. L. (1974) *J. Biol. Chem.* **249**, 7623-7629
- Shepherd, R. E. & Gollnick, P. D. (1976) *Pflügers Arch.* **362**, 219-222
- Weiner, B. J. (1971) *Statistical Principle in Experimental Design*, 2nd edn., pp. 216-240, McGraw-Hill, New York