Increased Dietary Protein Modifies Glucose and Insulin Homeostasis in Adult Women during Weight Loss\textsuperscript{1,2}

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ABSTRACT Amino acids interact with glucose metabolism both as carbon substrates and by recycling glucose carbon via alanine and glutamine; however, the effect of protein intake on glucose homeostasis during weight loss remains unknown. This study tests the hypothesis that a moderate increase in dietary protein with a corresponding reduction of carbohydrates (CHO) stabilizes fasting and postprandial blood glucose and insulin during weight loss. Adult women (n = 24; >15% above ideal body weight) were assigned to either a Protein Group [protein: 1.6 g/(kg \cdot d); CHO <40% of energy] or CHO Group [protein: 0.8 g/(kg \cdot d); CHO >55%]. Diets were equal in energy (7100 kJ/d) and fat (50 g/d). After 10 wk, the Protein Group lost 7.53 ± 1.44 kg and the CHO Group lost 6.96 ± 1.36 kg. Plasma amino acids, glucose and insulin were determined after a 12-h fast and 2 h after a 1.67 MJ test meal containing either 39 g CHO, 33 g protein and 13 g fat (Protein Group) or 57 g CHO, 12 g protein and 14 g fat (CHO Group). After 10 wk, subjects in the CHO Group had lower fasting (4.34 ± 0.10 vs 4.89 ± 0.11 mmol/L) and postprandial blood glucose (3.77 ± 0.14 vs. 4.33 ± 0.15 mmol/L) and an elevated insulin response to meals (207 ± 21 vs. 75 ± 18 pmol/L). This study demonstrates that consumption of a diet with increased protein and a reduced CHO/protein ratio stabilizes blood glucose during nonabsorptive periods and reduces the postprandial insulin response. J. Nutr. 133: 405–410, 2003.

KEY WORDS: • insulin • amino acids • leucine • obesity • syndrome X

Dietary requirements for amino acids remain controversial. Most studies are focused on criteria to define a minimum requirement to maintain short-term nitrogen balance. This concept is particularly useful for a limiting amino acid such as lysine, which serves as an essential amino acid for peptide structures and has limited use as a metabolic substrate (1,2). At the other end of the spectrum, the branched-chain amino acids (BCAA) are essential amino acids for protein synthesis and also participate in critical metabolic processes (3,4). These differences in roles among amino acids suggest that a single definition of requirements may not be adequate to encompass the full range of human needs for each of the nine indispensable amino acids.

The three BCAA, leucine, valine and isoleucine, support numerous metabolic processes ranging from the fundamental role as substrates for protein synthesis to metabolic roles as precursors for synthesis of alanine and glutamine (5,6) and as a modulator of the insulin-signaling pathway (7–9). The potential for the BCAA to participate in each of these metabolic processes appears to be in proportion to their availability.

Experimental evidence comparing the priority of use of the BCAA for each of these individual processes is limited, but suggests that the first priority is for aminoacylation of tRNA for protein synthesis (10), whereas their contribution to the production of alanine and glutamine or their effect on the signaling pathway is dependent on increasing intracellular concentrations (5,11,12). The potential effect of these amino acids on metabolic processes under physiologic conditions remains to be explored.

The interrelationship between BCAA and glucose metabolism was first reported to be associated with the glucose-alanine cycle (5,6). These investigators found that there was a continuous flux of BCAA from visceral tissues through the blood to skeletal muscle where transamination of the BCAA provides the amino nitrogen to produce alanine from pyruvate with a corresponding movement of alanine from muscle to liver to support hepatic gluconeogenesis. Although the importance of the glucose-alanine cycle has been debated, Ahlborg et al. (5) reported that it accounted for >40% of endogenous glucose production during prolonged exercise.

More recently, the overall contribution of dietary amino acids to glucose homeostasis received further support on the basis of quantitative evaluations of hepatic glucose production. Jungas et al. (13) provided an elegant argument that amino acids serve as a primary fuel for the liver and the primary carbon source for hepatic gluconeogenesis. Other investigators (14,15) extended this thinking with the findings that endogenous glucose production in the liver is a critical factor in the
maintenance of blood glucose. After an overnight fast, gluconeogenesis provides >70% of hepatic glucose release, with amino acids serving as the principal carbon source (16). These studies provide further evidence for a linkage between dietary protein and glucose homeostasis.

