

## Plasma Amino Acids and Insulin Levels in Obesity: Response to Carbohydrate Intake and Tryptophan Supplements

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We assessed the plasma amino acids, glucose, and insulin responses of obese and lean control subjects to midafternoon carbohydrate snacks. After a standard 400 kcal lunch, eight lean and nine obese subjects received, at 2 PM, a 30 g sucrose snack; blood samples were obtained at hourly intervals until 6 PM. Each subject participated in four similar studies in which the carbohydrate snack was consumed alone or with 250, 500, or 1,000 mg of L-tryptophan (Trp), offered as a capsule. The obese group exhibited elevated plasma levels of the branched-chain amino acids, phenylalanine and tyrosine, and the levels of these amino acids declined much less in response to carbohydrate intake than in lean controls. As a consequence, the plasma ratio of Trp:large neutral amino acids (Trp/LNAA ratio), which normally rises after carbohydrate consumption, showed virtually no change in the obese group. The plasma Trp/LNAA response of this group did not reach control values even when carbohydrate intake was increased to 50 or 75 g. Peak plasma Trp concentrations and Trp/LNAA ratios after 250, 500, and 1,000 mg Trp doses were also significantly lower in the obese. Since brain Trp uptake is strongly correlated with the plasma Trp/LNAA ratio, which in turn determines the rate of brain serotonin synthesis, the blunted Trp/LNAA response to carbohydrate intake in the obese could contribute to alterations in the serotonin-mediated regulation of food intake.

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**P**LASMA AMINO ACID concentrations normally exhibit marked variations throughout the day in response to food intake and to food-induced hormone release<sup>1</sup>; the branched chain amino acids can be up to sixfold higher after a high-protein meal than after a carbohydrate meal. Studies measuring plasma amino acid levels in obesity reported increased concentrations of the branched-chain amino acids in the postabsorptive state and a blunted fall in these amino acids in response to a glucose challenge.<sup>2,3</sup> Since plasma tryptophan (Trp) levels are reportedly normal or even below normal in the obese,<sup>3</sup> the result is an abnormally low plasma tryptophan:large neutral amino acids (Trp/LNAA) ratio, as well as a decrease in the rise in this ratio that normally occurs after ingestion of carbohydrates.<sup>4</sup> Since this ratio determines how much of the precursor amino acid Trp becomes available to brain neurons synthesizing serotonin,<sup>5,6</sup> the alterations in plasma amino acid levels associated with obesity could affect serotonin-mediated brain neuro-transmission.

An impairment in central serotonergic function could compromise nutrient choice and the regulation of food intake. When rats<sup>7,8</sup> or obese people<sup>9,10</sup> are treated with serotonin agonists and offered a choice of foods of different carbohydrate content, they diminish the proportion of their total caloric intake represented by carbohydrates, while sustaining protein intake. It has been proposed that this response could be part of a physiological feedback system, by which mammals regulate macronutrient intake.<sup>11,12</sup> Carbohydrate meals, by increasing the plasma Trp/LNAA ratio (a phenomenon mediated by insulin), increase brain tryptophan

uptake, and consequently, serotonin release. Serotonin acts then physiologically as the serotonergic agonists do, inhibiting carbohydrate intake relative to that of protein in subsequent meals. A decreased rise in the Trp/LNAA ratio after eating carbohydrates could, consequently, contribute to the specific carbohydrate hunger reported by many obese persons characterized as carbohydrate cravers.

The present study explored the response of plasma amino acids, insulin, and glucose to carbohydrate intake in a group of obese subjects. We also assessed the effect of Trp administration on the plasma amino acid levels and Trp/LNAA ratios.

### MATERIALS AND METHODS

Nine obese and eight lean subjects were studied. The obese group was drawn from a larger set of patients who participated in a previous study to assess patterns of food choice. In that study, all had been categorized as carbohydrate cravers,<sup>9</sup> and on-line records of food intake had shown that they preferentially consumed carbohydrate-rich snacks in the afternoon, between 2 PM and 4 PM. Thus, in the present study we used that time period for exploring the plasma hormone and substrate levels after carbohydrate consumption. Both lean and obese volunteers were healthy and free of medication, and had normal blood cell counts, transaminases, and bilirubin values. The study protocol was approved by the Committee on the Use of Humans as Experimental Subjects of Massachusetts Institute of Technology, and written consent was obtained from all volunteers.

