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Malnutrition during lactation changes growth hormone mRNA expression in offspring at weaning and in adulthood

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Abstract

Mothers' nutrition during lactation programs growth in their offspring. We studied the contribution of the growth hormone (GH) for this programming, evaluating GH mRNA expression. Lactating dams were grouped as follows: C, control diet with 23% protein; PR, 8% protein-restricted diet; and ER, energy-restricted diet, receiving the control diet in restricted quantities of the PR group's ingestion. Some pups were killed at weaning; the others received the control diet until they were sacrificed as adults. Pituitary GH mRNA was analyzed by Northern blot analysis. At weaning, the ER and PR animals had lower GH mRNA levels (−29% and −18%, respectively) and lower length as well as body weight. Ninety-day-old PR offspring showed a lower body length (−5%), whereas ER offspring showed a higher one (+5%); however, at 180 days, the lengths were not different. Both 90- and 180-day-old animals showed body weight differences against control animals, with PR offspring showing a lower (−10%) and ER offspring showing a higher (+12%) body weight. GH mRNA was higher in ER offspring at 90 and 180 days (+19% and +22%, respectively); it was lower in PR offspring at 90 and 180 days (−19% and −17%, respectively). Thus, we showed a direct relation between GH mRNA expression and length as well as body weight. We suggest that malnutrition during lactation may program GH mRNA expression patterns in adulthood and that these changes could be responsible for differences in growth patterns.

Keywords: GH; Malnutrition; Lactation; Programming; Rat

1. Introduction

Programming is defined as the influence of environmental changes occurring in a critical period early in life on physiological or pathological processes in adulthood [1]. In humans, epidemiological data confirm that the programming effect shows an association between low birth weight and the development of a metabolic syndrome [2], characterized by Type 2 diabetes mellitus, obesity and hypertension [3]. Several studies showed an abnormality in growth hormone (GH) secretion, characterized by lower mean levels, in children who suffered intrauterine growth retardation [4–8]. Studies on children have linked low birth weight with elevated serum IGF-I [7] or elevated urinary IGF-I excretion after catch-up growth [5].

Previously, we showed that malnutrition in lactating rats was associated with changes in body weight [9,10] and thyroid dysfunction in their adult offspring [11,12], reinforcing the concept of programming. The offspring of energy-restricted (ER) mothers had a lower body weight until weaning. However, after weaning, those animals gained more weight than did the offspring of the control (C) and protein-restricted (PR) mothers. In contrast, the PR offspring presented a lower body weight from birth to 6 months [9,10].

We evaluated several changes in hormonal regulation and milk composition during lactation in malnourished dams that could affect neonatal hormonal regulation. One of the most striking changes is maternal thyroid dysfunction [13], accompanied by a higher iodine [14] and T_3 [15] transfer

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through the milk in the PR dams. Despite these adaptive changes, pups still have a lower serum T_4 in the middle of lactation but a higher serum T_3 at the beginning and at weaning in the PR group [15]. ER animals had a different serum thyroid hormone pattern during lactation, characterized by a lower T_3 at weaning [15]. As thyroid hormone is important for GH production, especially early in life [16,17], we supposed that malnutrition and the associated thyroid dysfunction could cause important changes in neonatal GH production that can program its future regulation through adulthood.

We also detected that serum leptin was higher in the pups of malnourished dams at the end of lactation [10]. Recently, we observed that these offspring from malnourished dams presented a higher expression of the leptin receptor (Ob–Rb) in the anterior pituitary gland in their adult life [18]. In addition, those animals developed a resistance to the leptin anorectic effect [19]. As leptin seems to stimulate GH secretion [20,21], it is possible that this hormone can also be associated with GH changes in neonatal programming by malnutrition.

Programming of GH could be relevant for the hypothesis of fetal origin of diseases since a lower GH level is associated with a higher fat/lean mass [22] and a higher GH level is associated with the opposite relation but, notwithstanding, can compromise the cardiovascular function in the long term and precipitate to diabetes mellitus [23].

In this study, we aimed to evaluate whether maternal protein or energy restriction during lactation affects GH mRNA expression in the pituitary gland in the adult life of offspring.

2. Materials and methods

2.1. Animals and treatments

Wistar rats were kept in a room with controlled temperature (25±1°C) and with an artificial dark–light cycle (lights on from 7:00 a.m. to 7:00 p.m.). Three-month-old female rats were caged with one male rat at a proportion of 2:1. After mating, each female rat was placed in an individual cage with free access to water and food until delivery. The use and handling of experimental animals followed the principles described in the "Guide for the Care and Use of Laboratory Animals" [24].

