Hepatic Histidase Gene Expression Responds to Protein Rehabilitation in Undernourished Growing Rats$^{1,2}$

Armando R. Tovar, Adriana Santos, Ali Halhalí, Héctor Bourges and Nimbe Torres$^3$

Departamento de Fisiología de la Nutrición, Instituto Nacional de la Nutrición, “Salvador Zubirán” México, D.F. México, 14000

ABSTRACT We studied the effect of nutritional rehabilitation with a 6, 18 or 50% casein diet in undernourished rats on histidase (Hal) expression. Undernutrition was induced by feeding rats a 0.5% casein diet for 5 wk. Over this period, growth, serum total proteins and insulin-like growth factor-I (IGF-I) levels were significantly lower than those of rats that freely consumed an 18% casein diet. During this period, undernutrition also significantly reduced Hal activity and Hal-mRNA concentration. Nutritional rehabilitation for 21 d with a 6% casein diet did not change any of these variables. Nutritional rehabilitation with an 18 or 50% casein diet for 1 d initiated the restoration of Hal activity and mRNA concentration. After 10 d of consuming 18 or 50% casein diets, Hal activity was 5- and 14-fold, and mRNA concentration was 8.5- and 23-fold higher, respectively, than in the protein-undernourished group (PU). During this period, body weight, total serum proteins and IGF-I levels were also significantly ($P < 0.05$) higher than those of the PU group. At the end of 21 d of rehabilitation with an 18 or 50% casein diet, Hal activity was 14- and 31-fold higher and Hal mRNA concentration was 10- and 24-fold higher, respectively, than in the PU group. In conclusion, our data showed that rehabilitation of undernourished rats with a 6% casein diet was not sufficient to re-establish growth indicators, Hal activity or gene expression, and that nutritional rehabilitation with an 18 or 50% casein diet effectively re-established body weight, biochemical variables and the capacity of histidase gene expression to eliminate the excess of protein. J. Nutr. 128: 1631–1635, 1998.

KEY WORDS: • gene expression • histidase • protein rehabilitation • rats • undernutrition

Protein-energy malnutrition in young children is one of the most important health problems in nonindustrialized countries (Brown and Pollitt 1996). Undernutrition triggers a number of biochemical, enzymatic and hormonal adaptations. These include an increase in the recycling of amino acids for protein synthesis and a decrease in amino acid catabolism, thus reducing urea synthesis and urinary nitrogen excretion (Torun and Chew 1994). Consumption of a protein-free or low protein diet as well as starvation produces a reduction in the rate of protein synthesis in most of the tissues including liver, skeletal muscle and intestine (Essén et al. 1992, McNurlan et al. 1979, Millward and Waterlow 1978, Wenerman et al. 1987). Because protein is not stored in the body, the ingestion of different amounts of dietary protein produces different levels of amino acid oxidation. When protein intake is below requirement, amino acids are preferentially channeled to protein synthesis, whereas when the protein intake is above requirement, the excess amino acids are catabolized by specific amino acid–degrading enzymes.

Histidase (Hal;$^4$ histidine ammonia-lyase EC 4.3.1.3) is the rate-limiting enzyme in the degradation of histidine, which is an indispensable amino acid in growing animals including humans. This cytoplasmic enzyme catalyzes the irreversible nonoxidative deamination of L-histidine, generating urocanic acid and ammonia (Mehler and Tabor 1953, Peterkofsky 1962). The activity of this enzyme increases with the protein content of the diet (Kang-Lee and Harper 1979). The changes in activity are associated with changes in the Hal-mRNA abundance. The dietary regulation of Hal expression is at the pretranslational level and occurs only in liver (Torres et al. 1998). The activity of several hepatic amino acid–degrading enzymes is low in rats fed low protein or protein-free diets (Aebi and Berger 1980) or in undernutrition (Rao et al. 1965). However, it is important to determine whether histidase gene expression is preserved after a period of chronic undernutrition. If this capacity is not preserved, the activity of this and perhaps several other amino acid–degrading enzymes would be low, producing toxicity due to accumulation of amino acids, especially with consumption of high protein diets. Understanding how the regulation of gene expression of amino acid–degrading enzymes occurs under these conditions is essential for elucidating the mechanisms by which body nitrogen is spared. Protein restriction increases, decreases or produces no changes in the expression of particular hepatic genes, indicating that dietary protein produces selective effects (Straus et al. 1994).

The purpose of this work was to evaluate the histidase gene expression in undernourished growing rats (0.5% casein) and...
after nutritional rehabilitation with three different concentrations of dietary protein (6, 18 and 50% of casein), equivalent to nutritional rehabilitation with a deficient, adequate or excessive protein diet. In this study, body weight, tail length and serum total protein and insulin-like growth factor-I (IGF-I) levels were measured as indicators of nutritional status.

