

# Effect of vitamin B<sub>6</sub> on leucine-induced changes in human subjects<sup>1</sup>

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**ABSTRACT** Disturbances in the tryptophan-niacin pathway seen in endemic pellagra among sorghum eaters have been ascribed to high dietary intake of leucine. Vitamin B<sub>6</sub> plays an important role in several steps of this pathway. Therefore, studies on possible metabolic interrelations between excess dietary leucine and vitamin B<sub>6</sub> were undertaken in normal healthy human subjects. The results indicated that vitamin B<sub>6</sub> could successfully counteract the effects of leucine on quinolinic acid excretion in urine, and on in vitro nicotinamide nucleotide synthesis by erythrocytes, and also could correct the abnormalities of 5-hydroxytryptamine metabolism induced by excess leucine. These observations suggest that vitamin B<sub>6</sub> nutritional status may have a contributory role in the pathogenesis of endemic pellagra. *Am. J. Clin. Nutr.* 29: 177-181, 1976.

The occurrence of endemic pellagra among sorghum eaters has been reported from some parts of India, and the results of several studies have demonstrated that the high amount of the essential amino acid—leucine—present in sorghum is etiologically related. Administration of leucine to normal human volunteers has been found to increase the urinary excretion of quinolinic acid (1), to inhibit the capacity of erythrocytes to synthesize NAD in vitro (2), and to depress the activity of quinolinate phosphoribosyl transferase—a key enzyme in NAD synthesis in rats (3). It has recently been reported that pyridoxine deficiency in man leads to increased excretion of quinolinic acid, in addition to increased xanthurenic acid, and that these changes are corrected by the administration of pyridoxine (4). In women taking steroidal oral contraceptives, which are known to lead to pyridoxine deficiency, as well as in a subject given deoxypyridoxine—which is a pyridoxine antagonist—increased excretion of quinolinic acid in urine has been reported (5).

A number of enzymes in the tryptophan-niacin-ribonucleotide pathway are vitamin B<sub>6</sub>-dependent, and disturbances in this pathway are a feature of pyridoxine deficiency. Although pellagra is a disease arising from deficiency of nicotinic acid, it is not unusual to see signs of associated deficiency of other

vitamins of the B complex group. Studies on possible metabolic interrelationships between excess dietary leucine and pyridoxine were, therefore, undertaken to determine whether pyridoxine nutritional status had a contributory role in the pathogenesis of endemic pellagra among sorghum eaters.

## Materials and methods

Six apparently normal and healthy male volunteers, ages between 25 and 35 years, were investigated in a metabolic ward. The subjects were stabilized on a standard diet for a period of 7 days. The standard diet provided about 2500 calories and 45 g of protein daily, all the protein being derived from vegetable sources. The diet was calculated to contain 1.5 to 1.8 mg of vitamin B<sub>6</sub> and 3.5 to 4.0 g of leucine. After the period of stabilization on a standard diet, two 24-hr urine samples were collected. The subjects were then given 5 g of leucine/day for a period of 10 days and urine samples were collected again. Leucine was administered orally in the form of a suspension in water with glucose syrup as a sweetening agent in a single dose, after breakfast. Leucine was withdrawn for the next 10-day period and after collection of the urine samples during the last 2 days, 5 g of leucine were given daily together with 50 mg pyridoxine orally for the next 10 days and final urine samples were collected for 2 days. Pyridoxine was administered twice daily in tablet form, the first dose being given 2 to 3 hr after

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leucine administration. Quinolinic acid and nicotinic acid in urine were determined by microbiological assay (6).

A similar experimental design was followed for studies on 5-hydroxytryptamine (5-HT) metabolism. Five grams of leucine were given to volunteers for either 5 days or 10 days and platelet 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in urine were estimated initially, after giving leucine for either 5 or 10 days, after withdrawing leucine, and finally, after giving leucine together with pyridoxine. Blood was drawn under fasting conditions for 5-HT estimation and platelet 5-HT was determined. Platelet 5-HT was determined by the method of Weissbach (7); 5-HIAA in urine was estimated by the method of Korf and Valkenburgh-Sikkema (8).

Six normal subjects were taken up for studying levels of preformed nucleotides and the in vitro synthesis of nicotinamide nucleotides by erythrocytes. Fasting blood was analyzed initially for the nucleotides and after leucine administration (10 g/day) for a period of 10 days. After this period, the subjects were continued on 10 g of leucine/day, but in addition received pyridoxine, 50 mg/day by the parenteral route, for the next 10 days; at the end of this period blood was again drawn for nucleotide estimation. Erythrocytes were separated and prepared as described by Leder and Handler (9). Total nicotinamide nucleotides were estimated fluorometrically by the method of Levitas et al. (10). The in vitro synthesis of nucleotides from nicotinic acid was studied by the method of Preiss and Handler (11).

