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Long-Term Alterations in Growth Hormone and Insulin Secretion after Temporary Dietary Protein Restriction in Early Life in the Rat

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ABSTRACT

Protein malnutrition early in life stunts subsequent physical growth in both humans and rats, but the mechanism(s) is unknown. To test the hypothesis that temporary early life dietary protein restriction produces long-term alterations in the growth hormone (GH) neuroendocrine axis, we examined the effects of 3 wk of exposure to dietary protein restriction in male rats postweaning (3–6 wk of age) on spontaneous and GH-releasing factor (GRF)-stimulated GH secretion at 12 wk of age. In comparison to rats weaned onto a normal diet (23% protein), rats weaned onto a low (4%) protein diet failed to catch up growth when transferred to the normal diet between 6 and 12 wk of age. Spontaneous 6-h GH secretory profiles of adult rats fed the low protein diet early in life showed a 41% reduction in mean GH peak amplitude and a significant suppression in overall mean 6-h plasma GH concentrations (37.5 ± 4.5 *versus* 56.9 ± 5.9 μg/L; $p < 0.02$). The magnitude of the GH response to 1 μg of rat GRF(1–29)NH_2 i.v. challenge was augmented during the GH trough period in these rats (165.7 ± 30.4 *versus* 43.9 ± 17.6 μg/L; $p < 0.01$). Although basal plasma IGF-I levels and glucose tolerance of protein-deprived rats were normal at 12 wk of age, the insulin response to ip glucose was severely blunted [insulin integrated area under the curve: 303.0 ± 32.7 *versus* 778.3 ± 105.0 pmol/L/75 min; $p < 0.01$]. These results demonstrate that temporary protein malnutrition early in life *1*) blunts spontaneous pulsatile GH secretion, *2*) augments GH responsiveness to GRF challenge, and *3*) reduces the insulin secretory response to glucose in adulthood. Our findings suggest that dietary protein in early life is an important determinant for CNS control of GH secretion as well as for the development of pancreatic β-cell sensitivity to glucose. Such alterations in the GH neuroendocrine axis, together with the subnormal insulin secretion, likely contribute to the lack of catch-up growth in this model. (***Pediatr Res* 38: 747–753, 1995**)

Abbreviations

GH, growth hormone
GRF, growth hormone-releasing factor
SRIF, somatostatin
SLI, somatostatin-like immunoreactivity
IRI, immunoreactive insulin
AUC, area under the curve

Undernutrition in early life impairs subsequent physical growth in both humans (1–4) and rats (5), but the mechanism(s) is not yet known. Exposure to early postnatal undernutrition, in general, and protein malnutrition, in particular, has deleterious effects on the development and function of various organs, and leads to lower protein/DNA ratios in several tissues (5–10). Such persisting cellular hypoplasia may result in insufficient cellular machinery for hormonal biosynthesis.

GH and insulin are important growth-promoting hormones which exhibit interrelated metabolic actions. In the rat, a limited period of protein malnutrition early in life results in a persistent impairment in the insulin secretory response to glucose (5), a decreased β-cell mass (11), and an increased peripheral sensitivity to insulin (12). In general, GH shares protein anabolic properties with insulin but counteracts the effects of insulin on glucose and lipid metabolism (13). The elevation in GH levels that occurs during pubertal acceleration of linear growth in the human leads to reduced peripheral sensitivity to insulin accompanied by a compensatory increase in glucose-stimulated insulin secretion (13), whereas GH-deficient patients are hypersensitive to the actions of insulin (14). Moreover, it has been suggested that the production of IGF-I, an important mediator of GH's action on peripheral tissues, is negatively affected by early life protein-energy

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malnutrition, preventing it from reaching the supranormal levels usually associated with compensatory or catch-up growth in the rat (15).

We have recently reported that short-term dietary protein restriction in the adult rat impairs spontaneous pulsatile GH secretion and have suggested that the blunted GH release may contribute to the growth retardation observed in this model (16). To date, there are no studies that have investigated the effects of temporary exposure to protein malnutrition in early life on the GH neuroendocrine axis in adulthood. In this context, it was tempting to speculate that alterations in GH secretion might account for both the growth deficits as well as some of the long-term consequences on carbohydrate metabolism characteristic of early life temporary protein restriction. To test this hypothesis, we examined the effects of 3 wk of exposure to dietary protein restriction in male rats postweaning on spontaneous GH, insulin, IGF-I, and glucose secretion at 12 wk of age.