MATERIALS AND METHODS

Animals, groups and diets. Male Wistar rats, weighing 75–90 g, obtained from the Experimental Research Department and Animal Care Facilities at the National Institute of Nutrition, México D. F., were housed individually in wire stainless steel cages at 22°C with a 12-h light:dark cycle and with free access to water. Rats were divided into two groups as follows: 1) a protein-undernourished group (PU) with free access to a 0.5% casein diet for 5 wk (n = 50) according to the protocol described by Philbrick and Hill (1974) and 2) a control group (C), which had free access to an 18% casein diet for 5 wk (n = 5). At the end of the 5-wk period, five rats from each group were killed, livers were removed immediately and samples were frozen in liquid nitrogen for RNA extraction; the rest of the tissue was used for measurement of histidine activity. Blood was also collected, and serum was separated and frozen at −20°C for determination of IGF-I and total proteins. The remaining undernourished rats were divided into three groups for 3 wk of nutritional rehabilitation with free access to diets containing different concentrations of protein as follows: 6% casein (low protein diet); 18% casein (amount of protein that meets the rat protein requirement); and 50% casein (high protein diet). Five rats from each group were killed on d 1, 10 and 21. It has been reported that pathologic lesions and other associated symptoms of undernutrition reverse within 21 d (Essén et al. 1992). Livers were weighed and samples of liver and serum were obtained and frozen as described above. Body weight, tail length and food consumption were measured daily.

Diets. Diets were administered dry form and contained (g/kg diet) 5.60, 180 or 500% vitamin-free casein; 50 corn oil, 50 mineral mix, 10 vitamin mix, and cornstarch and cellulose in a proportion of 1:1 were added to complete 1 kg of diet. The detailed composition of the diets, including minerals and vitamin has been previously reported (Torres et al. 1998). The ingredients were obtained from Teklad, Madison, WI. The 6% casein diet was supplemented with 0.2% L-methionine and 0.4% L-threonine to improve the nutritional quality of casein. The diets were isocaloric (401 kcal/100 g). The protocol of this study was approved by the Ethical Committee in Animal Experimentation of the National Institute of Nutrition.

Histidase activity. Liver (1 g) was washed with ice-cold saline, blotted and homogenized in 4 mL of an ice-cold solution containing 0.005 mol/L NaOH in 0.14 mol/L KCl with a polytron homogenizer (PT2000 Kinematica, 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 measurement of histidine activity. The activity was assayed as described by Spolter and Baldwin (1963). 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 mol/L pyrophosphate buffer, pH 9.2. An enzyme unit was defined as the formation of 1 nmol of urocanic acid/min. The protein concentration was measured by biuret assay using bovine serum albumin as standard.

Northern blot analysis. Total RNA was isolated from liver according to Chomczynski and Sacchi (1987). For Northern analysis, 20 μg of total RNA was electrophoresed in a 0.8% agarose gel, containing 37% formaldehyde, transferred to a nylon membrane filter (Hybond- N+) and cross-linked with a UV crosslinker (Amersham, Buckinghamshire, UK). The cDNA probe was a 1.95-kb polymerase chain reaction (PCR) product amplified from rat liver HSDNA kindly provided by R. R. McNees of The Hospital for Sick Children, Toronto, Canada (Taylor et al. 1990). The forward and reverse primers used for the PCR reaction were 5′-ATGCTTAGTGACTCGTGGCGTGGCGC3′ and 5′-TTAAAGATCGTCAGACTCTG3′, respectively. The PCR product was purified with Gene Clean and labeled with Redivue [α-32P] DCTP (110 TBq/mmol) using the Rediprime DNA labeling kit (Amersham). 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 mol/L sodium chloride/0.015 mol/L sodium citrate), 0.1% SDS at room temperature for 20 min and then twice for 15 min with 0.1× SSC/0.1% SDS at 65°C. Digitized imaging and quantification of radioactivity (cpm) of the bands were done by using the Instant Image (Packard Instrument, Meriden, CT). Membranes were also exposed to Extascan film, (Kodak de México, Guadalajara, Mexico) at −80°C with an intensifying screen.

Serum total proteins and IGF-I concentrations. Total serum protein concentration was measured in a Synchro CX analyzer (Beckman, Palo Alto, CA). IGF-I contained in the serum was separated from its binding proteins by an acid/ethanol extraction as previously described (Crawford et al. 1992, Daughaday et al. 1980) and determined by using a Nichols RIA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). Intra-assay variation for 110 and 248 μg/L serum average concentrations (n = 20 each) was 2.9 and 2.5%, respectively. Interassay variation for 99 and 173 μg/L serum average concentrations (n = 23 each) was 9.8 and 11.4%, respectively.

