

Amino Acid Interrelationships: The Effect of a Load of Leucine on the Metabolism of Isoleucine¹ (34772)

SADASHIV V. PHANSALKAR, PATRICIA M. NORTON, L. EMMETT HOLT, JR.,² AND SELMA E. SNYDERMAN²

Department of Pediatrics, New York University School of Medicine, New York, New York 10016

During an investigation of the plasma aminogram of the infant, an interrelationship between the branched chain amino acids became apparent (1, 2). The effect of either leucine withdrawal or loading on the plasma levels of isoleucine and valine was particularly striking. The withdrawal of leucine from the diet was followed, within 24 hr, by a significant increase in the levels of isoleucine and valine as well as the anticipated reduction in the leucine level; the levels were three to four times the control values. Conversely, when a load of leucine was given, a marked elevation of plasma leucine and a depression of the isoleucine and valine levels to approximately one-third of the preload value occurred within 3 hr. The levels of the other plasma amino acids were not affected by this withdrawal or loading. A number of possible mechanisms for these consistent deviations were considered. They did not seem to be due to competition for cellular transport systems since this would result in shifts in just the opposite direction, and a load of leucine would then be accompanied by an elevation, not a depression, of the isoleucine and valine levels. Competition for renal tubular reabsorption might bring about such alterations in plasma levels. However, when a load of leucine was administered, there was no increase in excretion of isoleucine and valine that might have accounted for the fall in plasma values, and leucine withdrawal was not accompanied by decreased isoleucine and valine excretion which could be responsible for the elevated levels of these amino acids. All the urinary changes paralleled the plas-

ma rather than affording an explanation for the observed deviations. Change in the rate of protein synthesis was also considered, but the fact that these alterations only involved certain of the amino acids made this unlikely.

These findings are consistent with the premise that a deficiency of one of the branched chain amino acids might depress the metabolic activity of the other two and conversely a load might have a stimulating effect. This was first investigated with leucine loading. Rats to which uniformly labeled ¹⁴C-isoleucine was administered were given a load of leucine and the amount of ¹⁴CO₂ in the expired air was determined before and after such loading.

Methods. The subjects were 26 weaning male rats of the Sprague-Dawley strain who were trained to consume an amino acid gel diet (3) every morning between 9 and 11 a.m. They were placed in a glass metabolism cage through which carbon dioxide-free air was passed at a constant rate. Expired carbon dioxide was collected in a mixture of monoethanolamine and ethylene glycol monomethyl ether (1:2; v/v) and radioactivity was determined according to the method of Jeffay and Alvarez (4). All samples were counted in a liquid scintillation spectrophotometer with an average efficiency of 85% cpm over the original dpm. The rats were fed 2.0 g of diet containing 8.0 μ Ci of L-isoleucine-(U)-¹⁴C in gel form and 12 hr later the expired air was collected for two separate hourly periods. It had been previously determined that the rate of ¹⁴CO₂ production was relatively constant by this time. Then the experimental rats (14) received 250 mg/100 g of L-leucine in gel form while control rats (12) received only the gel. After a 0.5-hr

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interval, determination of expired $^{14}\text{CO}_2$ was carried out for the next 4 hr.

Results and Discussion. There was a marked increase in the amount of $^{14}\text{CO}_2$ expired by each rat that received the leucine load. This increase was most pronounced during the first hour after the load was administered, but continued for the duration of the study. The $^{14}\text{CO}_2$ expired by the control rats continued to fall during this same period. Since the rate of $^{14}\text{CO}_2$ production varied in individual rats, the results are expressed as the change (cpm/hr); values for each rat are compared to the difference in $^{14}\text{CO}_2$ expired between the two hourly periods prior to the load. They are expressed graphically in Fig. 1. Results obtained in the first hour after the

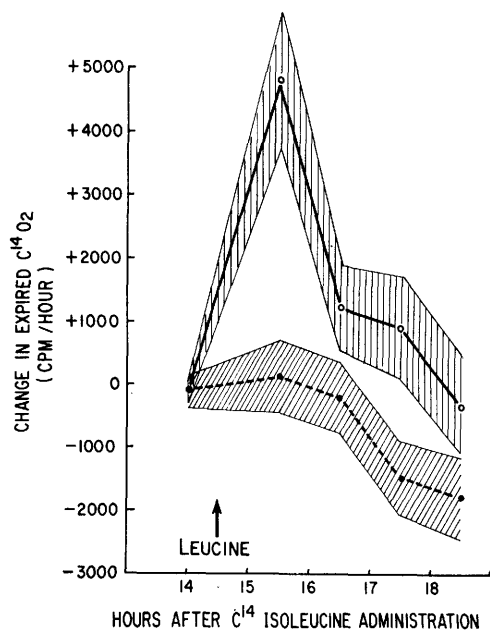


FIG. 1. The increase in $^{14}\text{CO}_2$ production resulting from a load of L-leucine to rats previously given L-isoleucine-(U)- ^{14}C : (---), average control; and (—), average experimental result; (shaded areas), standard error above and below the average.

load are statistically significant, $T = 3.50$, $p < 0.005$.

These results, together with the previously obtained data on plasma amino acids, demonstrate that an excess of one amino acid (leucine) can stimulate the metabolism of another (isoleucine). This is of particular interest in that although these amino acids are structurally related, the various steps in their metabolism are dependent on different enzyme systems. There are a number of factors known to induce enzyme activity such as increased level of substrate and certain hormones, but this seems to be the first demonstration that an excess of one amino acid can stimulate the activity of enzymes involved in the degradation of another amino acid. This stimulation of the metabolism of isoleucine by a load of leucine offers an explanation for the previously observed interrelationship between the plasma levels of leucine and isoleucine in the human infant.

Summary. The effect of a load of leucine on the metabolism of isoleucine was studied. Rats were fed L-isoleucine-(U)- ^{14}C and were given a load of leucine in gel form 12 hr later when the rate of $^{14}\text{CO}_2$ expired had become constant. This resulted in a significant increase in $^{14}\text{CO}_2$ expired, in every instance. This stimulation of the metabolism of isoleucine by a load of leucine offers an explanation for the previously observed depression of plasma isoleucine level after a load of leucine in human infants.

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