METHODS

Animals and experimental procedure. Twenty-four male Sprague-Dawley pups and their lactating mothers were obtained from Charles River Canada (St. Constant, Quebec, Canada). Upon arrival, the rats were housed on a 12-h light, 12-h dark cycle (lights on between 0600 and 1800 h) in a temperature (22 ± 1°C)- and humidity-controlled room. Free access to regular Purina rat chow (Ralston-Purina, St. Louis, MO) and tap water was given throughout lactation. At 3 wk of age, the young rats were weighed and caged in groups of six, evenly matched for body weight. Twelve young rats (control group) were weaned onto the regular diet which consisted of 23% protein, 59% carbohydrates, 5% fat, 8% nonnutritive bulk, 1% vitamins, and 4% minerals, hereafter referred to as the normal diet. Twelve other rats (experimental group) were weaned onto a low protein isocaloric pelleted diet (ICN Biochemicals, Cleveland, OH) with the following composition: 4% protein, 78% carbohydrates, 5% fat, 8% nonnutritive bulk, supplemented with 1% vitamins and 4% minerals. The two diets were given *ad libitum* for 3 wk, and both groups of animals were raised under identical environmental conditions. At 6 wk of age, rats fed the low protein diet were transferred to the normal diet, and both groups of rats continued to consume the normal diet until the end of the experiment, at 12 wk of age. All animals were weighed weekly until 9–10 wk of age. At this age, both groups of rats were implanted with chronic intracardiac venous cannulae under sodium pentobarbital (50 mg/kg, intraperitoneally) anesthesia, using a previously described technique (17). After surgery, the animals were placed directly in isolation test chambers and given free access to regular Purina rat chow and tap water until their body weights returned to preoperative levels (approximately 1 wk). During this time, and thereafter, the rats were weighed and handled daily. Between 11 and 12 wk of age, a series of experiments were carried out. All animal-based procedures were approved by the McGill University Animal Care Committee.

On the day of each experiment, food was removed 1.5–2 h before the start of sampling and returned at the end. Blood samples (0.4 mL) were withdrawn every 15 min for periods of 6 h (1000–1600 h) from all rats. All blood samples were immediately centrifuged and the plasma separated and stored at −20°C for subsequent measurement of GH. The red blood cells were resuspended in isotonic saline and returned to the animal after removal of the next blood sample to prevent hemodynamic instability.

In the first experiment, we assessed the effects of early life exposure to the 4% protein diet on spontaneous pulsatile GH secretion and basal plasma IGF-I concentrations during adult life. Six-hour plasma profiles were obtained from five rats of the experimental group and from five rats of the control group.

In the second experiment, we examined the long term effects of early dietary protein restriction on pituitary GH responsiveness to GRF. Five rats from the experimental group and five rats from the control group were administered rat GRF(1–29)NH_2 i.v. at two different time points during the 6-h sampling period. The times of 1100 and 1300 h were chosen because these reflect typical peak and trough periods of GH secretion, respectively, as previously documented in this laboratory (17, 18). The GRF peptide (lot CH-23-25-31-10-16; kindly provided by Dr. P. Brazeau, Notre-Dame Hospital, Montreal, Canada) was diluted in normal saline just before use and administered at a concentration of 1 μg/0.3 mL. To document the rapidity of the GH response to GRF, an additional blood sample was obtained 5 min after each injection of the peptide.

In the third experiment, we investigated the glucose tolerance and insulin secretory response to glucose challenge. Rats from both groups were fasted overnight (15–17 h before the test). On the test day, basal blood samples (0.4 mL) were drawn every 15 min between 1030 and 1130 h. At 1130 h, rats of both groups were injected intraperitoneally with a 50% (wt/vol) glucose solution at a dose of 2 g of glucose/kg of body weight. Blood samples were obtained every 15 min for an additional 3-h period (1130–1430 h) after the injection, for subsequent measurement of glucose and insulin.

At 12 wk of age, both groups of rats were killed by rapid decapitation. The pituitary gland was removed, weighed, homogenized in 2 mL 0.05 M $NaHCO_3$-Na_2CO_3 buffer, pH 9.6, and centrifuged at 2000 × *g* for 30 min. The supernatant was removed and stored at −20°C until subsequent assay for GH. Portions of the pancreas (body and tail) were also quickly removed onto dry ice and weighed. Individual fragments were added to 2 mL of 2 N acetic acid, placed in a boiling water bath for 5 min to inactivate degradative enzymes, homogenized, and then centrifuged at 2000 × *g* for 30 min. The supernatant was removed and frozen at −20°C until subsequent assay for insulin and somatostatin.

Hormone assays. Plasma GH concentrations were measured in duplicate by double antibody RIA using materials supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Hormone Distribution Program (Bethesda, MD). The averaged plasma GH values are reported in terms of the GH reference preparation (rGH-RP-2). The standard curve was linear between 0.62 and 320 μg/L. All samples with values

above 320 μg/L were reassayed at dilutions ranging from 1:2 to 1:10. The intra- and interassay coefficients of variation were 7.6 and 9.6%, respectively, for duplicate samples of pooled plasma containing a mean GH concentration of 7.4 μg/L.