Recent reports have highlighted the critical need to enhance regulation of blood glucose in overweight adults (17) and during weight loss (18). This study tests the hypothesis that a moderate increase in dietary protein with a corresponding reduction of carbohydrates (CHO) improves glucose and insulin homeostasis during weight loss. We propose that leucine is a critical substrate in the relationship of dietary protein to glucose homeostasis. Currently, the minimum leucine requirement for nitrogen balance is reported to be <38 mg/(kg · d) (19,20), whereas positive oxidative balance requires intakes of 89 mg/(kg · d) (20). We evaluated the effect of changing the dietary CHO/protein ratio from 3.5 to 1.4 and with doubling of dietary leucine on plasma BCAA, plasma levels of gluconeogenic precursors alanine and glutamine, insulin response to meals and maintenance of fasting and postprandial blood glucose.

**SUBJECTS AND METHODS**

Women (n = 24; 45–56 y old) with body weights >15% above ideal body weight (21) were recruited from the University of Illinois community. Subjects were screened using a medical history and a 24-h diet recall; subjects with known medical conditions, routine use of medications or smokers were excluded from the study. Subjects selected for the study had minimal daily physical activity, had maintained a stable body weight during the past 6 mo and consumed a diet that contained 12–17% of energy as protein. These conditions were selected as representative of average U.S. food intake (22) and to standardize prestudy conditions. All protocols and consent forms were reviewed and approved by the Institutional Review Board of the University of Illinois Urbana-Champaign.

After the initial screening period, subjects had an additional baseline evaluation period that included a 3-d weighed dietary record. Subjects were then given a test meal providing 1.67 MJ with the macronutrient composition similar to the respective diet treatments. The test meals were designed to represent common breakfast meals and to provide energy levels similar to standard oral glucose tolerance tests (24,25). The test meals were designed to be diet

| TABLE 1 |

| Energy and macronutrient compositions of weight loss diets¹ |
|-------------------------|------------------|-------------------|-------------------|-------------------|
| Daily intakes² | | | | |
| Macronutrients | Energy | CHO | Protein | Fat | Leu | Ile | Val | Phe | Thr |
| MJ/d | | | | | | | | | |
| Protein group | 6.98 ± 0.19 | 171 ± 7 | 125 ± 3 | 54 ± 2 | 9.89 ± 0.19 | 5.99 ± 0.11 | 6.42 ± 0.12 | 5.42 ± 0.11 | 5.03 ± 0.09 |
| CHO group | 6.94 ± 0.17 | 239 ± 5 | 68 ± 2 | 48 ± 2 | 5.39 ± 0.10 | 3.20 ± 0.06 | 3.67 ± 0.07 | 3.12 ± 0.06 | 2.67 ± 0.05 |

| Test meal³ | | | | | | | | | |
| Energy | CHO | Protein | Fat | Leu | Ile | Val | Phe | Thr |
| MJ | | | | | | | | |
| Protein group | 1.69 | 39 | 33 | 13 | 2.70 | 1.71 | 1.92 | 1.69 | 1.36 |
| CHO group | 1.57 | 57 | 10 | 12 | 0.66 | 0.36 | 0.41 | 0.44 | 0.28 |

¹ Weight loss diets were designed to be equal in energy and fat. The protein group had a daily intake of protein of 1.5 g/(kg · d) and a ratio of CHO/protein = 1.4 and the CHO group had a protein intake of 0.8 g/(kg · d) and CHO/protein = 3.5.

² Values represent intakes from 3-d weighed records; means ± SEM, n = 12.

³ Values represent defined intakes fed at test meal.
Dietary protein modifies glucose homeostasis

**RESULTS**

Daily intakes of macronutrients were determined from weekly 3-d weighed food records (Table 1). The Protein Group consumed 6.98 ± 0.19 MJ/d with 125 g of protein and 171 g of carbohydrates. The CHO Group consumed 6.94 ± 0.16 MJ/d with 68 g of protein and 240 g of carbohydrates. After consuming the respective diets for 10 wk, subjects in the Protein Group lost 6.96 ± 1.36 kg of body weight and subjects in the CHO Group lost 7.53 ± 1.44 kg of body weight and subjects in the CHO Group had higher fasting glucose but lower fasting insulin than the Protein Group (P < 0.05) for the Protein Group with a corresponding increase in the nitrogen-transporting mole-

Plasma concentrations for the indispensable amino acids did not differ between treatment groups after an overnight fast (Table 2). Subjects in the CHO Group had higher fasting blood levels for alanine and for the sum of alanine plus glutamine. After the test meal, changes in plasma amino acids reflected the indispensable amino acid content of the respective diets. The Protein Group received a meal containing 33 g of protein (Table 1) and plasma concentrations of amino acids remained significantly above fasting levels 2 h after the completion of the meal (Table 2). The magnitude of the increases varied among the amino acids with the BCAA increasing most (68–108%) and threonine increasing least (22%). In the CHO Group, the test meal provided 10 g of protein; 2 h after the meal, most amino acids exhibited concentrations that were not different from values after a 12-h fast. Alanine and phenylalanine concentrations tended to be greater (P = 0.61 and 0.78, respectively), whereas glutamine concentration was > 19% lower than the fasting concentration (P < 0.05).