Statistical analysis. Results are presented as means ± SEM. Statistical analysis was done by two-way ANOVA. Significant differences among groups were determined by Fisher’s protected least-square difference test. When the error variance in the groups was found to be heterogeneous, a logarithmic transformation of the data was carried out before ANOVA analysis. Differences were considered significant at P < 0.05; (Statview statistical analysis program, V.4.3, Abacus Concepts, Berkeley, CA).

RESULTS

Food intake. Food intake of the PU group was 5.6 ± 0.4 g/d at the beginning of the experiment and 3.92 ± 0.32 g/d (P < 0.01) at the end of wk 5 of treatment. During the same period, the control group consumed 20.0 ± 3.6 g/d. After 3 wk of nutritional rehabilitation, the group fed the 6% casein diet consumed 9.5 ± 1.5 g/d, whereas the food intake of the groups fed 18 and 50% casein diet was significantly higher, 18 ± 0.2 and 17.3 ± 0.2 g/d, respectively.

Body and liver weights, tail length, serum total proteins and IGF-I concentrations. After 5 wk of treatment, body weight of rats with free access to a 0.5 or 18% casein diet was 53.7 ± 0.62 and 272.0 ± 11.6 g, respectively. When PU rats were nutritionally rehabilitated with 18% casein, weight gain resumed within 24 h at a rate of 1.53 ± 0.23 g/d, after 21 d of rehabilitation, rats gained weight at a rate of 5.5 ± 0.8 g/d. Rats rehabilitated with 50% casein gained 5.3 ± 0.8 g in the first 24 h; after 21 d of rehabilitation, rats were gaining weight at a rate of 6.7 ± 1.0 g/d, whereas the group of rats rehabilitated with 60% casein showed a growth rate of 0.4 ± 0.06 g/d.

PU rats also had significantly lower liver weight, tail length and serum total proteins than the control group (Table 1). Nutritional rehabilitation of undernourished rats for 3 wk with 6, 18 or 50% casein diets significantly improved all of these variables. Rehabilitation with 50% casein was the most effective (P < 0.01), indicating that diets with protein concentration >18% can reverse the effects of undernutrition on the growth rate and in the biochemical variables.

PU rats had 95% lower (P < 0.01) serum IGF-I concentration than the control group. After 3 wk of nutritional rehabilitation with the 6% casein diet or after 1 d with the 18 or 50% casein diet, serum IGF-I concentration remained unchanged, whereas after 10 d of rehabilitation with 18 or 50% casein diet, serum IGF-I concentration increased ~12-fold. Rehabilitation for 3 wk with the 18 or 50% casein diet increased serum IGF-I concentration significantly, 21.2- and 26-fold, respectively, compared with the PU group, leading to the restoration of IGF-I concentration to the values of the control.
group. These results indicate that IGF-I production after previous undernutrition is highly responsive to diets containing ≥18% casein and that it requires at least 3 wk to reach normal values.

**Hepatic histidase (Hal) activity.** Hal activity in the PU group was 92% lower than that in the control group (P < 0.01). Hal activity in rats rehabilitated with the 6% casein diet for 3 wk did not differ from the PU group. After 1 d of rehabilitation with the 18% casein diet, Hal activity was 3.7-fold higher than that of the PU group. After 10 and 21 d of consuming this diet, Hal activity was 5- and 14-fold greater than in the PU group. Hal activity of rats rehabilitated with the 50% casein diet for 1 d did not differ from that in rats fed the 18% casein diet; however, protein rehabilitation for 10 and 21 d with 50% casein increased Hal activity 14- and 31-fold with respect to the PU group (Fig. 1).

**Histidase mRNA concentration (Hal-mRNA).** The low Hal activity observed in the PU and 6% casein groups was also associated with low Hal-mRNA abundance (Fig. 2). Hal-mRNA concentration was 90% lower (P < 0.01) in these groups than in the control group. Hal-mRNA abundance increased over time in rats fed 18 or 50% casein diets, reaching its maximum value in those fed 50% casein after 10 d of rehabilitation. Hal-mRNA concentration in rats fed 18% casein after 1, 10 and 21 d of rehabilitation was 6.8-, 8.5- and 9.9-fold higher than in the PU group (Fig. 2).

**DISCUSSION**

This study demonstrated several metabolic adaptations that occur in the liver of nutritionally rehabilitated rats after a period of undernutrition. As expected, protein undernutrition produced a reduction in growth, serum total proteins and IGF-I levels. The decrease in serum IGF-I is possibly mediated by the low supply of amino acids in undernutrition because provision of amino acids regulates IGF-I mRNA and IGF-I gene transcription in rat hepatocytes (Pao et al. 1993). In turn, the reduction in growth observed in the PU group was probably mediated in part by the decrease in IGF-I levels as has been previously reported (Keteslegers et al. 1995).

