Effect of Various Oral Glucose Doses on Plasma Neutral Amino Acid Levels

R. Martin-Du Pan, C. Mauron, B. Glaeser, and R. J. Wurtman

Six healthy, nonobese, fasting subjects each received, on different days 0, 6, 12.5, 25, or 50 g of glucose (Glucoza) in a total volume of 100 ml. Blood was taken at intervals and assayed for plasma levels of the branched-chain amino acids (valine, isoleucine and leucine); the other major large neutral amino acids (LNAA) (methionine, phenylalanine, tyrosine and tryptophan); and, in some cases, insulin and glucose. Insulin levels were significantly elevated 30 min after consumption of 12.5, 25, or 50 g of glucose, and were higher after the 50 g dose than after 12.5 g. Changes in plasma glucose concentrations were small and did not correlate with glucose dose. Mean percent reductions of LNAA tended to exhibit dose-dependence, most clearly observed after 120 min. In some subjects as little as 6 g of glucose transiently decreased LNAA concentrations. Branched-chain amino acids were most sensitive, decreasing by 35%–41% after 50 g of glucose. Plasma tryptophan concentrations fell only by 23%, hence the ratio of plasma tryptophan to other plasma LNAA (which affects brain serotonin synthesis) increased significantly.

PLASMA CONCENTRATIONS of large, neutral amino acids (LNAA) in humans exhibit diurnal fluctuations that are generated by the cyclic ingestion of food and are dependent on meal composition: if the initial daily postfasting meal (i.e., breakfast) is rich in carbohydrates and poor in proteins, it causes an insulin-mediated reduction in plasma LNAA other than tryptophan; however if its protein content is greater than about 10% of total calories its direct contribution of LNAA to the blood stream can overcome the insulin effect, and cause LNAA levels to rise.

Although some data are available on the amounts of carbohydrate that must be consumed to elevate serum insulin levels, apparently none have been published on the dose-response relationships between carbohydrate intake and the insulin-mediated fall in plasma LNAA. Such data could be useful in predicting and explaining meal-induced changes in central nervous system functions, inasmuch as plasma concentrations of various LNAA are known to influence brain tryptophan and tyrosine levels, and thereby affect the synthesis of serotonin and the catecholamines. In the rat, ingestion of a carbohydrate-rich, protein-free meal is rapidly followed by an insulin-mediated reduction in plasma LNAA, but little or no change in plasma tryptophan; this increases the ratio of plasma tryptophan to the sum of the plasma concentrations of other LNAA, thereby facilitating tryptophan’s uptake across the blood-brain barrier, increasing brain tryptophan levels; enhancing the substrate saturation of the enzyme tryptophan hydroxylase, and accelerating serotonin’s synthesis. Neuronal catecholamine synthesis can similarly depend on brain tyrosine levels, which vary with the ratio of plasma tyrosine to the sum of the other LNAA. The increase in brain serotonin that follows consumption of a carbohydrate meal may affect subsequent food intake: Data from studies on rats and humans suggest that the release of neuronal serotonin following carbohydrate consumption, or following the administration of drugs like fenfluramine, suppresses the elective consumption of carbohydrates but not protein, i.e., when subjects are allowed a choice of foods. If a similar mechanism coupling carbohydrate consumption to serotonin synthesis actually operates in humans, it becomes important to determine the amount of carbohydrate needed to activate it by changing the plasma tryptophan/LNAA ratio, and the extent to which the mechanism is altered in diseases (like diabetes or obesity) which suppress the secretion of insulin or impair its receptor-mediated metabolic effects. The present study shows that only relatively small quantities of glucose are needed to lower plasma LNAA in fasting healthy young adults.

MATERIALS AND METHODS

Following a protocol approved by the MIT Committee on the Use of Humans as Experimental Subjects, 6 healthy volunteer outpatients, aged 25–33 yr. participated in the study; 3 were males and 3 females. Mean weight was 60 kg for the women and 70 for the men; all subjects were within 10% of their ideal body weight as determined by Metropolitan Life Insurance Company tables. No subject was taking any medication and none had a family history of diabetes mellitus. Subjects were asked to take meals rich in carbohydrate content (about 300 g/day) for 3 days before beginning the study. On 5 separate days, each subject fasted from 8 p.m. till 9 the next morning and then received, at the M.I.T. Clinical Research Center.

From the Laboratory of Neuroendocrine Regulation, Massachusetts Institute of Technology Cambridge, Massachusetts.

Received for publication October 22, 1981.

Supported in part by grants from the National Institutes of Health, and The National Aeronautics and Space Administration.