## Results

### *Urinary excretion of quinolinic acid and nicotinic acid*

Data on quinolinic acid and nicotinic acid excretion in urine are presented in Table 1. The 24-hr quinolinic acid excretion before giving leucine was 8.79 mg, which increased to 16.04 mg after leucine administration ( $P <$

0.01). When leucine was withdrawn, the value decreased to 10.17 mg/day. After leucine and vitamin B<sub>6</sub> were administered together, the excretion was 7.59 mg/day. In all of the six subjects studied there was a clear cut increase in urinary quinolinic acid excretion after leucine administration. After withdrawal of leucine, in five of six subjects the quinolinic acid excretion decreased and was equal to basal excretion; on administration of leucine with pyridoxine, quinolinic acid excretion decreased further in all but one subject. After leucine administration, urinary excretion of nicotinic acid decreased to a value which was 68.4% of the pre-leucine level. With the withdrawal of leucine the excretion increased to 95% of the basal value. When pyridoxine was administered along with leucine, excretion of nicotinic acid remained essentially unchanged from pre-leucine values.

### *Nucleotide synthesis*

Data on preformed total nucleotides and the in vitro synthesis of nucleotides by erythrocytes are indicated in Table 2. Total preformed nucleotide levels were not altered by any of the experimental procedures. However, the in vitro synthesis decreased significantly after leucine administration in all of the subjects investigated ( $P < 0.02$ )—an observation similar to that made earlier (2). With the administration of vitamin B<sub>6</sub> along with leucine, there was a considerable increase in the in vitro synthesis of nucleotides in every subject.

TABLE 1  
Effect of leucine and leucine with vitamin B<sub>6</sub> on excretion of quinolinic acid and nicotinic acid in urine in six normal human subjects

Experimental period	Quinolinic acid in urine (mg/24 hr)	Nicotinic acid in urine (mg/24 hr)
Period I: basal	8.79 $\pm$ 1.207	0.65 $\pm$ 0.095
Period II: after 5 g leucine/day for 10 days	16.04 $\pm$ 2.016	0.44 $\pm$ 0.066
Change from period I	+7.26 $\pm$ 1.625*	-0.20 $\pm$ 0.164
Period III: 10 days after withdrawal of leucine	10.17 $\pm$ 2.547	0.61 $\pm$ 0.032
Period IV: after 5 g leucine and 50 mg pyridoxine/day for 10 days	7.59 $\pm$ 1.310	0.59 $\pm$ 0.025
Change from period III	-2.57 $\pm$ 1.579	-0.04 $\pm$ 0.077

\*  $P < 0.01$  using paired  $t$  test. All values are mean  $\pm$  SE.

TABLE 2  
Effect of leucine and leucine with vitamin B<sub>6</sub> on erythrocyte nicotinamide nucleotides in six normal human subjects

Nucleotides in RBC	Period I: basal	Period II: after 10 g leucine/ day for 10 days	Period III: after 10 g leucine + 50 mg pyridoxine/day for 10 days
Preformed total nucleotides (mg/100 ml RBC)	5.78 ± 0.48 <sup>a</sup>	4.91 ± 0.60	4.78 ± 0.36
Synthesized nucleotides (mg/100 ml RBC)	12.72 ± 1.22	6.54 ± 1.22	15.49 ± 2.26
Decrease in synthesized nucleotides from period I to period II		-6.16 ± 1.79 <sup>b</sup>	
Increase in synthesized nucleotides from period II to period III			+6.79 ± 1.56 <sup>c</sup>

<sup>a</sup> All values are mean ± SE. <sup>b</sup>  $P < 0.02$ . <sup>c</sup>  $P < 0.01$ . (Paired *t* test.)

TABLE 3  
Effect of leucine and leucine with vitamin B<sub>6</sub> on platelet 5-HT and urinary 5-HIAA

Experimental period		Platelet 5-HT (ng/mg protein)	Urinary 5-HIAA (mg/24 hr)
Period I	Basal	578 ± 11.8 <sup>a</sup> (8) <sup>b</sup>	2.59 ± 0.194 (9)
Period II	After 5 g leucine/day for 5 days	493 ± 29.3 (8)	1.67 ± 0.093 (9)
	Decrease from period I	85 ± 30.4 <sup>c</sup>	0.92 ± 0.203 <sup>d</sup>
Period III	After withdrawal of leucine for 5 days	501 ± 22.3 (6)	2.50 ± 0.246 (7)
Period IV	After 5 g leucine + 50 mg vitamin B <sub>6</sub> /day for 5 days	482 ± 25.4 (6)	2.19 ± 0.194 (7)
	Decrease from period III	20. ± 21.2	0.31 ± 0.154

<sup>a</sup> All values are mean ± SE. <sup>b</sup> Numbers in parentheses are the number of subjects. <sup>c</sup>  $P < 0.05$ . <sup>d</sup>  $P < 0.01$ . (Paired *t* test.)

### 5-HT metabolism

Results of the studies relating to 5-HT metabolism are presented in Table 3. The platelet 5-HT level registered a significant fall after leucine was administered in six of eight subjects, both at the end of 5 days and in all of the four subjects who were also studied at the end of 10 days. Since the results at the end of 10 days were essentially similar to those of the 5-day study, the results of the 10-day study are not included in Table 3. Platelet 5-HT levels fell by 14.7% at the end of 5 days and by 41.3% at the end of 10 days after

leucine administration. On withdrawal of leucine, the changes in platelet 5-HT levels were equivocal. When leucine was given along with pyridoxine, in three of the six subjects there was an increase in platelet 5-HT and in the other three there was a marginal decrease. However, the extent of reduction in these three was of a much smaller order than what was observed with leucine alone. The platelet 5-HT decreased by 4% at the end of 5 days and 7.5% at the end of 10 days.

The excretion of 5-HIAA was also significantly decreased in all subjects after leucine administration, the reduction ranging be-



tween 35 and 37%. After withdrawal of leucine in six of seven subjects studied, there was an increase in 5-HIAA excretion and the mean value was not significantly different from that of the basal period. When leucine was given along with pyridoxine, there was only a marginal decrease in all except one subject. The reduction in urinary 5-HIAA excretion was only 12%.

### Discussion

Data presented here indicate that in the development of pellagra due to excess dietary leucine, pyridoxine nutritional status may have an important contributory role to play, since they show that at least three of the metabolic changes brought about by excess leucine administration can be prevented or minimized by the simultaneous administration of pyridoxine. The data on quinolinic acid excretion suggest that vitamin B<sub>6</sub> may have a role in the metabolism of quinolinic acid. It has been reported that administration of leucine to rats results in a reduction in the activity of quinolinate phosphoribosyl transferase, which is a key enzyme in the synthesis of NAD from quinolinate (3). This enzyme so far has not been shown to require vitamin B<sub>6</sub> as coenzyme (12). However, the source for the purification of the enzyme quinolinate phosphoribosyl transferase was mushrooms. These data also suggest that there may be another step in tryptophan metabolism beyond the cleavage of 3-hydroxykynurenine side chain, hitherto unrecognized, which may be pyridoxine-dependent.


It must, however, be pointed out that the amounts of pyridoxine used here are large and it is, therefore, possible that the effects seen are pharmacological effects. The possibility that leucine is metabolized at a faster rate than normal under the influence of large amounts of pyridoxine, so that it has little time to bring about the metabolic changes, also needs to be considered. It may be relevant in this context to point out that the activity of the enzyme leucine amino-transferase is lowered in pyridoxine-deficient states (13).

Mental changes seen in pellagra, including alterations in the EEG pattern, have been ascribed to alterations in the metabolism of

the neurohormone 5-HT (14-16). Levels of 5-HT (serotonin) in platelets and levels of 5-HIAA in urine and cerebrospinal fluid have been found to be low in pellagrins with mental depression (17). The results of the present investigation indicate that vitamin B<sub>6</sub> supplements can overcome to a great extent the fall in 5-HT and urinary 5-HIAA induced by leucine. These results are somewhat similar to those observed in women using oral contraceptives. Women taking the estrogen-progesterone combination type of pills often develop mild depression which is relieved by pyridoxine administration (18). In fact Rose and Braidman (19) have shown that steroid hormones promote the conversion of tryptophan to nicotinic acid by inducing the enzyme tryptophan oxygenase. Also, it has been shown that because of induction of other pyridoxal-dependent enzymes, less pyridoxal phosphate may be available for biosynthesis of 5-HT (19).

Excess leucine has been reported to have a similar effect on tryptophan oxygenase activity. Leucine may thus divert tryptophan into the niacin-ribonucleotide pathway, leading to low levels of 5-HT in platelets and low 5-HIAA in urine. Leucine may also increase the vitamin B<sub>6</sub> requirements. This possibility is suggested by the observation that administration of vitamin B<sub>6</sub> prevents the leucine-induced changes in serotonin metabolism to a considerable extent. 5-Hydroxytryptophan decarboxylase is known to be a pyridoxine-dependent enzyme and the observation of Robins et al. (20) that this enzyme may be rate-limiting in the synthesis of 5-HT in the human brain acquires significance in this context.

The results of some studies on pellagrins whose staple is sorghum have shown that in about one-third of the subjects studied, erythrocyte glutamic oxaloacetic transaminase levels are low (21) and that orolingual manifestations as well as peripheral neuritis seen in these cases often respond to pyridoxine administration (unpublished observation). The vitamin B<sub>6</sub> content of sorghum is much lower than that of other staples like rice and wheat, and diets based predominantly on jowar may therefore be expected to be inadequate with respect to this vitamin (unpublished observations). The results of the study reported here

indicate that low levels of pyridoxine may contribute to the development of the disease. Studies are now underway to determine the clinical response of pellagrins to pyridoxine administration. 

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