Soy protein, casein, and zein regulate histidase gene expression by modulating serum glucagon

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Tovar, Armando R., Claudia Ascencio, and Nimbe Torres. Soy protein, casein, and zein regulate histidase gene expression by modulating serum glucagon. Am J Physiol Endocrinol Metab 283: E1016–E1022, 2002.—Glucagon has been postulated as an important physiological regulator of histidase (Hal) gene expression; however, it has not been demonstrated whether serum glucagon concentration is associated with the type and amount of protein ingested. The purpose of the present work was to study the association between hepatic Hal activity and mRNA concentration in rats fed 18 or 50% casein, isolated soy protein, or zein diets in a restricted schedule of 6 h for 10 days, and plasma glucagon and insulin concentrations. On day 10, five rats of each group were killed at 0900 (fasting), and then five rats were killed after being given the experimental diet for 1 h (1000). Rats fed 50% casein or soy diets showed higher Hal activity than the other groups studied. Rats fed 50% zein diets had higher Hal activity than rats fed 18% casein, soy, or zein diets, but lower activity than rats fed 50% casein or soy diets. Hal mRNA concentration followed a similar pattern. Hal activity showed a significant association with serum concentrations of glucagon. Serum glucagon concentration was significantly correlated with protein intake. Thus the type and amount of protein consumed affect Hal activity and expression through changes in serum glucagon concentrations.

Amino acid metabolism; insulin; rat

Amino acids are utilized mainly for protein synthesis and as precursors of some nitrogenous compounds. The excess amounts of amino acids are not stored; therefore, they must be oxidized through amino acid-degrading enzymes (6). Most of these enzymes are localized in the liver, with the exception of the branched-chain amino transferase (20). Histidase (Hal; histidinammonia-lyase, EC 4.3.1.3), the amino acid-degrading enzyme of histidine, is the first enzyme of the catabolic pathway of this amino acid. Hal is localized in liver and skin (22) and catalyzes the oxidative deamination of histidine to form urocanic acid. Hal gene expression is regulated in the liver by the dietary protein content at transcriptional level. The increased expression of Hal enables the liver to catabolize and thereby to eliminate excess histidine after the protein requirement is met, resulting in adequate plasma levels of this amino acid even after the ingestion of a high-protein diet (21). However, a high-histidine diet does not induce Hal mRNA, indicating that the protein content in the diet, instead of the histidine content of the diet, is responsible for Hal induction.

It is not known whether different types of protein induce to the same extent the expression of hepatic amino acid-degrading enzymes, including histidase. Each dietary protein contains a specific amino acid pattern that may or may not meet the amino acid requirements of an individual. When a protein is low or deficient in at least one amino acid, it is considered a low-quality protein. Proteins deficient in or devoid of one or more amino acids are naturally present in different types of plant food proteins, including cereals, vegetables, fruits, and nuts (23). A disproportion of dietary amino acids that occur in low-quality proteins can be artificially created using an amino acid-imbalanced diet. These diets are made by supplementing indispensable amino acids to a low-protein diet except for one. The result of the ingestion of an imbalanced diet is a reduction in food intake, which in turn increases the expression of amino acid-degrading enzymes, including Hal (19). The higher the imbalance, the higher the induction of the Hal gene. Thus the protein quality may have an important role in the gene expression of amino acid-degrading enzymes.

Hormonal changes have been assumed to be the physiological signal responsible for Hal induction. This rationale is proposed from experiments in which infusion of amino acids stimulates the release of pancreatic glucagon (9, 12), and it has been associated with an increased degradation of amino acids and urea excretion (4). Furthermore, the injection of rats with glucagon or glucocorticoids stimulates the activity, amount of protein, and Hal mRNA expression (2). Thus it is likely, but not demonstrated, that diets with different protein quality modulate the expression of amino acid-degrading enzymes through changes in the secretion of insulin and glucagon. It has been hypothesized that the dietary content of arginine and lysine affects the concentration of insulin and glucagon.
in plasma (15). Consumption of a vegetable protein diet with a high arginine-to-lysine (arginine/lysine) ratio probably increases plasma glucagon concentration, and this in turn may modify amino acid-degrading enzyme gene expression. Casein and soy protein, from animal and plant origin, respectively, contain all the amino acids needed to meet the amino acid requirement to support adequate growth rates in rats; however, the arginine/lysine ratio is 0.47 and 1.20 for casein and soy protein, respectively. This difference could modify in a protein-specific manner the insulin and glucagon status, inducing the genes of amino acid-degrading enzymes to a different extent. On the other hand, zein, a maize protein that is devoid of lysine and deficient in tryptophan, represents a naturally imbalanced protein. This imbalance might also alter the plasma concentrations of glucagon and insulin, thus affecting the expression of the amino acid-degrading enzymes.