Dr. Martin-Du Pan is the recipient of a fellowship from Stiftung zur Förderung der Ernährungsforschung in der Schweiz.

Address reprint requests to Dr. R. J. Wurtman, Laboratory of Neuroendocrine Regulation, Massachusetts Institute of Technology Room E25-604, Cambridge, MA 02139.

© 1982 by Grune & Stratton, Inc.

0026-0495/82/1109-0014$01.00/0
a load of 0, 6, 12.5, 25, or 50 g of glucose (caffeine-free glucola)
dissolved in water in a total volume of 100 ml; the order of doses was
randomized. Venous blood samples were drawn from the antecubital
vein through an indwelling catheter at 0, 60, and 120 min for all
doses and additionally at 30 and 180 min for the 12.5, 25, and 50 g
doses. Blood was collected in heparanized tubes; after centrifugation
plasma was stored at −20°C until analyzed. It was not possible to
obtain blood for the last dose in subject 2 (0 g of glucose).

Glucose was analyzed spectrophotometrically after reaction with
0-toluuidine in hot acidic acid (Sigma Bulletin #35:15). Insulin was
measured using a commercial radioimmunoassay kit by a double
antibody method (Bio-RIA, Louisville, KY). Plasma tryptophan
was measured fluorometrically on 20 μl plasma aliquots by the
method of Denckla and Dewey, as modified by Lehman.16,17
Branched-chain amino acids and aromatic amino acids were mea-
sured in 500 μl aliquots of plasma, deproteinized with sulfosalicylic
acid,2 using a Beckman amino acid analyzer (Beckman Instruments,
Palo Alto, Calif., Model 119C).

Date, given as means ± SEM, were analyzed by two-way analysis
of variance; factors compared were dose and time. The Scheffe test
was employed to test the statistical significance of differences: p <
0.05 was considered significant. Areas under the curves were evalu-
ated by the method of trapezoid approximation. Student t tests for
paired data were used to evaluate the significance of changes in
individual amino acids. Amino acid concentrations after treatments
for each subject were also expressed as a percentage of that day's
pretreatment levels (Fig. 3); mean percent changes were then
calculated for each treatment and time interval (e.g., Fig. 2).

RESULTS

Following ingestion of 12.5, 25, or 50 g of glucose, mean plasma glucose concentrations rose after 30 min
from 83 ± 2.2 mg % to 105 ± 8.6, 122 ± 11.0, and
114 ± 7.9 mg % respectively, and returned to baseline
after 60–120 min (Fig. 1). Mean insulin concentra-
tions increased from 9 ± 0.9 μU/ml to 21.3 ± 3.8, 44 ±
5.0, and 58 ± 6.9 μU/ml, respectively, after 30 min.

Glucose consumption caused a dose-related depres-
sion of plasma LNAA concentrations (expressed as
percents of pretreatment levels) after 60 and 120 min
(Figs. 2 and 3; Table 1). Significant changes in LNAA
were not observed when subjects consumed the glu-
cose-free control solution. The decreases observed 60
and 120 min after subjects received the 25 and 50 g
doses were significantly greater than those following
the 0 and 6 g doses (p < 0.05). Because of the high
intra-individual and inter-individual variations in
basal LNAA concentrations (especially for the branched-chain amino acids) significant dose-related
decreases in mean absolute LNAA concentrations
occurred only at 120 min after the largest glucose
doses. The mean percent decreases in LNAA concen-
trations at this time were however, highly correlated
with glucose doses (r = 0.95; p < 0.01; Fig. 3) as well
as with insulin levels (r = 0.96; p < 0.01).

Among individual amino acids (Table 1) absolute
iso-leucine concentrations decreased significantly with
time after 12.5–50 g of glucose; leucine, tyrosine and
phenylalanine decreased significantly after 25 and 50
g, while methionine and tryptophan decreased signifi-
cantly only after 50 g. Branched-chain amino acids
concentrations decreased by 35–41% 120 min after
subjects received 50 g of glucose; methionine by 31%,
phenylalanine and tyrosine by 29–31%, and trypto-
phan by only 23% (Table 1). Because of this smaller
reduction in plasma tryptophan (Fig. 2), the trypto-
phan/LNAA ratio became elevated (Fig. 2), as has
been noted previously after carbohydrate consump-
tion;2 the changes were statistically significant after 25
or 50 g of glucose (Table 1). The tryptophan/LNAA

Fig. 1. Mean concentra-
tions ± SEM of plasma insulin
(μU/ml) and glucose (mg/100
ml) after 6 fasting subjects
ingested 0, 12.5, 25 or 50 g
of glucose. The symbols used are
the following: ■, 12.5 g of
 glucose; ○, 25 g of glucose ■,
 50 g of glucose.
ratio calculated 120 min after treatment was significantly correlated with glucose doses ($r = 0.87$; $p < 0.05$). In contrast, the tyrosine/LNAA ratio did not change significantly with any glucose dose.