Plasma IGF-I concentrations were measured in duplicate by double antibody RIA using a modification of previously described methods (19, 20). To decrease the interference of IGF binding proteins in the assay, the samples were prepared by acid-ethanol extraction followed by cryoprecipitation. In brief, 200 μL of an acid ethanol solution [87.5% ethanol and 12.5% 2 N HCl (v/v)] was added to 50 μL plasma. After incubation for 30 min and centrifugation, 100 μL of supernatant was neutralized with 40 μL of 0.855 M Tris and kept at −20°C for 1 h. After cryoprecipitation and recentrifugation, the supernatant was further diluted 1:40 in a buffer containing 0.9% NaCl, the acid-ethanol solution, and 0.855 Tris-base (1:4:2 v/v). The RIA was performed in a buffer (pH 7.5) containing 200 mg/L protamine sulfate, 4.14 g/L sodium phosphate monosodic, 0.2 g/L sodium azide, 3.72 g/L EDTA, and 0.25% ultrapure BSA (A-4378; Sigma Chemical Co., St. Louis, MO). The IGF-I/SmC rabbit antiserum (UB3-189) was obtained from the NIDDK Hormone Distribution Program (gift of Drs. L. Underwood and J. Van Wyk). Recombinant human IGF-I (Lilly, Indianapolis, IN) was iodinated by the chloramine-T method. The averaged IGF-I values are reported in terms of the recombinant human IGF-I reference preparation (Lilly) that was used for the standard curve of the assay. The standard curve was linear between 0.1 and 25 μg/L. All samples were run in a single assay.

IRI was measured by a dextran-coated charcoal method using guinea pig anti-porcine insulin serum (21). Purified crystalline rat insulin (Lot. 615-JE 6-9; Lilly, kindly provided by Dr. R. Chance) served as a reference standard. The sensitivity of the assay was 27.5 pmol/L, and the intra- and interassay coefficients of variation were 8.2 and 10.3%, respectively, for duplicate samples of pooled plasma containing a mean IRI concentration of 976.3 pmol/L. Plasma glucose was measured by an automated glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Palo Alto, CA).

SLI was assayed by a previously reported double antibody RIA (22). On the day of the assay, the pancreatic samples were recentrifuged at 900 × *g* for 10 min and then diluted at concentrations ranging from 1:100 to 1:200 and neutralized with 5 N NaOH using phenol red as a pH indicator. The SLI values are reported in terms of the somatostatin-14 reference standard (lot 83-57-36-60-70; kindly provided by Dr. N. Ling, Salk Institute, La Jolla, CA). The intra- and interassay coefficients of variation were 8 and 12%, respectively.

Statistical analysis. The plasma GH profiles of individual rats were analyzed using the Cluster Analysis Program for endocrine pulse detection (23). Briefly, a *t* statistic of 4.1 was selected to maintain a maximal false-positive rate of 1% or less by using test cluster sizes of one in the prepeak nadir, peak, and postpeak nadir. Analysis of variance and *t* tests for unpaired and paired data, as appropriate, were used for statistical comparisons between and within experimental groups. The integrated area under the insulin and glucose response curves was calculated using the linear trapezoidal method. The results are expressed as the mean ± SE. $p < 0.05$ was considered significant.

RESULTS

Body weight gain. At weaning (3 wk of age), rats of the experimental group weighed 41.5 ± 0.5 g compared with 41.0 ± 0.8 g for the control group. After weaning, rats fed the normal diet continued to gain weight throughout the experimental period, with a mean body weight gain of 139.1 ± 3.2 g over the 3-wk period. In contrast, rats weaned onto the 4% protein diet (experimental group) gained almost no weight from weaning to 6 wk of age (4.2 ± 0.7 g; $p < 0.001$) (Fig 1). When transferred to the normal control diet, these rats immediately resumed growing and gained weight at a rate similar to that of the control group (Fig. 1); however, they remained significantly smaller than the control rats throughout the 6–12-wk period and never caught up in weight to the control group. (Final weight: 314.3 ± 5.6 *versus* 421.7 ± 6.5 g; $p < 0.001$) (Fig. 1).)

Effects of early life dietary protein restriction on spontaneous pulsatile GH secretion and basal plasma IGF-I levels in adulthood. Normally fed adult animals exhibited the typical pulsatile pattern of GH secretion characteristic of the male rat (Fig. 2*A*). Two major episodes of GH secretion were observed during the 6-h sampling period with most individual peak GH values greater than 150 μg/L; in the intervening trough periods, plasma GH values were usually below 1.2 μg/L. Adult rats fed the 4% protein diet between 3 and 6 wk of age continued to show the pulsatile pattern of GH release; however, the amplitude of the spontaneous GH pulses was decreased in these animals compared with normal diet-fed controls (Fig. 2*B*).