Therefore, the purpose of the present study was to investigate the effect of different types of protein (casein, soy, and zein) with different amino acid profiles on Hal activity and gene expression and to determine whether these changes are associated with the plasma concentration of glucagon and insulin generated by the ingestion of these diets. This study will provide evidence of how the protein quality of the diet modifies the amino acid catabolism through changes in glucagon concentrations.

MATERIALS AND METHODS

Reagents and chemicals. Nylon membrane filters (Hybond-N+), Rediprime DNA labeling system, and deoxyctydine 5'-[α-32P]triphosphate (110 TBq/mmoll) were purchased from Amersham (Buckinghamshire, UK) and Gene Clean II from Bio 101 (La Jolla, CA). The vitamin-free casein and the remainder of the ingredients were obtained from Teklad (Madison, WI). Zein was obtained from ICN Pharmaceuticals (Costa Mesa, CA), and the isolated soy protein (Supro 710) was kindly donated by Protein Technologies International (México).

Animals. Male Wistar rats were obtained from the Experimental Research Department and Animal Care Facilities at the Instituto Nacional de Ciencias Médicas y Nutrición and were housed individually in wire stainless steel cages at 22°C with a 12:12-h light-dark cycle and free access to water.

Dietary treatments. Sixty rats, weighing 80–90 g, were randomly divided into six groups of 10 rats each: 1) 18% casein diet (C 18), 2) 50% casein diet (C 50), 3) 18% soy diet (S 18), 4) 50% soy diet (S 50), 5) 18% zein diet (Z 18), and 6) 50% zein diet (Z 50). To study changes in Hal expression and hormonal levels, a dietary regimen of a meal-restricted schedule was selected to synchronize food intake. Body weight and food intake were registered daily. Rats were fed in a restricted schedule of 6 h (900 to 1500) for 10 days. On day 10, five rats of each group were anesthetized with carbon dioxide and killed by decapitation at 0900 (fasting), and then five rats were killed after an opportunity to ingest the experimental diet for 1 h (1000). Blood was collected, and serum was obtained by centrifugation at 2,000 g and kept at −80°C until analysis. The liver from each rat was immediately dissected and weighed, and a tissue sample was quickly frozen in liquid nitrogen for RNA extraction; the rest of the tissue was used to measure Hal activity. The protocol was approved by the Ethics Committee in Animal Experimentation of the Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán.”

Diets. Diets were administered in dry form and contained (g/kg diet) 180 or 500 of vitamin-free casein, isolated soy protein, or zein, 50 corn oil, 50 mineral mix, and 10 vitamin mix. Cornstarch and sucrose, in a 1:1 proportion, were added to complete 1 kg of diet. Caloric density was 4.01 kcal/g in all experimental diets. The detailed composition of the diets, including minerals and vitamins, has been previously reported (21).

Insulin and glucagon RIA. Serum insulin and glucagon were determined by RIA with rat insulin and glucagon kits (Linco Research, St. Charles, MO). The sensitivity for the rat insulin assay was 0.1 ng/ml, and the intra- and interassay coefficients of variation were <5% and <5%, respectively. The sensitivity of the glucagon assay was 20 pg/ml, and the intra- and interassay coefficients of variation were <2.12% and <5%, respectively. Immune complexes were counted with a Cobra II gamma counter (Packard Instruments, Meriden, CT).

Hal activity. One gram of liver was washed with ice-cold saline, blotted, and homogenized in 4 ml of an ice-cold solution containing 5 mmol/l of NaOH in 14 mmol/l of KCl with a polytron (PT2000 Kinematica, Lucerne, Switzerland) at the lowest setting. The homogenates were centrifuged for 60 min at 105,000 g, and the clear supernatant was stored at −80°C before Hal activity was measured. The activity was assayed as described (16a). The method is based on the spectrophotometric measurement of the appearance of urocanic acid at 277 nm. The reaction was linear for 10 min at 25°C in 0.1 M pyrophosphate buffer, pH 9.2. An enzyme unit was defined as the formation of 1 μmol of urocanic acid/min. The protein concentration was measured by means of the biuret assay, with bovine serum albumin as standard.