Meal responses for the BCAA and the two nonessential amino acids, alanine and glutamine, reflected fundamental differences in metabolism between the groups (Table 2). After the higher protein meal, the sum of the BCAA increased by 76% (248 ± 10 μmol/L, P < 0.05) for the Protein Group with a corresponding increase in the nitrogen-transporting mole-

**FIGURE 1** Plasma glucose concentrations in fasting women assigned to a moderate protein diet (Protein Group) or a high carbohydrate diet (CHO Group) during 10 wk of weight loss. Values are means ± SEM, n = 12. Means without a common letter differ, P < 0.05. The linear decline in the CHO Group was significant (R² = 0.982; P = 0.0086).

**FIGURE 2** Plasma glucose (A) and insulin (B) concentrations after a 12-h fast and 2 h after a 1.67 MJ test meal in women assigned to a moderate protein diet (Protein Group) or a high carbohydrate diet (CHO Group) during 10 wk of weight loss. Values are means ± SEM, n = 12. Means without a common letter differ, P < 0.05.

**FIGURE 3**
DISCUSSION

The potential for amino acids to interact with glucose metabolism is well established; however, the effect of prolonged modification of protein intake on glucose homeostasis is unknown. In the 1970s, researchers reported that amino acid availability supported glucose metabolism during prolonged aerobic exercise (5) or during intravenous infusions (31). Subsequently, quantitative measures of amino acid flux established the importance of liver gluconeogenesis in the maintenance of blood glucose during nonabsorptive periods (13,15,16). The present study evaluated the potential of moderate changes in the diet CHO/protein ratio to affect the metabolic balance between glucose and insulin homeostasis and the availability of gluconeogenic amino acids. After 10 wk of diet modification, increasing dietary protein and reducing the CHO/protein ratio minimized postprandial and fasting changes in blood glucose during weight loss.

Maintenance of blood glucose within the normal range of 4.4–6.0 mmol/L requires a precise balance between hepatic glucose release (16) and peripheral tissue glucose use (32). The liver regulates glucose release by balancing the disposal of exogenous dietary glucose with endogenous production from gluconeogenesis and glycogenolysis. The balance achieved by the liver among absorption, de novo synthesis and stored glycogen is dependent on diet composition and stage of absorption (14,16,33,34). Similarly, the use of blood glucose by peripheral tissues is a balance among both insulin dependent and insulin independent tissues and varies widely, depending on glucose availability, hormone status and tissue energy needs. This balance between hepatic glucose release and peripheral clearance must be able to extend from minimum needs of ~80 to 120 g/d for obligate glycolytic tissues such as the brain, nerve tissue and blood cells to levels >400 g/d during conditions of high dietary carbohydrate intakes.

To test the influence of the dietary CHO/protein ratio on glucose homeostasis, one approach would be to examine glucose absorption curves and peak insulin levels. This approach would be essential to evaluate the meal response to a carbohydrate load including the potential to reduce hepatic endogenous production (16) or to increase insulin-driven peripheral clearance (35). In nondiabetic subjects, the balance of these responses maintains blood glucose within normal ranges. On the other hand, another key regulatory challenge occurs during periods between meals when exogenous glucose is not available. During these periods, the body must rebalance hepatic glucose production and peripheral clearance to protect blood glucose from hypoglycemic responses. The two most likely periods for hypoglycemia would be in the morning after an overnight fast or at the end of a postprandial period when insulin is elevated and blood glucose returns to nonabsorptive levels (24,36). These two “nonabsorptive” periods were the focus of the current study.

After 10 wk of controlled dietary intakes, subjects consuming a diet with adequate protein [0.8 g/(kg · d)] and a high carbohydrate diet (CHO Group). The insulin response was determined as the 2-h postprandial value minus the fasting value for each subject. Values represent means ± SEM, n = 12. Means without a common letter differ, P < 0.05.