Cluster analysis of GH secretory dynamics (Fig. 3) revealed a 41% reduction in mean GH peak amplitude (197.3 ± 67.1 *versus* 335.4 ± 79.4 μg/L) and a significant suppression in

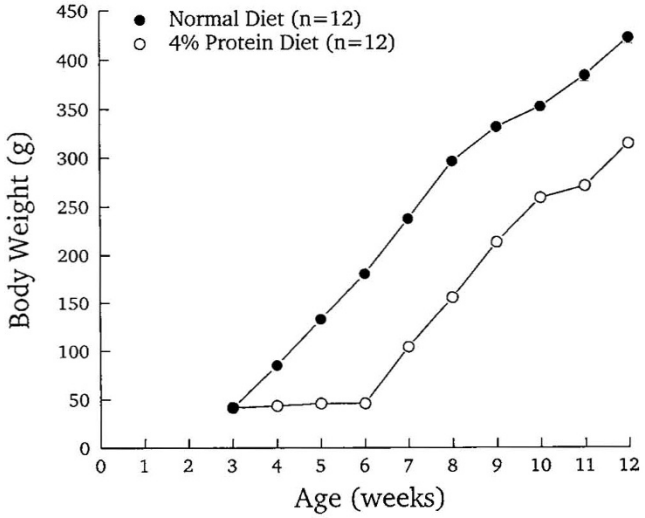

Figure 1. Growth curves of rats fed the 4% protein diet (*open circles*) or the normal diet (*closed circles*) between 3 and 6 wk of age. From 6 to 12 wk of age, both groups were maintained on the normal diet. Values are the mean ± SE; the SE are within the area of the symbols. The number of rats in each group is shown in parentheses.

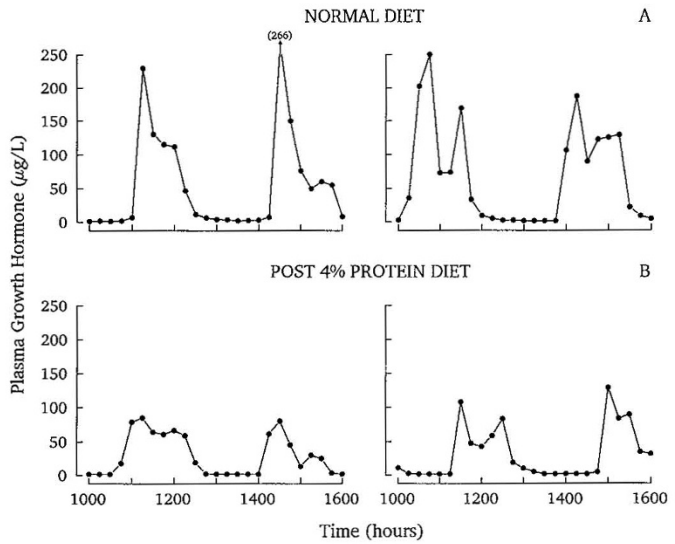

Figure 2. Individual representative 6-h plasma GH profiles in two adult rats fed the normal diet (*A*) compared with those in adult rats fed the 4% protein diet between 3 and 6 wk of age (*B*). Rats exposed to dietary protein restriction in early life exhibited an attenuation in amplitude of the spontaneous GH secretory bursts, although the typical ultradian rhythm of GH secretion was preserved.

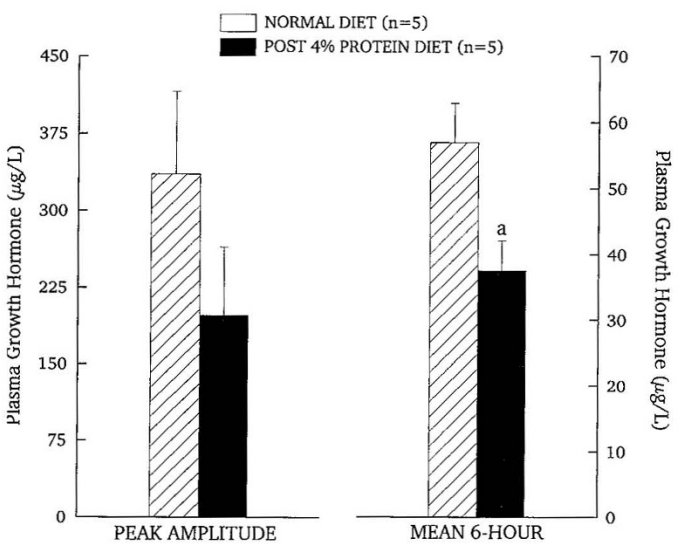

Figure 3. Cluster analysis of effects of dietary protein restriction early in life on pulsatile GH secretion. Rats fed the 4% protein diet between 3 and 6 wk of age exhibited a reduction in GH peak amplitude and overall mean 6-h plasma GH levels in adulthood. Each *bar* represents the mean ± SE. The number of animals in each group is shown in parentheses. *a*, $p < 0.02$ compared with normally fed rats.

