



Addition of leucine precursors to the diet of leucine-starved mice¹

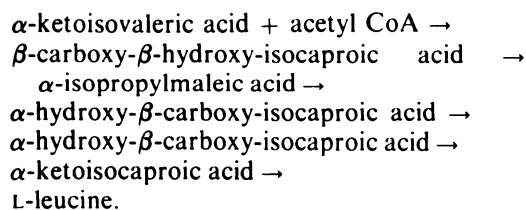
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ABSTRACT Leucine-starved mice placed on a diet supplemented with the immediate precursor of leucine, α -ketoisocaproic acid, regain lost weight. This weight gain is similar to that observed when the leucine-starved mice are provided with leucine in their diet. Mice on a leucine-free diet supplemented with α -ketoisovaleric acid, the first compound in the leucine biosynthetic pathway, continued to lose weight as quickly as mice on leucine-deficient diets. *Am. J. Clin. Nutr.* 28: 947-949, 1975.

On a protein-deficient diet, animals experience a decreased growth rate, decreased weight, and decreased reproductive activity (1). Animals maintained on a leucine-deficient diet become weak and inactive, have difficulty walking, lack personal cleanliness, and have a thinning of their hair (2). Food consumption does not decrease but animals do lose weight.

The effects of leucine starvation have been known for some time. With the elucidation of the leucine synthetic pathway in bacteria it is of interest to know if leucine-starved animals will recover lost weight when fed leucine precursors (3-5).

The bacterial leucine biosynthetic pathway is:



The first step involves a condensing enzyme (3). The next two steps involve isomerization by a single enzyme (4). The remaining two steps (4, 5) involve an NAD-linked

dehydrogenase and then a decarboxylase (5). The final product of the leucine operon enzymes is α -ketoisocaproic acid which, in combination with two glutamine and pyridoxal phosphate-dependent nonspecific transaminases, yields L-leucine.

Methods

All mice were housed in plastic cages with bed-o-cob bedding and fed synthetic amino acid diet without L-leucine (Nutritional Biochemical) and water ad libitum. All mice were of NIH albino strain. Mice begun on the special diet ranged in weight from 15 to 45 g. Each mouse was subsequently weighed every 7-10 days.

The mice were divided randomly into two groups. Group 1 was provided with distilled water without supplement and group 2 was provided with a 1% solution of L-leucine (Nutritional Biochemical) in distilled water. When mice in group 1 had lost 30-40% of their original weight, the mice in this group were then reassigned to one of three subgroups on the basis of the supplement to be in their drinking water: the drinking water of the mice in subgroup 1a contained no supplement; the drinking water for the mice in subgroup 1b was supplemented with 1% solution of α -ketoisovaleric acid (Sigma); and the drinking water for the mice in subgroup 1c was supplemented with 1% solution of α -ketoisocaproic acid (Calbiochem).

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Results

Figure 1 demonstrates the effect of the leucine-deficient diet on mouse weight. Animals fed the leucine-deficient diet had difficulty walking, became unkempt and weak, and displayed a hyperactive behavior. Their hair became thin, dull, and ruffled. Animals were quite lethargic just prior to death. Mice on the leucine-deficient diet lost weight continuously until all were dead. Those mice with 1% leucine in their drinking water, however, maintained their original weight or gained weight. Animals with a leucine supplement reproduced normally in contrast to those mice with no leucine in their diet (group 1). This latter group did not reproduce. The average weight at death in the group of mice on the leucine-deficient diet was 44% of the original weight. The original weight of a mouse fed only the leucine-deficient diet without leucine supplement tended to determine length of survival, i.e., larger mice lived longer than did smaller ones. Percent of original weight lost by the time of death was quite similar for each animal.

Figure 2 demonstrates weight recovery in leucine-starved mice fed a 1% solution of α -ketoisocaproic acid. Animals that had lost 30–40% of their original weight were able to regain most of that lost weight within 14 days

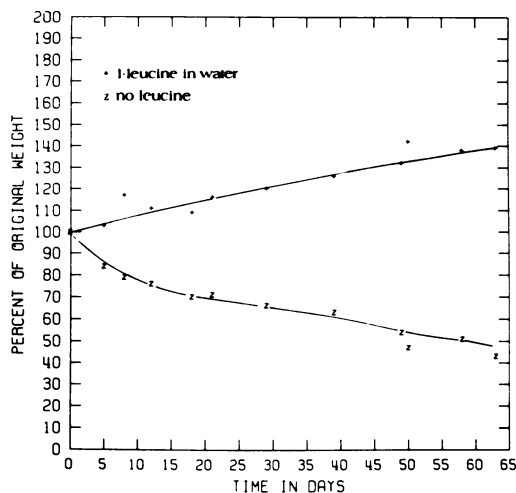


FIG. 1. Plot of percent original weight of mice when started on special diet without leucine in water (group 1) and special diet with 1% leucine in water (group 2); as a function of time. End of group 2 plot marks time when mortality on diet was 100%.

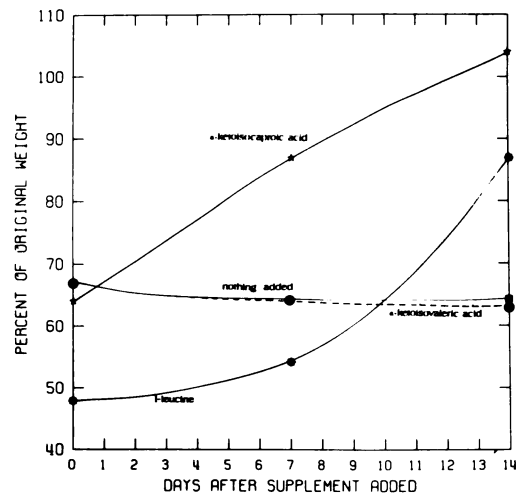


FIG. 2. Plot of percent original weight recovered or lost of leucine-starved mice with the following added to their water: α -ketoisovaleric acid (●); α -ketoisocaproic acid (★); L-leucine (●); and nothing (■).


with the addition of L-leucine or α -ketoisocaproic acid to their diets. No difference was found in overall recovery between these two supplements to the diet. Animals given α -ketoisovaleric acid continued to lose weight at the same rate as animals fed only the leucine-deficient diet and eventually died.

Discussion

Mice fed a special diet without leucine progressively lost weight and deteriorated until death. No mouse survived without a source of leucine. Starved mice provided with the immediate precursor of leucine, α -ketoisocaproic acid, recovered lost weight as well as did those provided with L-leucine. The mouse, therefore, does not need L-leucine in its diet to survive as it can synthesize leucine from the immediate precursor, α -ketoisocaproic acid. In contrast, feeding of the first compound in the leucine biosynthetic pathway, α -ketoisovaleric acid, does nothing to prevent or to prolong the ultimate demise of animals fed a diet deficient in leucine, an essential amino acid.

It has been found that diets high in leucine content mimic protein-deficient diets as the excess leucine inhibits use of two other essential amino acids, valine and isoleucine (6, 7). A diet made up to 1% leucine appears to meet the needs of the animals when balanced with

equal quantities of the branched chain amino acids.

Finally, mammals have many metabolic deficiencies, such as the need for certain amino acids and vitamins. Such deficiencies can be considered to be inborn genetic defects. The inability of the mouse to synthesize leucine may, therefore, provide a system in which to study potential gene therapy techniques for the correction of this and similar defects. 

References

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