Northern blot analysis. Total RNA was isolated from the liver according to a method described in Ref. 5. For Northern analysis, 20 μg of RNA were electrophoresed in a 1% agarose gel containing 18% formaldehyde, transferred to a nylon membrane filter (Hybond-N+), and cross-linked with a UV cross-linker (Amersham). The cDNA probe was a 1.95-kb PCR product amplified from rat liver histidase cDNA (18). The forward and reverse primers used for the PCR reaction were 5'-ATGCCCTAGGTACACGGTG-3' and 5'-TTAAA-GATCTGCGACTCTG-3', respectively. The PCR product was purified with Gene Clean and labeled with Redivue [α-32P]dCTP (110 TBq/mmoll) by use of the Rediprime DNA labeling kit. Membranes were prehybridized with rapid-hyb buffer (Amersham) at 65°C for 30 min and then hybridized with the cDNA probe (53.3 MBq/l) for 2.5 h at 65°C. Membranes were washed once with 2× SSC (1× SSC = 0.15 M sodium chloride and 15 M sodium citrate containing 0.1% SDS) at room temperature for 20 min and then twice for 15 min with 0.1× SSC containing 0.1% SDS at 65°C. Digitized images and quantitation of radioactivity (counts/min, or cpm) of the bands were done by use of the Instant Imager (Packard Instrument, Meriden, CT). Membranes were also exposed to Extascan film (Kodak) at −70°C with an intensifying screen.

Statistical analysis. Results are presented as means ± SE. The type of protein × protein concentration interaction was assessed statistically by two-way ANOVA followed by Fisher's protected least squares difference test to determine significant differences among groups. P values for interactions are reported, and the significance level was set at P < 0.05. Associations between variables were tested using the Spearman rank correlation test. Differences were considered statistically significant at P < 0.05. We used the Statview
RESULTS

Effect of casein, soy, and zein on growth and food intake. Rats fed 18% casein or 50% soy showed similar growth rates, 3.29 ± 0.20 and 3.18 ± 0.13 g/day, respectively. Rats fed 50% casein grew at the highest growth rate (4.11 ± 0.27 g/day). Rats fed 18% soy showed lower growth rate (2.44 ± 0.30 g/day) than rats fed 18% casein or 50% soy. Rats fed 18 or 50% zein diet showed growth failure (~1.57 ± 0.2 and ~1.48 ± 0.1 g/day, respectively) (Table 1). Two-way ANOVA revealed a significant effect of the type of protein and the protein content on growth rates (P < 0.0001), but there was no significant interaction of type of protein × protein content on growth rates. As shown in Table 1, food intake was similar in rats fed 18 or 50% casein. Rats fed 50% casein also showed no differences in food consumption from rats fed 18 or 50% soy diets, whereas those fed zein consumed ~50% less than rats fed casein or soy (P < 0.0001). Rats fed 50% casein or soy diets consumed 150 and 180% more dietary protein than rats fed 18% casein or soy diets, respectively (P < 0.0001). Rats fed 18 or 50% zein diets ingested significantly less protein than the corresponding groups fed casein or soy diets (P < 0.0001). Rats fed 50% casein diet showed significantly higher daily body weight gain (g/day) per gram of diet consumed than rats fed 18% casein or soy diets (P < 0.0001). Rats fed the 50% soy diet showed similar body weight gain to rats fed 18 or 50% soy or casein diets, whereas rats fed zein diets had negative growth rate per gram of diet ingested (Table 1).

Liver weight. As shown in Table 1, liver weight of rats fed zein diets was significantly lower than that of rats fed soy or casein diets (P < 0.0001). Rats fed the 50% casein diet showed the highest liver weight of all the groups. Rats fed 18% casein showed a similar liver weight with rats fed the 50% soy diet. The two-way ANOVA revealed a significant effect of the type of protein (P < 0.0001), protein content (P < 0.0001), and interaction between the type of protein × protein content and the liver weight of rats (P < 0.02).