overall mean 6-h plasma GH levels (37.5 ± 4.5 *versus* 56.9 ± 5.9 μg/L; $p < 0.02$) in rats fed the low protein diet compared with control-fed rats. There were no significant differences in GH trough levels within the limits of detection of our GH RIA (1.2 ± 0 *versus* 1.2 ± 0.02 μg/L), or in interpeak interval (3.7 ± 0.9 *versus* 2.8 ± 0.4 h), suggesting that the normal pulsatile pattern of GH secretion was preserved in these animals despite the attenuation in overall GH release.

Although the basal (at 1000 h) levels of plasma IGF-I in adult rats subjected to early life dietary protein restriction (680 ± 60 μg/L) were lower than those observed in rats fed the normal diet (770 ± 40 μg/L), the difference did not reach statistical significance ($p > 0.20$).

Long-term effects of early life exposure to 4% protein diet on GH responsiveness to GRF. Figure 4 illustrates the mean GH response to GRF administered at 1100 and 1300 h in both groups of rats. Animals fed the normal diet exhibited the typical malelike time-dependent pattern of GH responsiveness to GRF (18), with high GRF-induced GH release observed at the time of a spontaneous GH peak (564.6 ± 91.6 μg/L) and only a limited response during the trough period (43.9 ± 17.6 μg/L; $p < 0.02$) (Fig. 4*A*). Although adult rats fed the 4% protein diet between 3 and 6 wk of age continued to exhibit a time-dependent difference in their ability to respond to GRF (646.6 ± 79.8 *versus* 165.6 ± 30.4 μg/L; $p < 0.02$), the magnitude of their GH response during the trough period was augmented when compared with GRF-induced GH release in control-fed rats at the same time points (Fig. 4*B*).

Figure 5 summarizes the pituitary GH responsiveness to GRF in the two groups of rats. Compared with rats fed the normal diet, 4% protein-fed rats released 3–4-fold more GH at 1300 h (165.7 ± 30.4 *versus* 43.9 ± 17.6 μg/L; $p < 0.01$). Although GRF-induced GH release in these rats was also higher at 1100 h (646.6 ± 79.8 *versus* 564.6 ± 91.6 μg/L), this difference did not reach significance.

Effects of dietary protein restriction in early life on glucose tolerance and insulin secretory response to glucose in adulthood. The adult basal fasting levels of glucose (6.3 ± 0.3 mmol/L) and insulin (55.1 ± 0 pmol/L) in those rats subjected to early life dietary protein restriction were similar to the basal levels of glucose (6.3 ± 0.1 mmol/L) and insulin (58.5 ± 3.4 pmol/L) observed in the control group fed the normal diet (Fig. 6). After the glucose challenge, plasma glucose values increased to a peak of 29.0 ± 1.0 mmol/L in the low protein-fed group which did not differ from the peak glucose response

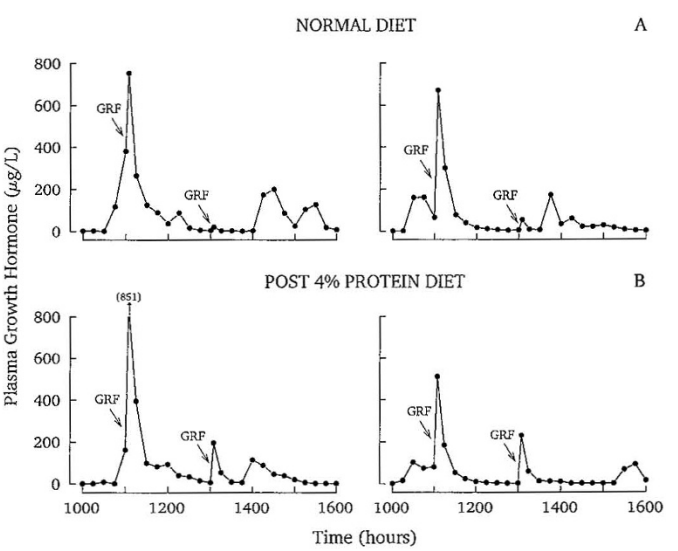

Figure 4. Adult plasma GH responses to 1 μg of GRF administered during peak (1100 h) and trough (1300 h) periods of GH secretion in individual rats fed either the normal diet (*A*) or the 4% protein diet (*B*) during 3–6 wk of age. The magnitude of the GH response to GRF challenge was augmented at trough times in rats fed the low protein diet. *Arrows* indicate the times of i.v. injections.

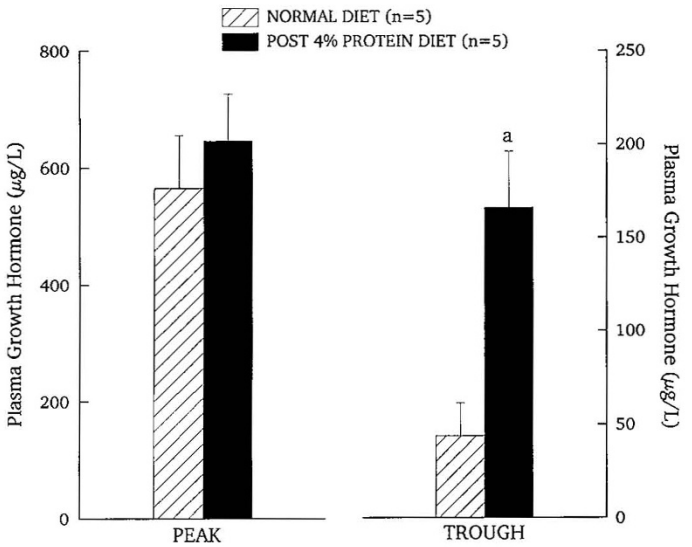

Figure 5. Summary of GH responsiveness to GRF in normal diet-fed rats and adult rats subjected to temporary dietary protein restriction early in life. Both groups exhibited a time-dependent difference in their ability to respond to GRF, with GRF-induced GH release being significantly greater during a time of peak GH secretion than during a trough period. However, rats fed the 4% diet showed a 3–4-fold augmentation in GH responsiveness to GRF at trough times compared with normally fed controls. Each *bar* represents the mean ± SE. The number of animals in each group is shown in parentheses. *a*, $p < 0.01$ compared with GH release during trough periods in rats fed the normal diet.

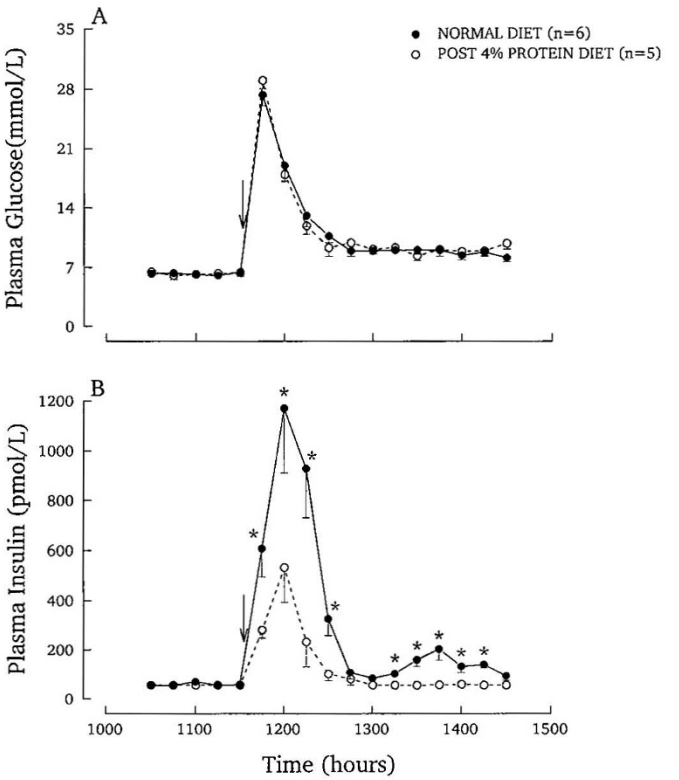

Figure 6. Mean plasma glucose (*A*) and insulin (*B*) response to ip glucose challenge (2 g/kg of body weight) at 12 wk of age in rats fed either the 4% protein diet (*dashed line*) or the normal diet (*solid line*) during 3–6 wk of age. Although the glucose response was similar in both groups, protein malnutrition early in life resulted in a severe impairment of the insulin secretory response to glucose in adulthood. *Arrows* indicate the time of injection. *Vertical lines* indicate the SE. The number of rats in each group is shown in parentheses. *$p < 0.05$ or less *vs* normally fed rats.

observed in the control group 26.9 ± 1.5 mmol/L (Fig. 6*A*). Subsequently, plasma glucose levels declined rapidly in both groups to values which again were not significantly different from each other. There was no significant difference in the integrated area under the glucose response curve between the two groups (19.1 ± 0.8 *versus* 18.6 ± 1.4 mmol/L/75 min).

In contrast to the normal glucose tolerance, the insulin response to intraperitoneal glucose was markedly blunted at 15, 30, 45, and 60 min in those rats fed the 4% protein diet in early life, when compared with rats fed the normal diet (Fig. 6*B*). The integrated area under the insulin response curve (AUC) in these animals was 2–3-fold lower than that of normally fed controls (AUC over 75 min: 303.0 ± 32.7 *versus* 778.3 ± 105.0 pmol/L/75 min; $p <$ 0.01). Moreover, whereas control-fed animals showed a second smaller insulin secretory response at 105–135 min after the intraperitoneal glucose injection, this was absent in rats fed the 4% protein diet in early life (see Fig. 6*B*).

Pituitary wet weight and pituitary GH concentration in rats fed the 4% protein diet in early life. The pituitary wet weight (11.3 ± 0.4 *versus* 14.1 ± 0.7 mg; $p < 0.01$) and pituitary GH content (520.5 ± 41.7 *versus* 631.8 ± 48.2 μg/fragment; NS) of rats exposed to the 4% protein diet early in life were lower than those of normal diet-fed rats. However, when expressed in relation to body and organ weights, respectively, the relative pituitary wet weight (3.5 ± 0.1 *versus* 3.3 ± 0.1 mg/100 g of body weight) and pituitary GH concentration (46.2 ± 3.7 *versus* 44.6 ± 3.0 μg/mg of wet weight) in 4% protein-fed rats were similar to those in rats fed the normal diet.

Pancreatic immunoreactive somatostatin and insulin concentration in adult rats exposed to dietary protein restriction in early life. Both pancreatic SLI (0.21 ± 0.03 *versus* 0.18 ± 0.03 ng/mg of wet weight) and IRI (12.5 ± 3.2 *versus* 9.0 ± 3.7 ng/mg of wet weight) concentrations were not different in adult rats exposed to the 4% protein diet early in life when compared with those of normally fed control animals.

DISCUSSION

The results of the present study demonstrate that temporary dietary protein malnutrition early in life leads to persisting alterations in GH and insulin secretion, which likely contribute to the lack of catch-up growth observed in this model.

Rats weaned onto a low (4%) protein diet between 3 and 6 wk of age exhibited a reduction in GH peak amplitude and mean 6-h plasma GH concentrations at 12 wk of age when compared with rats weaned onto a normal diet (23% protein). Despite this attenuation in overall GH release, the normal ultradian rhythm of GH secretion was preserved in these animals. These findings extend our earlier observations in the adult rat that short-term dietary protein restriction impairs pulsatile GH secretion (16).

The size of the pituitary gland was significantly lower in adult rats subjected to temporary protein restriction early in life than in normally fed controls. Although pituitary GH content was also lower in the adult rats weaned onto the 4% protein diet, when expressed in relation to body and organ weight, respectively, both pituitary wet weight and GH concentrations were similar in the two groups. These data indicate a relatively

preserved pituitary GH pool, and suggest that the pituitary gland might recover from the general cellular hypoplasia induced by limited protein-energy malnutrition early in life (5). It remains to be determined whether there are permanent changes in somatotroph number and/or somatotroph cell size after temporary early life dietary protein restriction.

The secretion of GH from the pituitary is regulated by a delicate interplay between stimulatory (GRF) and inhibitory (SRIF) hypothalamic hormones (18). In the present study, adult rats subjected to temporary early life protein restriction exhibited a 3–4-fold augmentation in GH responsiveness to GRF challenge, at trough times, when compared with that of normal diet-fed rats. Both SRIF and GRF have been shown to be sensitive to changes in nutritional status (21, 22, 24, 25). In particular, Bruno *et al.* (25) reported a dramatic reduction in hypothalamic preproGRF mRNA levels in protein-deprived adult rats. It is therefore possible that early postnatal protein deprivation produced a long-term alteration in hypothalamic GRF gene expression and/or release. A reduction in hypothalamic GRF signaling to pituitary somatotrophs could conceivably alter GRF receptor sensitivity resulting in up-regulation of the somatotroph GRF receptor, and perhaps account for both the augmentation in GH responsiveness to GRF and the attenuation in spontaneous GH pulses that we observed.