Among 4 of the subjects (numbers 1, 3, 5, and 6), as little as 6 g of glucose may have been sufficient to reduce plasma LNAA at one or more of the times tested (Fig. 4A,B); subject 2 probably required 12.5 g, while subject 4 may have needed 25 g. Among subjects 2 and 4 there was a clear inverse linear relationship between the rise in plasma insulin after 12.5–50 g of glucose and the fall in LNAA (as assessed by calculating areas under the curve for each dose); this relationship was not robust for the other subjects. Even though the 50-g glucose dose caused a greater elevation in insulin than the 25-g dose in 4 of the 6 subjects, it was not consistently associated with a greater percent reduction in plasma LNAA (Figs. 2 and 4); the 50-g dose was associated with lower absolute concentrations of the amino acids than the 25-g dose after 120 min (Table 1); however, for unknown reasons, zero-time plasma LNAA concentrations were also lower on days that subjects received 50 g than when they took the 25-g dose.

**DISCUSSION**

These data show that glucose consumption causes dose-related decreases in plasma LNAA concentrations among healthy young fasting subjects. In some subjects as little as 6 g of glucose may be sufficient to decrease LNAA; larger doses (25–50 g) are needed to increase the plasma TRP/LNAA ratio, and thus to affect brain tryptophan and serotonin levels.

Previous studies have compared the effects on plasma insulin of glucose doses of 25 g or more.$^{4,18,19}$ Our observations (Figs. 3 and 4) indicate that even lower doses can elicit the secretion of sufficient insulin to activate the receptors that affect the flux of LNAA between the plasma and such tissues as skeletal muscle.$^{20}$ The insulin-dependent uptake of the branched-chain amino acids into muscle, and their subsequent transamination or incorporation into protein, constitute the major mechanisms for retarding the increases in their plasma levels that would otherwise occur after protein ingestion, inasmuch as these compounds are metabolized only marginally in the

---

*Fig. 2. Mean % decrease in plasma (LNAA) and TRP concentrations, 60 and 120 min after administration of 0, 6, 12.5, 25 and 50 g of glucose to 6 fasting subjects. As TRP does not decrease as much as the other LNAA, there is an increase in the ratio of TRP to the LNAA. The symbols used are the following: ○, 0 g of glucose; ⬤, 6 g of glucose; ⬦, 12.5 g of glucose; □, 25 g of glucose; ◊, 50 g of glucose.*
liver. \textsuperscript{21,22} In contrast, dietary phenylalanine, tyrosine, and methionine are removed from the blood stream both by insulin-mediated tissue uptake and hepatic metabolism.\textsuperscript{21,22} This difference probably explains the greater amplitude of the daily rhythms in plasma branched-chain amino acids and their greater percent decreases after glucose consumption.\textsuperscript{23-25} It may also have contributed to the large interindividual variations that we observed in LNAAs concentrations. These variations could also be explained by the presence of volunteers of both sexes in our study, females tending to have lower LNAAs concentrations than males,\textsuperscript{26} or by differences in weight among the subjects, since all received the same amount of glucose. Although the subjects were fasted for 13 hr before each experiment and were asked not to engage in unusual physical

![Graph](image_url)

**Fig. 3.** Correlation between glucose dose and the mean % decrease in plasma LNAAs measured 120 min after the ingestion of 0, 6, 12.5, 25, or 50 g of glucose by 8 subjects. The correlation ($r = 0.95$) is statistically significant at $p < 0.01$. Symbols are as in Fig. 2.