Insulin and glucagon. Insulin and glucagon concentrations were measured in the fasting condition and 1 h after being fed. Rats fed 50% casein diet showed higher fasting serum insulin concentration than rats of the other groups studied (P < 0.05). After 1 h of food consumption, rats fed 18 or 50% casein showed significantly higher serum insulin concentration than rats fed either 18 or 50% soy or zein diets (P < 0.0001). Because of the high fasting insulin levels of rats fed the 50% casein diet, the increase in serum insulin concentration after 1 h of food consumption was 1.3-fold, whereas rats fed the 18% casein diet had lower fasting insulin levels than rats fed the 50% casein diet and showed an increase of serum insulin concentration of 3.5-fold after 1 h of food ingestion. No significant difference in serum insulin concentration was observed between rats fed 18 and 50% casein diets after 1 h of food ingestion. Consumption for 1 h of soy protein or zein diets also increased serum insulin concentration but to a lesser extent, even in the groups of rats fed 50% soy or zein diets. After 1 h of food ingestion, rats fed 18 or 50% soy diets showed 65 and 53% lower serum insulin concentration than rats fed 18 or 50% casein, respectively (Fig. 1A), whereas no significant difference was observed between rats fed soy or zein diets (Fig. 1A).

Interestingly, fasting serum glucagon concentrations were higher in rats fed 18 or 50% soy diets than in rats fed 18 or 50% casein or zein diets. After 1 h of food consumption, rats fed 50% casein or soy diets showed a six- and twofold increase in serum glucagon concentration. Rats fed 50% casein or soy diets showed significantly higher concentrations of glucagon than the other groups (Fig. 1B). Rats fed 18% casein or soy protein diets, as well as rats fed 18 or 50% zein diets, also increased serum glucagon concentration 2.4-, 0.38-, 1.9-, and 0.6-fold, respectively, compared with fasting levels. However, serum glucagon concentration was not significantly different after 1 h of food ingestion among rats fed 18% soy, casein, and zein, as well as 50% zein diets (Fig. 1B).

To establish whether serum glucagon concentration depended on the amount of protein ingested, we carried out associative analyses between these variables. Serum glucagon concentration after 1 h of food ingestion of rats fed casein, soy, and zein diets correlated significantly with the protein intake (ρ = 0.706, P = 0.0007). Thus the higher the amount of protein ingested, the higher was the serum glucagon concentration (Fig. 2). Because of the different content of amino acids in each diet, an association analysis was per-

Table 1. Growth rates, liver weight, food and protein intake, and growth rate-to-food intake ratio in rats fed casein, soy, and zein diets

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<thead>
<tr>
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<th>Casein</th>
<th>Soy</th>
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<tr>
<td></td>
<td>18%</td>
<td>50%</td>
<td>18%</td>
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<tr>
<td>Growth rate, g/day</td>
<td>3.3 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.1 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Liver wt, g</td>
<td>4.1 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.1 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Food intake, g/day</td>
<td>12.9 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.7 ± 0.3&lt;sup&gt;c,b&lt;/sup&gt;</td>
<td>10.1 ± 0.8&lt;sup&gt;c,b&lt;/sup&gt;</td>
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<tr>
<td>Protein intake, g/day</td>
<td>2.3 ± 0.1&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>5.8 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 0.1&lt;sup&gt;c,b&lt;/sup&gt;</td>
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<tr>
<td>Wt gain, g/day/intake, g/day</td>
<td>0.3 ± 0.01&lt;sup&gt;c,b&lt;/sup&gt;</td>
<td>0.4 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2 ± 0.01&lt;sup&gt;c,b&lt;/sup&gt;</td>
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Values are means ± SE; n = 10. Values within a row with different letter superscripts are significantly different (P < 0.05): a > b > c > d > e.
formed between serum glucagon concentration 1 h after feeding and the intake of individual amino acids. Arginine intake and serum glucagon concentration showed a significant correlation ($r = 0.57; P = 0.006$). However, the associative analyses between serum glucagon and other amino acids, such as leucine ($r = 0.44; P < 0.031$), methionine ($r = 0.77; P < 0.002$), and histidine ($r = 0.87; P < 0.0001$), also were significant.

Hal activity. Two-way ANOVA showed a significant effect of the type of protein ($P < 0.0001$), concentration of dietary protein ($P < 0.0001$), and the interaction of type of protein × dietary protein concentration ($P < 0.0001$) on Hal activity. Hal activity was ~3.2-fold higher in rats fed 50% casein or soy diet than in rats fed 18% casein or soy diet ($P < 0.0001$). However, rats fed 50% zein showed only a 78% higher Hal activity than rats fed the 18% zein diet ($P < 0.001$). Rats fed 50% casein and soy diets showed 89 and 107% higher Hal activity than rats fed 50% zein ($P < 0.05$). Rats fed the 18% zein diet did not show a significant difference in Hal activity compared with rats fed 18% casein or soy diets (Fig. 3).