The augmented GH responsiveness to GRF reported here differs from our earlier observation of diminished GRF-induced GH release in rats fed a low protein diet in adult life (16), but resembles the paradoxical enhancement of GH responsiveness to GRF found in food-deprived adult rats that exhibit high SRIF tone (26). Thus, an increased SRIF influence on pituitary somatotrophs could provide an additional explanation for the suppression in GH pulse amplitude and the enhanced GH response to GRF that we observed in adult rats exposed to protein deprivation in early life. On the other hand, an increase in GRF-induced GH release at GH trough times has also been found in rats passively immunized with anti-SRIF serum (18). Thus, it remains unclear whether the somatostatinergic system is altered in this model.

It is also possible that the lack of essential amino acid dietary components early in life altered other brain neurotransmitters which are known to modulate GH secretion via changes in hypothalamic SRIF and/or GRF (27). Protein deprivation during development has been shown to significantly affect a variety of neurotransmitter systems including β-endorphin (8, 28); this could provide another mechanism for the alteration in the GH neuroendocrine axis. Although the hypothalamic-hypophysial axis is evident by the eighth postnatal day in the rat (29), CNS control of GH does not mature until after puberty (30). Taken together, all of these findings suggest that the maturation process of the regulatory mechanisms governing GH secretion is dependent upon adequate dietary protein supply during early life.

At the level of the pancreas, experimental protein-calorie malnutrition has been shown to reduce both the size of the individual β-cell (31) and the total islet mass (32). It is believed that the persistent reduction of pancreatic β-cell mass (11) results in an inadequate insulin secretory response to glucose (5), which may impair the ability of the β-cells to withstand an environmental diabetogenic insult (33). Our finding of a significant decrease in both the rapid first phase and second phase insulin secretory response to glucose, in the face of normal pancreatic insulin concentrations, suggests an altered sensitivity of the β-cell to glucose in adult rats exposed to temporary dietary restriction early in life. These *in vivo* results are similar to those previously reported by Swenne *et al.* (34) *in vitro*, and confirm that dietary protein during the weaning period is an important determinant for the development of β-cell sensitivity to glucose in the rat.

The presence of a normal glucose tolerance, despite the low insulin response to glucose, indicates that sufficient insulin was secreted for metabolic needs in the protein-deprived animals, and is consistent with the findings of Escriva *et al.* (12) of an increased sensitivity to insulin in target tissues of adult rats subjected to temporary early life protein malnutrition. Inasmuch as GH is known to induce a peripheral resistance to insulin action (13), it is conceivable that the relative deficiency of GH release, shown in this animal model for the first time, provides a compensatory mechanism to enhance peripheral tissue sensitivity to insulin in these rats. The finding of normal pancreatic SRIF concentration in these rats suggests that intrapancreatic SRIF is not involved in this response.

The mechanism(s) whereby protein malnutrition early in life stunts subsequent physical growth is, as yet, unknown. In the present study, rats subjected to dietary protein restriction from weaning to 6 wk of age failed to catch-up growth when transferred to the normal diet between 6–12 wk of age. This contrasts with findings in adult rats which exhibit catch-up growth (and increased GH concentrations) after exposure to a short period (24–72 h) of fasting (35). Increased insulin secretion has been considered to be one of the mechanisms responsible for catch-up growth during recovery from either acute starvation (36) or acute infection in weanling rats (37). In the experiments reported here, the amount of insulin appears to have been sufficient for glucose homeostasis, but was clearly insufficient to induce catch-up growth during the 6–12-wk time period. It has been shown that insulin plays a role in regulating GH-binding sites in peripheral tissues (38), in addition to the known regulatory role of GH (39), and that protein-deprived adult rats which are hypoinsulinemic (16, 40) have a reduced number of peripheral GH-binding sites (41). Therefore, it is plausible that the relative deficiency of GH secretion observed in the present study, together with the persisting reduction in insulin secretion, results in a decrease in GH-binding sites in peripheral tissues and, as a consequence, these tissues lack the ability to increase IGF-I production to the supranormal levels associated with compensatory (42) or catch-up growth. Indeed, plasma IGF-I levels in the adult rats subjected to temporary early life protein-energy malnutrition in the present study failed to exceed normal levels, consistent with that previously reported for both tissue and serum IGF-I concentrations in this model (15). All of these findings suggest that the cooperative interaction between GH, insulin, and IGF-I, which is essential for the normal growth process, is altered in this model.

In conclusion, the results of the present study demonstrate that temporary dietary protein restriction early in life *1*) blunts spontaneous pulsatile GH secretion, *2*) augments GH respon-

siveness to GRF challenge, and *3*) reduces the insulin secretory response to glucose in adulthood. Our findings suggest that dietary protein in early life is an important determinant for CNS control of GH secretion as well as for the development of pancreatic β-cell sensitivity to glucose. Such alterations in the GH neuroendocrine axis, together with the subnormal insulin secretion, likely contribute to the lack of catch-up growth after temporary dietary protein restriction early in life.

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