On the first day of lactation, 18 dams were randomly assigned to one of the following three groups: (a) C group, with free access to a standard laboratory diet containing 23% protein; (b) PR group, with free access to an isoenergy and PR diet containing 8% protein; and (c) ER group, receiving a standard laboratory diet in restricted quantities, which were calculated according to the mean ingestion of the PR group. In this way, the amounts of food consumed by the ER and PR groups were about the same. Table 1 shows the composition of the diets, which follows recommended standards [25]. The PR diet was prepared in our laboratory using the control diet and replacing part of its protein with cornstarch. The amount of starch was calculated to make up for the decrease in energy content due to protein reduction. Malnutrition was started at birth (Day 0 of lactation) and was ended at weaning (Day 21).

Table 1
Composition of the control and low-protein diets

	Control diet[a]	Low-protein diet[b]
Ingredients (g/kg)		
Soybean+wheat	230.0	80.0
Cornstarch	676.0	826.0
Soybean oil	50.0	50.0
Vitamin mix[c]	4.0	4.0
Mineral mix[c]	40.0	40.0
Macronutrient composition (%)		
Protein	23.0	8.0
Carbohydrate	66.0	81.0
Fat	11.0	11.0
Total energy (kJ/kg)	17,038.7	17,038.7

[a] Standard diet for rats (Nuvilab, NUVITAL Nutrientes, Paraná, Brazil).

[b] The low-protein diet was prepared in our laboratory using the control diet and replacing part of its protein with cornstarch. The amount of the latter ingredient was calculated to make up for the decrease in energy content due to protein reduction.

[c] Vitamin and mineral mixtures were formulated to meet the AIN-93G recommendation for rodent diet and contain the recommended amount of iodine [25].

Within 24 h of birth, excess pups were removed such that only 6 male pups were kept per dam, because it has been shown that this procedure maximizes lactation performance [26]. Two pups of each dam were sacrificed on Day 21, giving a total of 12 animals per group. The other pups received a standard laboratory diet with 23% protein since weaning until they were 90 or 180 days old. At this time, offspring (2 from each dam) were killed with a lethal dose of pentobarbital and the pituitaries were excised and kept at −70°C, giving a total of 12 animals per group.

During lactation, body weight and nose–rump length were monitored everyday. After weaning, food intake, body weight and length were monitored every 4 days until the sacrifice. The amount of the diet ingested was the difference between the weight of food that remains in the food bin (Da) and the amount placed 4 days before (Di). These data were then used to calculate daily food intake according to the following formula: food intake (g)=(Di−Da/3)/4, where 3 represents the number of animals in each cage and 4 is the number of days.

2.2. GH mRNA expression evaluation

Anterior pituitaries were collected and two pituitaries were pooled as described previously [16,27]. Total pituitary RNA was isolated using the guanidine isothiocyanate–phenol–chloroform extraction method [28], and samples were then stored in liquid nitrogen until they were assayed. The total RNA concentration was estimated from the absorbance at 260 nm, and the RNA integrity was performed by observation of 28S and 18S ribosomal RNA (rRNA)

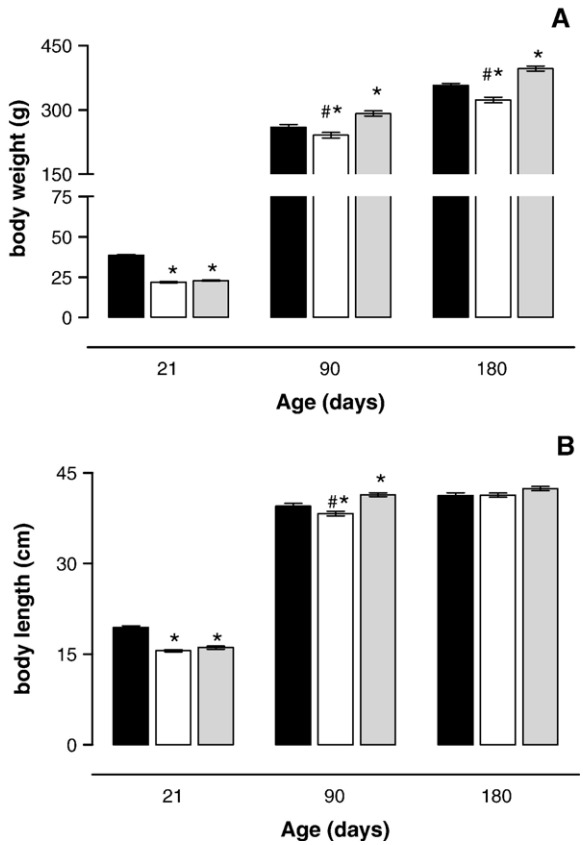

Fig. 1. Body weight (A) and length (B) of 21-, 90- and 180-day-old rats whose dams were fed a C (black bar), a PR (white bar) or an ER (gray bar) diet during lactation. Values represent the mean±S.E.M. of 12 pups per group. Significant differences between either of the diet-restricted groups and the C group (*) or between the two diet-restricted groups (#) were determined by a multiple-comparisons test.