The study consisted of 4 one-day experiments. On each of the study days, after an overnight fast, subjects received a 195 kcal breakfast at 8 AM, and a 400 kcal (55% carbohydrate, 15% protein, and 30% fat) lunch at noon. At 2 PM, a 30 g-sucrose, lemon-flavored snack was administered along with 0, 250, 500, or 1,000 mg of L-tryptophan, administered by capsule. Plasma glucose, amino acids, and insulin levels were measured at noon, and hourly between 2 PM and 6 PM. The order of each test was randomly assigned, and a 1-week period elapsed between tests. LNAA levels in deproteinized plasma samples were determined by high-performance liquid chromatography with postcolumn derivatization and fluorometric detection. Total tryptophan was measured by the method of Denckla and Dewey.<sup>13</sup> Plasma insulin was measured using a commercial RIA kit (Immuno Nuclear Co, Stillwater, MN). This assay uses a human

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Table 1. Data of the Groups Studied

|                 | Lean<br>(n = 8) | Obese<br>(n = 9) |
|-----------------|-----------------|------------------|
| Age (yr)        | 30 ± 4          | 39 ± 8           |
| Weight (kg)     | 60.8 ± 9.9      | 107.4 ± 26.0*    |
| Body Mass Index | 21.3 ± 2.3      | 38.7 ± 10.7*     |
| Obesity Index   | 0.98 ± 0.1      | 1.7 ± 0.4*       |

Values given are the mean ± SD.  
\*Groups differ, *P* < .01, Student's *t* test.

insulin antibody, and includes a preliminary PEG step to remove endogenous antibodies; its sensitivity in our laboratory is around 2 μU/mL, and the variability between assays is <7%.

To determine the amount of carbohydrate that obese subjects had to consume to attain Trp/LNAA ratios comparable to controls, the obese volunteers participated in two additional studies. Using the same schedule as described above, they received 50 g or 75 g carbohydrate snacks on two separate days.

Baseline plasma amino acid concentrations for lean and obese were compared by Student's *t* test for unpaired samples. The plasma substrate response curve was evaluated by a two-way ANOVA for unbalanced designs, with group and time as the main effects. The CLINFO and SAS statistical packages were used.

RESULTS

The anthropometric characteristics of the two groups studied are summarized in Table 1. Noon levels of plasma insulin (Fig 1) were higher in the obese, but this difference did not attain statistical significance. Noon values of branched-chain amino acids, tyrosine, and phenylalanine and the Trp/LNAA ratio (Table 2) were, on the other hand, significantly higher in the obese group. Two hours after lunch (2 PM), mean branched-chain amino acid concentrations were similar in both groups: 565.5 and 546.6 mmol/L in lean and obese, respectively (Fig 2). The day-to-day variability of noon amino acid and insulin values was approximately 15%. For example, the mean values of branched-chain amino acid levels at noon in each of the four test days

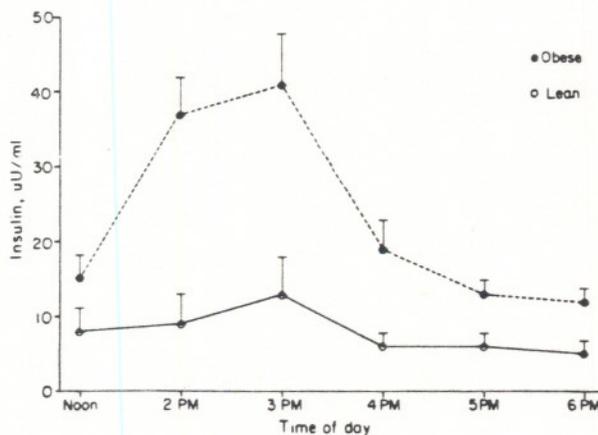


Fig 1. Plasma insulin levels after carbohydrate intake in lean and obese. Subjects received a 400 kcal lunch at noon and a 30 g sucrose snack at 2 PM. Values are mean ± SEM. Levels in the obese group at 2 PM, 3 PM, and 4 PM are significantly higher than in lean controls (*P* < .05).

Table 2. Plasma Amino Acid Levels at Noon

|                | Lean          | Obese          |
|----------------|---------------|----------------|
| Valine         | 185.2 ± 47.0  | 225.0 ± 38.1*  |
| Leucine        | 117.2 ± 11.2  | 135.7 ± 23.0*  |
| Isoleucine     | 55.8 ± 6.1    | 70.7 ± 21.3*   |
| Tyrosine       | 50.2 ± 9.8    | 71.1 ± 12.2*   |
| Phenylalanine  | 48.3 ± 6.4    | 60.7 ± 9.0*    |
| Tryptophan     | 62.7 ± 6.9    | 56.0 ± 12.1    |
| Trp/LNAA ratio | 0.122 ± 0.017 | 0.097 ± 0.012* |

Values represent μmol/L, mean ± SD of four measurements on different days.