<table>
<thead>
<tr>
<th>Glucose Dose (g)</th>
<th>Time after Glucose Ingestion (min)</th>
<th>Val</th>
<th>Iso</th>
<th>Leu</th>
<th>Met</th>
<th>Tyr</th>
<th>Phe</th>
<th>Trp</th>
<th>LNAAs</th>
<th>Trp/LNAAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>209 ± 15</td>
<td>59 ± 6</td>
<td>120 ± 11</td>
<td>24 ± 2</td>
<td>61 ± 3</td>
<td>54 ± 2</td>
<td>42 ± 4</td>
<td>528 ± 37</td>
<td>.079</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>190 ± 13</td>
<td>52 ± 6</td>
<td>109 ± 9</td>
<td>23 ± 4</td>
<td>55 ± 2</td>
<td>49 ± 1</td>
<td>38 ± 4</td>
<td>478 ± 32</td>
<td>.079</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>208 ± 13</td>
<td>52 ± 5</td>
<td>115 ± 9</td>
<td>24 ± 1</td>
<td>59 ± 5</td>
<td>53 ± 1</td>
<td>39 ± 3</td>
<td>512 ± 32</td>
<td>.075</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>220 ± 19</td>
<td>60 ± 7</td>
<td>113 ± 11</td>
<td>24 ± 4</td>
<td>57 ± 8</td>
<td>50 ± 3</td>
<td>46 ± 4</td>
<td>522 ± 47</td>
<td>.088</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>201 ± 15</td>
<td>51 ± 4</td>
<td>100 ± 12</td>
<td>21 ± 3</td>
<td>51 ± 7</td>
<td>46 ± 2</td>
<td>43 ± 4</td>
<td>470 ± 33</td>
<td>.092</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>200 ± 16</td>
<td>51 ± 5</td>
<td>101 ± 9</td>
<td>22 ± 3</td>
<td>50 ± 6</td>
<td>47 ± 3</td>
<td>41 ± 4</td>
<td>471 ± 37</td>
<td>.086</td>
</tr>
<tr>
<td>12.5</td>
<td>0</td>
<td>255 ± 47</td>
<td>65 ± 5</td>
<td>117 ± 10</td>
<td>27 ± 2</td>
<td>58 ± 4</td>
<td>54 ± 4</td>
<td>45 ± 2</td>
<td>574 ± 15</td>
<td>.079</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>226 ± 48</td>
<td>53 ± 4</td>
<td>96 ± 10</td>
<td>26 ± 1</td>
<td>52 ± 3</td>
<td>50 ± 3</td>
<td>40 ± 1</td>
<td>501 ± 66</td>
<td>.080</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>221 ± 98</td>
<td>48 ± 3 *</td>
<td>95 ± 9</td>
<td>23 ± 1</td>
<td>50 ± 3</td>
<td>46 ± 2</td>
<td>41 ± 1</td>
<td>482 ± 49</td>
<td>.084</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>294 ± 50</td>
<td>71 ± 6</td>
<td>130 ± 13</td>
<td>26 ± 3</td>
<td>63 ± 3</td>
<td>56 ± 3</td>
<td>47 ± 2</td>
<td>639 ± 79</td>
<td>.073</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>218 ± 29</td>
<td>48 ± 4 #</td>
<td>84 ± 10 *</td>
<td>21 ± 2</td>
<td>49 ± 2 #</td>
<td>47 ± 4</td>
<td>42 ± 2</td>
<td>466 ± 45</td>
<td>.091</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>212 ± 26</td>
<td>46 ± 4 #</td>
<td>91 ± 8</td>
<td>19 ± 2</td>
<td>46 ± 2 #</td>
<td>41 ± 2 #</td>
<td>41 ± 3</td>
<td>454 ± 40 *</td>
<td>.089 *</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>262 ± 40</td>
<td>61 ± 4</td>
<td>114 ± 11</td>
<td>26 ± 2</td>
<td>59 ± 4</td>
<td>50 ± 2</td>
<td>46 ± 2</td>
<td>573 ± 60</td>
<td>.080</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>215 ± 32</td>
<td>48 ± 2 #</td>
<td>93 ± 11</td>
<td>21 ± 2</td>
<td>43 ± 3 #</td>
<td>50 ± 4</td>
<td>40 ± 1</td>
<td>469 ± 52</td>
<td>.085</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>170 ± 30</td>
<td>36 ± 4 #</td>
<td>72 ± 10 *</td>
<td>18 ± 2 #</td>
<td>40 ± 4 #</td>
<td>37 ± 2 #</td>
<td>35 ± 2 #</td>
<td>372 ± 50 *</td>
<td>.094 *</td>
</tr>
</tbody>
</table>

**Table 1.** Plasma Amino Acid Levels after Glucose Ingestion

Sum of the LNAAs: total concentration of leucine, isoleucine, valine, methionine, phenylalanine, and tyrosine. TRP/LNAAs: ratio of tryptophan to the sum of the LNAAs, measured 0, 60 and 120 min after 6 nonobese, fasting subjects ingested 0, 6, 12.5, 25 or 50 g of glucose. All plasma amino acids, except tryptophan, were measured on a Beckman amino acid analyzer. Tryptophan was measured by the method of Denckla and Dewey.\textsuperscript{14} Data are expressed as means ± SEM. Data were analyzed by Student’s t test.

\* $p < 0.05$ compared to fasting basal values (time 0).