Serum glucagon concentration after 1 h of food ingestion of rats fed casein, soy protein, and zein diets showed a positive and significant correlation with hepatic Hal activity ($r = 0.49; P < 0.05$) (Fig. 4). No significant correlation was observed between serum insulin concentrations and hepatic Hal activity. Hal activity also showed a positive and significant correlation with protein intake ($r = 0.7; P < 0.001$).

Hal mRNA concentration. Similarly to Hal activity, Hal mRNA expression was induced in rats fed 50% casein or soy diets compared with those fed 18% casein or soy diets ($P < 0.05$). Hal mRNA abundance in rats fed 50% zein diets was higher than in rats fed 18% casein or soy diets.

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Fig. 1. Serum insulin (A) and glucagon (B) concentrations during fasting and 1 h after food ingestion in rats fed 18% or 50% casein (C18 or C50), soy protein (S18 or S50), or zein (Z18 or Z50) diets. Values are means ± SE; $n = 5$. Different letters indicate significant differences among groups ($P < 0.05$); a > b.

Fig. 2. Association between daily protein intake and serum glucagon concentration 1 h after food ingestion in rats fed 18 and 50% casein, soy protein, and zein diets after 10 days of treatment.

Fig. 3. Effect of the concentration and type of dietary protein on hepatic histidase (Hal) activity. Rats were fed casein, soy protein, or zein diets for 10 days. Values are means ± SE; $n = 10$. Bars with different letters are significantly different ($P < 0.05$): a > b > c.
casein or soy diets ($P < 0.05$), although it was significantly lower than in rats fed 50% casein or soy diets (Fig. 5).

DISCUSSION

Previous studies in rats fed diets containing graded concentrations of casein showed that, once the rat meets its protein requirement, the excess of amino acids contained in a high protein diet is oxidized. The degradation of the surplus amino acids is carried out by amino acid-degrading enzymes. We and others have demonstrated that the mechanism by which rats are adapted to consume high-protein diets is through the induction of genes coding for amino acid-degrading enzymes responsible for amino acid oxidation (11), including histidase (21). In this work, results showed that not only casein but also soy isolated protein, which are from an animal and a plant source, respectively, stimulated histidase gene expression similarly when they were added in high concentrations to the diet. Interestingly, rats fed the 50% zein diet also increased Hal activity and gene expression despite the reduction in food intake, although the induction was almost one-half of that observed with rats fed 50% casein or soy diets.

The differences in Hal gene expression by the three types of proteins used depend on the amino acid composition of each protein. Casein and soy protein meet the protein requirement of the rat when they are consumed in an 18% protein diet. When soy protein or casein diets are consumed in high concentrations (50%), the excess of amino acids is oxidized by amino acid-degrading enzymes because of the lack of a permanent amino acid reservoir in the body (6). In fact, rats fed 50% casein or soy protein diets induced Hal gene expression to a higher extent than rats fed an 18% casein or soy diet (Fig. 5). Hence, the activity and expression of the amino acid-degrading enzymes increase with elevated consumption of high-quality protein diets, as observed in this and other studies (1, 10). The exception is the branched-chain aminotransferase, the first enzyme in the branched-chain amino acid catabolism, which remains unaffected in most tissues by the protein content in the diet or by hormones, except in the skeletal muscle (20).

On the other hand, zein is a protein devoid of lysine and low in tryptophan, and for this reason this protein is classified as a low-quality protein. Thus the consumption of a low-quality protein produced some of the effects observed in rats fed an artificially imbalanced diet, such as depression in growth rate and decrease in food intake (19). In addition, rats fed zein diets showed low serum insulin concentrations (Fig. 1A) as a consequence of the low food intake, which can reduce body protein synthesis. This response, in turn, produces a continuous weight loss leading possibly to protein breakdown, mainly from skeletal muscle. Under this condition, amino acids from peripheral tissues are sup-

Fig. 4. Correlation between serum glucagon concentration after 1 h of food ingestion and hepatic Hal activity in rats fed 18 or 50% casein, soy protein, or zein diets after 10 days of consumption of the experimental diets.