**TABLE 2**

Blood amino acid values in women consuming either a moderate protein or high carbohydrate (CHO) diets during weight loss.1

<table>
<thead>
<tr>
<th>Protein group</th>
<th>Fasting</th>
<th>Test meal</th>
<th>Change</th>
<th>Fasting</th>
<th>Test meal</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>102 ± 5b</td>
<td>181 ± 9a</td>
<td>77</td>
<td>98 ± 4b</td>
<td>93 ± 4b</td>
<td>−6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>50 ± 3b</td>
<td>104 ± 6a</td>
<td>108</td>
<td>50 ± 2b</td>
<td>49 ± 2b</td>
<td>−2</td>
</tr>
<tr>
<td>Valine</td>
<td>171 ± 8b</td>
<td>287 ± 12a</td>
<td>68</td>
<td>174 ± 8b</td>
<td>158 ± 7b</td>
<td>−9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>41 ± 1.5c</td>
<td>66 ± 2.6a</td>
<td>61</td>
<td>43 ± 1.8c</td>
<td>51 ± 2.3b</td>
<td>18</td>
</tr>
<tr>
<td>Threonine</td>
<td>104 ± 7b</td>
<td>127 ± 11a</td>
<td>22</td>
<td>106 ± 12b</td>
<td>113 ± 11b</td>
<td>7</td>
</tr>
<tr>
<td>Alanine</td>
<td>324 ± 16b</td>
<td>485 ± 28a</td>
<td>50</td>
<td>388 ± 21b</td>
<td>452 ± 24a</td>
<td>16</td>
</tr>
<tr>
<td>Glutamine</td>
<td>378 ± 15b</td>
<td>513 ± 24a</td>
<td>36</td>
<td>449 ± 12a</td>
<td>363 ± 19b</td>
<td>−19</td>
</tr>
<tr>
<td>ΣBCAA</td>
<td>323 ± 5b</td>
<td>571 ± 9a</td>
<td>77</td>
<td>322 ± 5b</td>
<td>300 ± 4b</td>
<td>−7</td>
</tr>
<tr>
<td>Σala + gln</td>
<td>702 ± 16c</td>
<td>998 ± 26a</td>
<td>42</td>
<td>837 ± 16b</td>
<td>815 ± 22b</td>
<td>−3</td>
</tr>
</tbody>
</table>

1 Values represent means ± SEM of three trials for each of the 12 women (n = 12) at wk 2, 4 and 10. Means in a row without a common letter differ, P < 0.05.
CHO/protein ratio (> 3.5) had lower fasting and postprandial blood glucose than subjects consuming the diet with more protein [1.5 g/(kg · d)] and a lower CHO/protein ratio (< 1.4). As outlined above, regulation of blood glucose must be achieved through changes in either hepatic output or peripheral clearance, or both. In the CHO Group at 2 h after the test meal, lower blood glucose was associated with blood insulin levels higher than either the fasting concentration or those of the Protein Group. This response to the test meal seems reasonable because the CHO Group consumed more carbohydrates. However, although the direction of the insulin response to the test meal was expected, the magnitude of the insulin response appeared to be disproportionate to the carbohydrates consumed. Carbohydrate intake from the test meal was 46% higher for the CHO Group (57 g vs. 39 g), whereas the insulin response to the meal was 115% higher in the CHO Group (Fig. 2B) and the magnitude of the insulin response increased over the 10-wk feeding period (Fig. 3). These data are consistent with reduced insulin sensitivity in the CHO Group during the study. On the other hand, subjects in the CHO Group had lower fasting blood glucose than subjects in the Protein Group with similar fasting insulin levels. If insulin sensitivity is lower in the CHO Group, reduced peripheral sensitivity should produce higher fasting blood glucose. These data suggest that dietary changes in the CHO/protein ratio produce changes in endogenous glucose regulation that likely include changes in both peripheral glucose clearance and hepatic glucose production.

Estimates of the contribution of amino acid carbon to de novo glucose synthesis range from 0.5 to 0.7 g of glucose from 1 g of dietary protein (28,37). In addition to the direct conversion of amino acid carbon to gluconeogenic precursors, there is also the contribution of the BCAA to the glucose-alanine cycle (5,29). Because BCAA are catabolized in skeletal muscle, the amino nitrogen is transferred from the BCAA to α-keto glutarate, forming glutamate. The nitrogen is then transferred from glutamate to alanine via aminotransferase, or glutamate is further aminated to glutamine. The net effect is a direct stoichiometric relationship between catabolism of the BCAA in skeletal muscle and production of alanine or glutamine. The alanine and glutamine produced in muscle are released to the blood circulation and extracted by splanchnic tissues, predominately the liver and gut. Production of these nonessential amino acids affects glucose homeostasis by the recycling of glucose carbon. Production of alanine requires pyruvate in skeletal muscle (29,31); during catabolism of glutamine in the gut, at least 50% of the α-amino group of glutamine is converted to alanine via transamination (28,29). These metabolic pathways suggest that skeletal muscle uptake of BCAA is directly linked to the quantity of alanine and glutamine associated with the provision of 3-carbon substrates for hepatic gluconeogenesis.