\*Groups differ, *P* < .05, Student's *t* test.

were 358, 372, 345, and 381 μmol/L in lean controls and 424, 442, 454, and 444 μmol/L in the obese. Likewise, the response curve to the 30 g carbohydrate snack was consistent in each individual in all test days, and concurrent administration of tryptophan had no significant effect on plasma amino acid levels other than tryptophan.

The 30 g carbohydrate snack produced similar blood glucose response curves in lean and obese. Plasma insulin concentration was significantly higher in the obese group at 2 PM, 3 PM, and 4 PM (Fig 1). With the 30 g carbohydrate dose, peak hormone level was 41 ± 20 and 13 ± 4 μU/mL in obese and lean, respectively. At the end of the test at 6 PM, plasma insulin level was 5 ± 1 in lean and 12 ± 4 μU/mL in obese.

Administration of 30 g carbohydrate at 2 PM caused a significant fall in the plasma concentration of branched-chain amino acids in the control group, down to 282.1 ± 73 mmol/L at 5 PM. In contrast, the plasma BCAA level of the obese group at this same time point was 422.9 ± 73 mmol/L (*P* < .05) (Fig 2). Peak plasma Trp/LNAA ratio was attained one hour after carbohydrate intake in both groups, reaching 0.150 ± 0.031 in controls and 0.108 ± 0.017 in obese (*P* < .05) (Fig 3).

Response to Tryptophan Doses

Administration of 250 mg of tryptophan along with the carbohydrate snack caused an elevation of mean plasma Trp to reach, at 4 PM, 101.3 mmol/L in lean and 92.2 mmol/L in obese. Peak plasma Trp/LNAA ratio was 0.286 ± 0.078 in

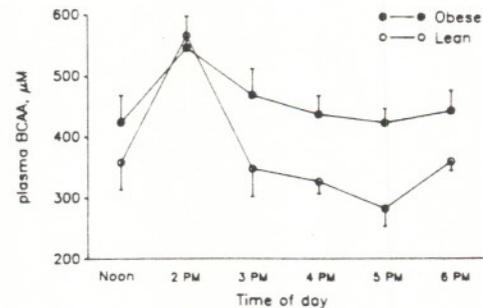


Fig 2. Plasma branched-chain amino acids (valine + leucine + isoleucine) after ingestion of a 400 kcal lunch at noon and a 30 g sucrose snack at 2 PM. Except for the 2 PM values, all differences between lean and obese are significant (*P* < .05). Values are mean ± SEM.

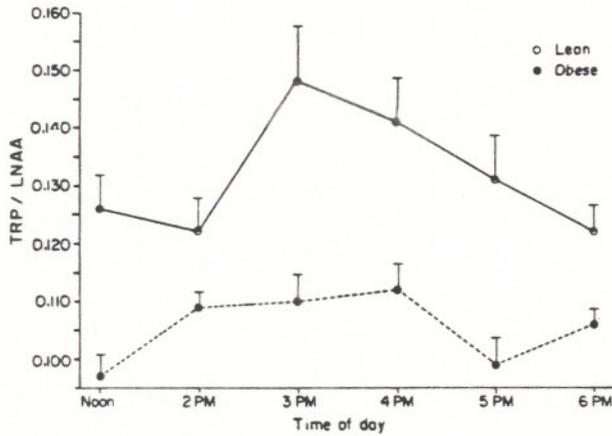


Fig 3. Plasma Trp/LNAA ratio after ingestion of a 400 kcal lunch at noon and a 30 g sucrose snack at 2 PM. Values are mean  $\pm$  SEM. All differences between lean and obese are statistically significant ( $P < .05$ ).

controls and  $0.159 \pm 0.032$  in obese. This peak response for each tryptophan dose is presented in Fig 4. None of the tryptophan doses used affected the branched-chain amino acid response to carbohydrate in either group.

Peak plasma tryptophan levels after ingestion of 500 mg and 1,000 mg tryptophan were significantly higher in lean than in obese. The plasma tryptophan response curve to the highest tryptophan dose used (1 g) is shown in Fig 5.

#### Response to Carbohydrate Intake in the Obese Group

Besides the 30 g carbohydrate test, two additional carbohydrate tests were performed in the obese group, at levels of 50 g and 75 g of intake. After ingestion of 50 g carbohydrate at 2 PM, obese subjects exhibited a fall in branched-chain amino acids from  $500.5 \pm 48$  to  $447.7 \pm 115$  mmol/L at 4 PM. The Trp/LNAA ratio increased from 0.090 to 0.100. After ingestion of 75 g carbohydrate, the peak Trp/LNAA ratio rose to  $0.103 \pm 0.015$ , a level still significantly lower than that attained by lean people after ingestion of 30 g carbohydrate (Fig 6).