![Graph showing correlation between serum glucagon concentration and hepatic Hal activity](http://ajpendo.physiology.org/)

Rho = 0.424, $p < 0.05$
plied mainly to the liver to maintain essential functions and plasma proteins (7). In fact, diets devoid of one indispensable amino acid increase the incorporation of amino acids into the liver compared with muscle (16). Nonetheless, the utilization of the limiting amino acids for body protein synthesis is high, and oxidation of individual amino acids other than limiting amino acids is increased (8). The catabolic condition induces amino acid-degrading enzyme gene expression, including Hal, increasing amino acid oxidation to yield energy as has been observed under some pathological conditions (3). Thus, in rats fed zein diets, the catabolic state and the reduced increase in protein intake stimulate Hal gene expression but to a lesser extent than in rats fed soy or casein diets. Glucocorticoids that are produced in catabolic states are also regulators of Hal gene expression. We have previously demonstrated that injections to rats with hydrocortisone also induce, although to a lower extent, the Hal gene (2). Further studies are required to analyze the effect in vivo of glucocorticoids on Hal gene expression under catabolic conditions.

The consumption of casein, soy protein, and zein diets was accompanied by an elevation of serum glucagon levels compared with fasting levels (Fig. 1B). However, the extent of increment of serum glucagon levels by these diets was not similar. The ingestion of an excess (50%) of high-quality protein, such as casein or soy protein, increased serum glucagon concentration to a higher extent than the excess of a low-quality protein such as zein due to its reduced intake. However, serum glucagon levels were significantly associated with the amount of protein ingested. Thus, rats fed zein diets ingested less protein and showed lower serum glucagon levels than rats fed soy or casein diets, which ingested approximately twice the amount of dietary protein. The elevation of serum glucagon by the dietary protein is supported by the fact that infusion of amino acids stimulates pancreatic glucagon secretion (9, 12). Several studies have been conducted to elucidate the mechanism by which amino acids stimulate glucagon secretion (14). Studies in pancreas ßTC6 cells have shown that glucagon gene expression is regulated by the availability of amino acids (13). We demonstrated previously (2) that administration of glucagon to rats stimulates Hal gene expression. Furthermore, the promoter sequence of the human Hal gene contains several cAMP-responsive cis-acting elements that include consensus sequences for transcriptional factors activated protein-1 and activating transcription factor (17). We are at present cloning the promoter sequence of the rat Hal gene, and preliminary results also reveal the existence of responsive elements to cAMP, indicating that glucagon can be a major regulator of the Hal gene (G. Aleman, N. Torres, and A. R. Tovar, unpublished results). Interestingly, in the present work, we observed for the first time that glucagon production due to the consumption of the different diets was significantly associated with the activity of hepatic histidase and that glucagon concentration was directly associated with protein intake.

These results support the physiological importance of glucagon in the expression of this enzyme.

It has been hypothesized that the low lysine and high arginine contents of soy protein decrease secretion of insulin and increase glucagon secretion compared with casein (15). We observed a significant correlation between dietary arginine concentration and serum glucagon concentration. However, we also found significant correlation with other amino acids, such as histidine, leucine, and methionine. These results indicate that secretion of glucagon after food ingestion is not absolutely associated with a single amino acid but rather with a group of amino acids, or with the total amino acid content.

On the other hand, serum insulin concentration increased in response to the type of protein consumed after 1 h of food ingestion, rather than to the amount of protein consumed. Consumption of casein diets, independent of their protein content, at least doubled serum insulin levels after 1 h of food ingestion. The response to glucagon and insulin by casein and soy protein diets altered the insulin-to-glucagon (insulin/glucagon) ratio. The insulin/glucagon ratio of rats fed 18 or 50% soy protein diets was ~44 and 50% lower, respectively, than the ratio of rats fed casein diets. The changes in this ratio will not only produce changes in amino acid catabolism but may also affect lipid and carbohydrate metabolism. We are conducting studies to demonstrate the lipogenic effect of casein diets. Also, in support of this evidence, piglets fed formula with casein and whey protein have higher postprandial insulin/glucagon ratios than animals fed formulas based on intact or hydrolyzed soy protein (24).

The results of this study improve our understanding of the physiological relevance of glucagon to amino acid catabolism. Adequate growth rates in young individuals and maintenance of body nitrogen balance are the result of rates of amino acid utilization for body protein synthesis and catabolism. Glucagon has a central role, and its concentration in blood depends, in part, on the type and amount of dietary protein. Hence, the concentration of glucagon is one of the fine-tuning regulators of nitrogen metabolism through the regulation of the amino acid-degrading enzyme gene expression.

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