Furthermore, undernutrition significantly reduced hepatic histidase activity and mRNA abundance. The poor availability of protein during undernutrition reduces protein synthesis (McNurlan et al. 1979, Millward and Waterlow 1978, Wenerman et al. 1987, Young and Schrimshaw 1968) to preserve...

When protein intake is reduced, there is a decrease in total body nitrogen or amino acid turnover (Toru Ân and Chew 1994). This is supported by the evidence that excretion of urinary nitrogen in humans is not further decreased when subjects are fed a protein-free diet for several days (Young and Schrimshaw 1968). It is estimated that during undernutrition, ~95% of amino acids are recycled (Toru Ân and Chew 1994).

FIGURE 2 Northern blot analysis of histidase expression in rats rehabilitated for 1, 10 and 21 d with diets containing 6, 18 or 50% casein diets, in control rats fed an 18% casein diet (C) and in protein- und nourished rats fed a 0.5% casein diet (PU). Panel A shows the autoradiography of the Northern blot analysis of 20 µg of total RNA from livers of rats in all groups. Panel B shows ethidium bromide stained gels of RNA samples as a control for RNA integrity. Panel C shows the densitometric analysis of autoradiographs. Values are mean ± SEM, n = 3. Bars with different letter superscripts are significantly different (P < 0.05).

branched-chain amino acids has been observed in growing rats fed a protein-free diet (Sketcher and James 1974).

The reduction in Hal activity was possibly mediated by repression of the expression of the Hal gene. Previous studies have shown that the expressions of glutaminase (Watford et al. 1994) and serine dehydratase (Ogawa et al. 1991) are reduced in animals fed low or protein-free diets. All of these studies, including this work, suggest a repression of genes encoding enzymes involved in the catabolism of amino acids to prevent the loss of body nitrogen under conditions of protein restriction.

Hormones are important in the adaptive metabolic process; they are the most important mediators of the expression of the amino acid–degrading enzymes. There is evidence that glucagon (Torres et al. 1998) and glucocorticoids (Lee and Harper 1971) are important mediators of Hal gene expression. During protein-energy malnutrition there is a reduction in plasma insulin and an increase in plasma cortisol and growth hormone (Lunn et al. 1973). Also, during severe kwashiorkor, plasma levels of glucagon are relatively low (Buchanan et al. 1976). It is not clear at this time how the specific hormonal milieu generated by protein undernutrition can repress the expression of Hal. The promoter region of the Hal human gene contains cAMP and glucocorticoid response elements (Suchi et al. 1995), although there is no information at the present time concerning the functionality of the Hal promoter.

Rehabilitation of undernourished rats with normal or high protein diets caused rapid growth (Table I). However, in those fed a low protein diet (6% casein), this effect was not observed even after 21 d of protein rehabilitation. During rehabilitation with adequate protein diets, an increase in amino acid uptake through system A is mediated by IGF-I and insulin (Hundal et al. 1994, Tovar et al. 1991), increasing protein synthesis and decreasing proteolysis in skeletal muscle (Fryburg et al. 1995), leading to the restoration of growth in undernourished rats.

Thus, the rapid stimulation of peripheral protein synthesis by IGF-I after the inclusion of amino acids in the diet (Jacob et al. 1996) can explain the rapid growth of the liver and the whole body during nutritional rehabilitation observed in animals fed diets that meet the protein requirement. Therefore, IGF-I is considered an anabolic agent that stimulates protein synthesis, improves nitrogen balance and produces an inhibitory effect on the capacity of urea synthesis by regulating the enzymes of the urea cycle (Grobe et al. 1997).

However, when amino acids provided by a high protein diet exceed the amounts needed for tissue protein synthesis, efficient channeling of the extra amino acids into degradative pathways is critical for preventing amino acid accumulation in the body (Harper 1984); the activity of the urea cycle enzymes increases with a concomitant increment of urea synthesis (Schimke 1962). Results in undernourished rats in this study demonstrated that the capacity to respond to dietary protein is preserved despite the initial severe protein malnutrition because Hal activity and Hal-mRNA expression increased after 24 h of nutritional rehabilitation with 18 or 50% casein diets. In conclusion, after prolonged protein undernutrition, Hal expression is repressed and does not respond to a low protein diet even after 3 wk of rehabilitation. However, Hal expression is re-established rapidly when dietary rehabilitation is carried out with a high protein diet, indicating that the mechanisms for sensing the excess of amino acids are still present in protein-malnourished rats.

LITERATURE CITED