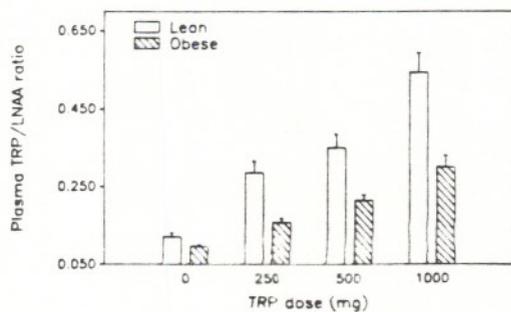


Fig 4. Peak plasma Trp/LNAA ratios after oral tryptophan administration. Subjects received the amount of L-tryptophan in capsule form, along with a 30 g sucrose snack, at 2 PM, two hours after the standard 400 kcal lunch. Values are mean  $\pm$  SEM. All differences between lean and obese are statistically significant ( $P < .05$ ).

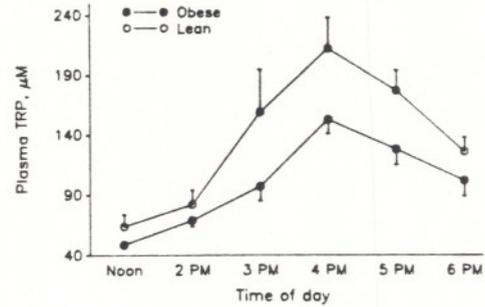


Fig 5. Dose-response curve of plasma tryptophan after ingestion of 1 g of L-tryptophan administered in capsule form, along with the 30 g sucrose snack at 2 PM. Values are in  $\mu$ mol/L, mean  $\pm$  SEM. Differences between lean and obese in values at 3 PM, 4 PM, and 5 PM are statistically significant ( $P < .05$ ).

The peak blood glucose level reached after the 75 g dose was  $145 \pm 13$  mg/dL, one hour after ingestion.

#### DISCUSSION

These data show that the plasma amino acid response to a 30 g carbohydrate snack differs significantly in obese and lean persons. It also shows that moderately obese subjects with normal glucose tolerance and plasma insulin levels that are not significantly elevated exhibit high plasma levels of the branched-chain amino acids and of phenylalanine and tyrosine. Our data also indicate that the normal rise in the plasma Trp/LNAA ratio caused by carbohydrate consumption is markedly blunted in the obese (Fig 3). The lack of a significant increase in that ratio is a consequence of persistently high levels of branched-chain amino acids, even after the meal-induced insulin release (Fig 2). For example, after ingestion of 30 g carbohydrate the amino acid valine fell only by 18%, as compared with 46% in the control group. As a result, the rise in the plasma Trp/LNAA ratio induced by carbohydrate ingestion was significantly less in the obese.

The abnormal amino acid response to carbohydrate intake is a manifestation of the insulin resistance associated with obesity. Early studies reporting elevated plasma levels of several amino acids in obese subjects<sup>2,14</sup> already suggested a

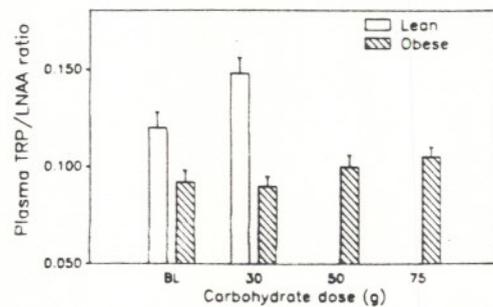


Fig 6. Peak plasma Trp/LNAA ratios in lean and obese after ingestion of a 30 g sucrose snack, and in obese after ingestion of 50 g and 75 g sucrose snacks, all administered at 2 PM in different days. BL, baseline (2 PM) levels. Values are mean  $\pm$  SEM. All differences between lean and obese are statistically significant ( $P < .05$ ).

link between this hyperaminoacidemia and decreased insulin sensitivity. The amino acids usually elevated are valine, leucine, isoleucine, phenylalanine, and tyrosine,<sup>2,3,14,15</sup> although high alanine concentrations in the postabsorptive state have also been reported.<sup>14</sup> In addition, abnormally low plasma tryptophan levels have been described in obese subjects with hyperinsulinemia.<sup>3</sup> In spite of the association between insulin resistance and elevated amino acid levels, there is no clear correlation between plasma insulin and amino acid concentrations,<sup>15,16</sup> and one study found normal valine disposal in obese persons with impaired glucose disposal.<sup>17</sup> The relationship between insulin resistance and amino acid metabolism is complex, and is affected by factors such as duration and severity of obesity and lean body mass.<sup>18</sup> Furthermore, it is possible that carbohydrate cravers constitute a subgroup of obese persons with higher branched-chain amino acid: insulin ratios than the general obese population, and that this is the basis for their impaired appetite behavior.

Although the plasma insulin levels in our obese group were not significantly different from controls (mainly because of the large variability), mean values were 60% higher in the obese. This difference in insulin concentration is similar to that reported by Forlani et al.<sup>15</sup> The mean noon plasma insulin level of our obese group (15  $\mu$ U/mL) is also identical to the mean fasting value reported by Glass et al in 17 obese,<sup>17</sup> and very similar to that of Pennetti et al in 74 obese women and 42 obese men.<sup>16</sup>

Our study found significant elevations in all the branched-chain amino acids, phenylalanine, and tyrosine at noon (Table 2). Plasma tryptophan levels tended to be lower in the obese, but the difference did not reach the statistical significance reported by Ashley et al.<sup>3</sup> These investigators found that the only amino acid significantly elevated in nondiabetic obese was tyrosine. Other neutral amino acids were elevated only in diabetic obese, who had impaired glucose tolerance or marked hypoinsulinemia or hyperinsulinemia. The authors also reported no differences between lean and nondiabetic obese in the 120-minute amino acid response to a 100 g oral glucose challenge. The contrast with findings presented here could be due to differences in the population studied, but they may also suggest that the plasma amino acid response to carbohydrate intake is different in the morning, after an overnight fast, than in midafternoon. Studies in normal individuals indicate that the time of day affects the plasma amino acid response to meal composition.<sup>19,20</sup> We chose the time of our study based on the pattern of snack consumption observed in this population of obese persons, to explore the possible biochemical correlates of their craving for carbohydrate foods.

Several lines of evidence support the role of central

serotonergic transmission in the regulation of appetite and food choice. The serotonin reuptake inhibitor fenfluramine selectively decreases carbohydrate intake in obese persons,<sup>10</sup> as does the serotonin precursor tryptophan in some obese individuals.<sup>9</sup> A feedback mechanism between diet composition and the serotonergic neurons, mediated by the plasma Trp/LNAA ratio, has been proposed. In this model, high-carbohydrate diets, by causing a marked rise in the plasma Trp/LNAA ratio, would increase brain tryptophan uptake and serotonin synthesis, which in turn acts by inhibiting specific hunger for carbohydrates.<sup>11</sup> Since the plasma Trp/LNAA ratio is correlated with brain tryptophan concentration and serotonin synthesis in animals<sup>6,21</sup> and presumably in humans, the blunted Trp/LNAA response to carbohydrate ingestion may contribute to the decreased central serotonergic transmission and carbohydrate craving described in some groups of obese persons. Our data showed that, at the time of day in which they tended to consume more starchy snacks, obese subjects were unable to raise their Trp/LNAA ratio to control levels, even when consuming twice as much carbohydrate.

Increases in brain tyrosine levels have been shown to enhance the synthesis of catecholamines in and the release from physiologically active neurons.<sup>22</sup> In contrast, increases in brain phenylalanine levels can have the opposite effect.<sup>23</sup> Hence, the possibility should be considered that the elevations in plasma levels of these two amino acids, as found in our study, might contribute to the behavioral and regulatory disturbances associated with obesity.

An unexpected finding in our study was the blunted response to oral tryptophan administration in the obese. Not only were peak plasma Trp/LNAA ratios significantly lower than in controls at all dose levels, but the *absolute* peak plasma tryptophan concentrations and the areas under the curve were also lower in the obese, suggesting that the plasma appearance rate of tryptophan after oral administration is different than in lean subjects. This difference persisted even when the tryptophan dose was corrected per body weight. Although we measured plasma levels for only four hours after administration, it seems unlikely that the different tryptophan response curve could be explained only by differences in gastric emptying and rate of absorption, since the plasma glucose response curves were very similar in lean and obese. Other possible differences may be related to the rate of first-pass removal of the amino acids by the liver, and in the subsequent oxidation in this organ. In any case, these results indicate that obese subjects may require larger doses of tryptophan in order to reach plasma levels comparable to lean controls. This different response should be considered in studies assessing the effects of tryptophan supplements on food intake or behavior in obese.

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