Changes in plasma concentrations of amino acids after the meal reflect diet composition and known metabolic responses in the gut, liver and peripheral tissues (1,28). After a mixed meal containing protein, there is an increased rate of disposal of amino acids for protein synthesis and amino acid degradation. Catabolism of individual amino acids is tissue specific. For example, after a meal, the gut is highly active in amino acid catabolism and disposes of most glutamine and threonine before they reach the portal circulation (28). At the other extreme, the gut and liver have minimal capacity to initiate amino acid degradation of the BCAA, resulting in increased movement of the BCAA through the blood to peripheral tissues (11,28,30). Plasma appearance of phenylalanine would be expected to be intermediate between Thr and BCAA, with minimal disposal in the gut and a relatively slow rate of catabolism in liver (28). Changes in the BCAA, Phe and Thr reflected these metabolic differences in tissue specificity, with postprandial plasma Thr increased by 21%, Phe by 60% and the sum of the BCAA by 93% in the Protein Group.

Postprandial changes in plasma levels of BCAA, alanine and glutamine after the test meal were consistent with our proposed relationship of dietary amino acids to glucose homeostasis. Specifically, we proposed that postprandial increases in BCAA are associated with increased production of alanine and glutamine and enhanced hepatic gluconeogenesis to maintain fasting blood glucose. We found that the higher protein meal produced anticipated increases in plasma BCAA with corresponding increases in alanine and glutamine. For the CHO Group, with dietary protein at levels designed to meet minimum needs for nitrogen balance, the 2-h postprandial values for BCAA were not different from fasting levels with no change in the sum of alanine plus glutamine.

Changes in plasma amino acid concentrations are not equivalent to quantitative measurements of amino acid flux. However, concentration differences for BCAA, alanine and glutamine reflect metabolic differences. Increases in BCAA levels in the blood relate directly to changes in intracellular concentrations (11,29,38). Similarly, increases in tissue concentrations of BCAA increase catabolism via the aminotransferase and dehydrogenase (11,39), and increased BCAA flux to muscle relates directly to increased production and release of alanine and glutamine (5,29,30,39). Summing each of the elements of the pathway, this study demonstrated that prolonged dietary modification resulting in increased postprandial levels of BCAA, and increased levels of alanine and glutamine can affect glucose homeostasis in free-living subjects.

The relationship of the dietary CHO/protein ratio to hepatic glucose production was also evident in subjects after fasting overnight. After 10 wk of consuming the energy-restricted diets, subjects receiving the higher CHO diet (239 g/d) had 12% lower fasting blood glucose and the level declined throughout the study (Fig. 1). Associated with the reduced blood glucose, subjects in the CHO Group had combined alanine plus glutamine levels that were 20% higher than those of the Protein Group. This increase in plasma alanine and glutamine associated with a diet containing more CHO and less protein was unexpected. Increases in peripheral production of alanine and glutamine are unlikely with lower dietary intake of the BCAA. A possible explanation could be increased rates of protein breakdown associated with the high CHO diet. A more likely explanation for the increased alanine and glutamine levels would be a reduction in the rate of hepatic clearance. This hypothesis is supported by reports that the rate of clearance of plasma alanine is proportional to plasma alanine concentration (40). Investigators have shown that high CHO feeding or elevated insulin result in down-regulation of hepatic gluconeogenesis on the basis of measurements of flux (16) as well as down-regulation of gene expression of key regulatory enzymes (41).

This study evaluated the effect of sustained dietary changes in the ratio of CHO to protein intake on plasma amino acid profiles and maintenance of blood glucose during energy restriction. We found that a diet with increased protein and reduced levels of CHO stabilizes blood glucose during nonabsorptive periods and reduces postprandial insulin response. Additional research utilizing substrate flux measurements is required to define the quantitative relationship between dietary intake of glucose and amino acids and hepatic vs. peripheral management of blood glucose levels. However, this
study supports the hypothesis that the ratio of dietary protein and carbohydrates can have a significant effect on metabolic balance and specifically on glucose homeostasis during weight loss.

LITERATURE CITED