\# $p < 0.02$ compared to fasting basal values (time 0).

\# $p < 0.01$ compared to fasting basal values (time 0).

\# $p < 0.001$ compared to fasting basal values (time 0).
activities before being tested, differences in nutritional state or physical activity might also have contributed to the variations.

We failed to detect a simple relationship between plasma insulin and plasma LNAA concentrations in subjects receiving different doses of glucose. The areas under the curve for the increases in insulin did not correlate well with the corresponding areas for decreases in LNAA. A number of factors might explain the discrepancy:

It could reflect the short time-course of our experiment, i.e., an inverse relationship between insulin and LNAA might have become apparent at 180 min after glucose consumption, but not at 120 min. However, the
measurement of areas under the curve after 180 min in 2 subjects did not support this hypothesis (data not shown).

Another possibility is that the dose-response characteristics of the insulin receptors controlling amino acid fluxes are such that the amounts of insulin released after 25 g glucose are sufficient to cause maximal effects.

Finally, insulin may not be the only factor responsible for the LNAA decrease. Glucose per se may lower the free amino acids as suggested by Adibi et al.\textsuperscript{27} for juvenile diabetics. In our study, blood glucose levels were slightly higher 30 min after 25 g of glucose than after 50 g, and the mean percent drop in LNAA was also greater 60 min after 25 g than after 50 g. Interestingly, subject 1, whose LNAA concentrations did not decrease 60 min after ingesting 50 g of glucose, in spite of a large insulin release, also failed to exhibit an increase in glucose after 30 min.

Our data confirm reports\textsuperscript{23,28} that dietary carbohydrate causes only relatively small decreases in total plasma tryptophan (i.e., free plus albumin-bound). This nonresponsiveness results from tryptophan's property, unique among amino acids, of travelling in the circulation largely bound to albumin.\textsuperscript{29} The binding is of the low-affinity, high-capacity type, and is competitive with non-esterified fatty acids (NEFA).\textsuperscript{30} Thus, when the insulin secreted after carbohydrate consumption lowers plasma NEFA levels (largely by stripping the NEFA off of albumin), it increases the ability of the albumin to bind more tryptophan. Hence, the plasma concentration of albumin-bound tryptophan rises, partly or totally compensating for the fall in the smaller "free" tryptophan pool. The tryptophan bound to albumin is almost as accessible to the brain as "free" tryptophan,\textsuperscript{31} probably because the affinity of the blood-brain barrier LNAA transport system for tryptophan is greater than the affinity of albumin for tryptophan.\textsuperscript{32} Hence carbohydrate consumption, which increases plasma total tryptophan levels while decreasing the concentrations of other LNAA, is able to elevate brain tryptophan and to enhance the synthesis and release of serotonin.\textsuperscript{3,4}

Enhanced release of serotonin is followed by diminished consumption of additional carbohydrates when animals\textsuperscript{12} or humans\textsuperscript{13} have a choice of what to consume. Our present data suggest that 25 g or more of dietary carbohydrate are needed to activate this mechanism (Fig. 2). A theoretically more effective way of activating it would be to administer the carbohydrate along with tryptophan itself. Perturbations of the mechanism by which carbohydrate intake affects brain function might exist in diseases like early-onset diabetes where insulin secretion is diminished, or obesity and adult-onset diabetes, where insulin receptors apparently function abnormally.\textsuperscript{14} Plasma levels of branched-chain amino acids are known to be very high in human diabetics, especially after a high-protein meal;\textsuperscript{12} moreover studies in diabetic rats have shown that the abnormal plasma amino acid pattern is associated with diminished brain uptake of tryptophan.\textsuperscript{33,34}

The possibility that diseases involving some insulin receptors also alter the hormone's effects on plasma LNAA, and thus brain serotonin, cannot now be evaluated; too few data are available concerning the sensitivity of the receptors affecting amino acid fluxes in diseases (like obesity) that are known to affect receptors mediating glucose or fatty acid uptake. One recent study\textsuperscript{35} showed that the capacity of obese, insulin-resistant (in relation to glucose) subjects to metabolize a valine load was normal; however, the valine was administered along with a relatively high dose of glucose, and so the possibility of diminished LNAA responses to submaximal insulin levels could not be assessed.

The likelihood that some people secrete quantities of insulin sufficient to reduce plasma LNAA after as little as 6 g of oral glucose, and that others require larger doses (Fig. 4a, b) raises the possibility that those requiring the higher doses might be manifesting early signs of metabolic disease.

REFERENCES

8. Fernstrom JD, Faller DV: Neutral amino acids in the brain:


