

LEUCINE METABOLISM IN THE BLACK BEAR

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Abstract: Metabolism of the essential amino acid leucine was studied in 3 black bears (*Ursus americanus*) in February while the bears were denning and not eating or drinking and in May while they were eating and drinking normally. (^{14}C) leucine was injected as a bolus intravenously, and timed samples of venous blood were obtained over an 8-day period. Total activity in plasma gradually increased in February and May, reaching a maximum at 12 hours. Total activity then gradually decreased to 50% of maximum after 8 days. Free ^{14}C leucine in plasma disappeared after 8–12 hours. Although free ^{14}C leucine was in plasma, traces of labeled glucose, pyruvate, and lactate were detected. In plasma proteins, activity was detected in the 1st sample at 30 minutes and gradually increased, describing a pattern similar to plasma total activity. Hydrolysis of plasma protein revealed that all counts were found in leucine. All ^{14}C counts after 12 hours could be accounted for in labeled leucine.

Leucine in summer and winter is rapidly removed from the plasma and incorporated into plasma proteins. Very small quantities of leucine are oxidized. Although plasma leucine is oxidized in winter, its level in blood plasma remains constant and similar to summer values. The data suggest that bears may synthesize this essential amino acid during dormancy.

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Bears undergo a prolonged period of starvation at near-normal body temperature during winter while denning. Despite no food or water for 100 days, while urea was continually produced (Nelson et al. 1975), no significant changes occurred in individual amino acid concentrations in plasma (Nelson et al. 1973). In contrast, in starving human beings allowed unlimited water, most plasma amino acids decreased after 40 days with the greatest decline in alanine (Felig et al. 1969).

More recent studies show that in denning bears, unessential amino acids such as alanine and serine are synthesized using glycerol derived from triglycerides (Ahlquist et al. 1984). The amino acids formed are not only incorporated into plasma proteins but are deaminated to form glucose, pyruvate, and lactic acid (Ahlquist et al. 1984).

However, protein synthesis also requires essential amino acids whose carbon structures cannot be synthesized by animals. Essential amino acids are also deaminated and catabolized, but they cannot be replaced except by dietary sources. This study traces the metabolism of an essential amino acid, leucine. Its concentration does not change during denning (Nelson et al. 1973), yet the bear does not eat during this period.

METHODS

Three captive adult male black bears were studied in February during hibernation and in May, after hibernation. In winter, bears were quartered in dens without food or water; in May they were given food and water ad lib. All bears were trapped as nuisance animals, 2 from Michigan's upper peninsula and 1

from northern Minnesota. Studies were performed at Mayo Clinic, Rochester, Minn.

Immobilization and maintenance under anesthesia were described previously by Nelson et al. (1973) and Ahlquist et al. (1984).

^{14}C -leucine was administered by single-pulse injection into the femoral vein (150 Ci to 2 bears, 100 Ci to the 3rd). Timed collections of blood were taken from the femoral vein contralateral to the site of isotope injection. Plasma was frozen immediately and stored at -20 C until assayed. Ion exchange chromatography was used to confirm the purity of the isotope and the absence of background levels of radiation in plasma specimens from all bears before the injection of leucine.

Mass isotope quantification of free amino acids in plasma and hydrolyzed protein using a Beckman 120B amino acid analyzer with a Beckman LS-100 C liquid scintillation counter were described previously (Ahlquist et al. 1984). This system was also used to determine ^{14}C in lactate, pyruvate, and glycerol. Plasma proteins were precipitated with trichloroacetic acid (TCA), washed, and a portion hydrolyzed with 6N HCl (Ahlquist et al. 1984) and fractionated by ion exchange to determine the ^{14}C containing compound(s). The *t*-test, with significance determined at the 5% level, was used for all statistical analyses.

RESULTS

In winter and spring, ^{14}C leucine was quickly cleared from the plasma (Fig. 1). Labeled lactate, pyruvate, and glucose were detected in plasma only when free labeled leucine was present (Fig. 2). All 3 were quickly cleared from plasma.

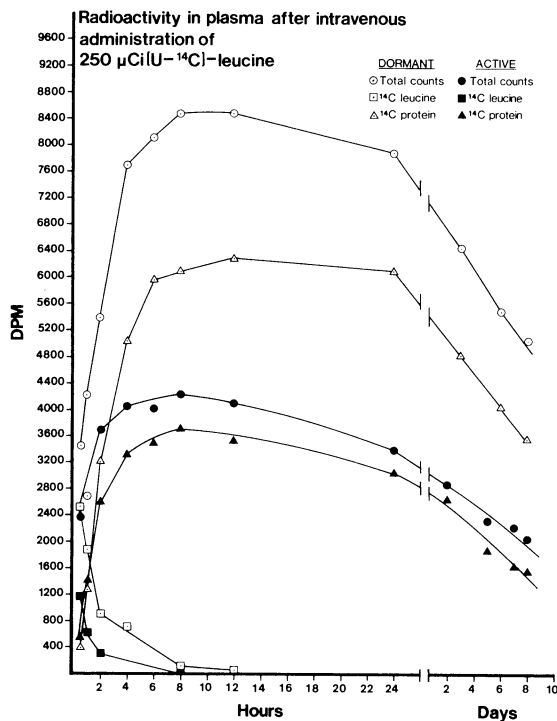


Fig. 1. ¹⁴C-activity in leucine and carbohydrate metabolites in bears (plasma).

Total ¹⁴C activity in plasma gradually increased, reaching a maximum in winter between 8 and 12 hours. In spring, the maximum was reached somewhat earlier, at 4 hours. Activity then gradually decreased, reaching 50% of maximum after 8 days (Fig. 1). The ¹⁴C activity found in plasma proteins described curves similar to those for total plasma ¹⁴C activity in winter and spring (Fig. 1). Most of the ¹⁴C given as free leucine, therefore, entered into plasma proteins.

The total activity of ¹⁴C found in plasma was compared with the sum of activity found in the methanol:ether extract, plasma proteins, free leucine, and other labeled substances. The sum of these measurements accounted for 110 ± 0.05% (SEM) in winter and 100 ± 0.02% (SEM) in spring. In winter and spring, after hydrolysis of plasma proteins, all activity was found in leucine (Table 1).

There were no significant differences between winter and spring in leucine concentration in plasma (Table 2); however, the concentration of plasma proteins in winter of 8.2 g/dl was significantly higher than the spring concentration of 7.4 g/dl (P < 0.001).

¹⁴C-ACTIVITY IN LEUCINE AND CARBOHYDRATE METABOLITES IN BEARS (Plasma)

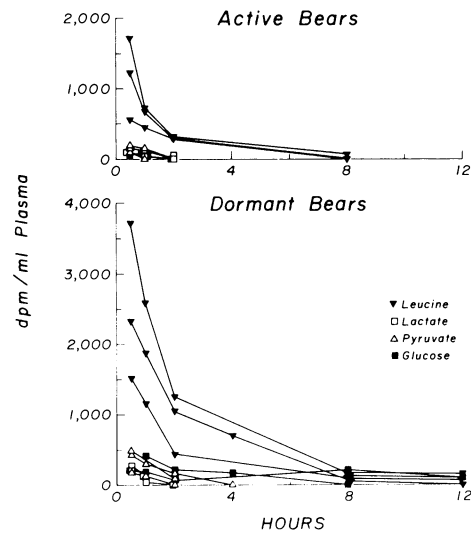


Fig. 2. Radioactivity in plasma after intravenous administration of (U-¹⁴C)-leucine.

DISCUSSION

The pattern of leucine metabolism was similar in winter and spring. The injected labeled leucine was quickly cleared from plasma but reappeared in plasma proteins. The only indication of oxidation was the appearance of labeled glucose, lactate, and pyruvate during the 1st 8 hours of study. This was at a time when free labeled leucine remained in plasma. The small amount of activity that appeared in glucose, lactate, and pyruvate was most likely due to oxidation of leucine to acetyl-CoA, which then entered the Krebs cycle. Oxaloacetate formed in the cycle was then converted to phosphoenolpyruvate, glucose, pyruvate, and lactate (Stryer 1981:415). When these carbohydrate intermediates were cleared from plasma, ¹⁴C was found only in leucine and in hydrolyzed plasma protein.

The data were consistent with the concept that leucine was conserved. When alanine, an unessential amino acid, was injected into bears, total plasma activity was highest immediately after injection, then gradually decreased. Its metabolites were easily detected. As alanine disappeared, labeled glucose, lac-

Table 1. Concentrations of amino acids in hydrolyzed protein during dormancy ($\mu\text{m}/100\text{ mg protein}$).^a

Amino acid	Mean and SEM
Aspartic acid	22.22 \pm 2.76
Threonine	16.45 \pm 1.69
Serine	19.22 \pm 2.15
Proline	15.56 \pm 1.77
Glutamic acid	33.30 \pm 4.58
Glycine	20.28 \pm 6.74
Alanine	21.02 \pm 2.86
Valine	22.81 \pm 2.74
Methionine	0.72 \pm 0.43
Isoleucine	6.04 \pm 0.50
Leucine	22.84 \pm 1.80 ^b
Tyrosine	8.18 \pm 1.32
Phenylalanine	11.42 \pm 1.38

^a Proteins sampled at 2 hours, 8 hours, and 6 days.

^b Only amino acid to show ¹⁴C activity.

tate, urea, and pyruvate appeared (Ahlquist et al. 1984). On the other hand, total ¹⁴C activity in plasma immediately after leucine was injected was the lowest observed. This suggests that leucine was taken up by tissues (most likely liver) and quickly incorporated into plasma proteins.

Further, once the labeled leucine was equilibrated in the body pool, its oxidation was so slow that activity could not be picked up in the metabolites. Plasma proteins were turning over, and specific activity of the plasma proteins decreased while concentrations remained constant. Thus, labeled leucine was replaced by unlabeled leucine. A continued turnover of body protein is suggested because the lean body mass of the bear remains constant during denning (Nelson et al. 1975, Lundberg et al. 1976). The fate of the labeled leucine once it left plasma proteins was unknown. The possibility that the bear was synthesizing leucine could not be ruled out. In adult rats, pigs, and humans a positive nitrogen balance can be maintained on low or leucine-free diets (Burroughs et al. 1940, Rose et al. 1955, Baker et al. 1966). However, these animals require dietary protein to achieve this. The bear maintained a normal leucine concentration in blood in spite of evidence of slow leucine catabolism. The question arises: can bears synthesize leucine while starving? Unfortunately, the experiment did not allow evaluation of the de novo synthesis of leucine in bears. However, if bears are able to replenish essential amino acids such as leucine

Table 2. Plasma amino acid concentrations (means and SEM) of dormant and active bears ($\mu\text{m}/100\text{ ml}$).

Amino acid	Dormant bears	Active bears
Aspartic acid	0.90 \pm 0.20	1.6 \pm 0.2
Threonine	13.05 \pm 1.56	12.5 \pm 1.3
Serine	9.01 \pm 0.29	12.7 \pm 1.0
Asparagine and glutamine	62.06 \pm 3.69	66.5 \pm 9.8
Proline	12.32 \pm 0.77	21.3 \pm 1.0
Glutamic acid	7.27 \pm 1.08	7.3 \pm 0.6
Citrulline	7.10 \pm 0.38	8.4 \pm 0.4
Glycine	28.71 \pm 1.83	30.9 \pm 4.8
Alanine	29.59 \pm 2.08	31.3 \pm 0.9
Valine	32.96 \pm 1.98	26.9 \pm 2.4
Methionine	3.57 \pm 0.23	4.1 \pm 0.3
Isoleucine	11.68 \pm 1.22	7.9 \pm 0.9
Leucine	17.32 \pm 0.01	14.5 \pm 1.7
Tyrosine	4.02 \pm 0.01	7.2 \pm 0.1
Phenylalanine	8.18 \pm 0.54	8.1 \pm 0.1

without eating, it may well be that a high biologic source of protein need not be eaten before denning to ensure survival.

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