bands in agarose gels stained with ethidium bromide. Total RNA was denatured with formaldehyde–formamide, electrophoresed in 1% agarose gel containing 2.2 M formaldehyde in 1× 3-*N*-morpholinepropanesulfonic acid buffer, blotted to a nylon membrane (Gibco, USA) by neutral capillary transfer and baked at 80°C in a vacuum oven. The membrane was prehybridized in 50% formamide hybridization solution and 100 μg/ml of sonicated salmon sperm DNA at 42°C for 4 h. Then, the membrane was probed with a full-length Randon Primer ^{32}P-labeled rat GH cDNA (Gibco) for 16 h at 42°C. The membrane was washed under high stringency conditions, subjected to autoradiography and quantified by phosphor imaging using ImageQuant software (Molecular Dynamics, USA) overnight. All blots were stripped and rehybridized with a ^{32}P-labeled RNA probe specific for 18S rRNA, synthesized by in vitro transcription (MAXIScript T_3/T_7 in vitro RNA transcription, Ambion, USA), to correct for the variability in RNA loading. The hybridization signal intensity was determined by a scanning densitometry system (STORM 840) and expressed in arbitrary units. The results are expressed as the mean±S.E.M. of GH mRNA/18S rRNA band ratios.

2.3. Statistical analysis

Data are represented as mean±S.E.M. Two-way analysis of variance followed by the Student–Newman–Keuls multiple-comparisons test was used for assessment of the significance of all data. Data are reported as arbitrary densitometry units, calculated by the quantification of GH mRNA/18S rRNA values, to evaluate GH gene expression. Differences were considered to be significant at $P<.05$.

3. Results

3.1. Evaluation of the pups at the end of lactation

3.1.1. Body weight and length

Pups whose dams were submitted to protein or energy malnutrition presented at weaning (21 days) significantly lower body weight [RP=−43%; RC=−41%; Fig. 1A] and body length [RP=−20%; RC=−17%; Fig. 1B] as compared with control pups.

3.1.2. GH mRNA expression

Fig. 2A presents a representative Northern blot of one experiment from a total of six experiments. Fig. 2B depicts lower GH mRNA levels in the pups from PR or ER dams as compared with control pups at the end of the lactation period (−29% and −18%, respectively; $P<.05$).

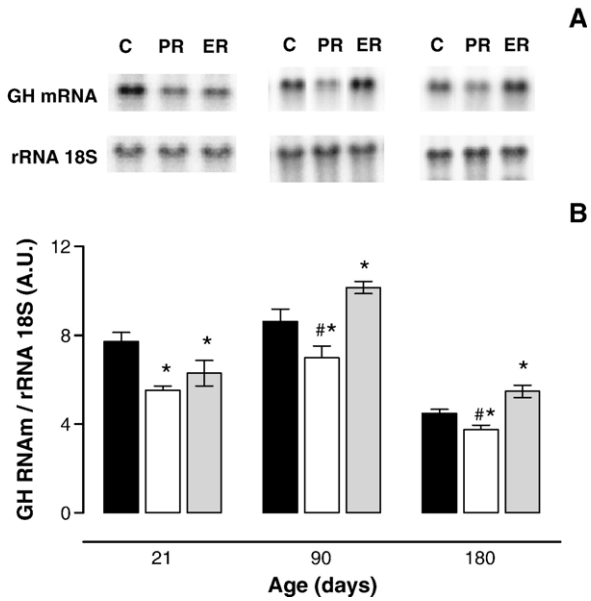

Fig. 2. (A) Northern blot analysis of pituitary GH mRNA levels of 21-, 90- and 180-day-old rats whose dams were fed a C (black bar), a PR (white bar) or an ER (gray bar) diet during lactation. (B) Quantitative representation of hybridization of rGH and 18S rRNA transcripts obtained by densitometry analysis of the exposed films and expressed in arbitrary units. Values represent the mean±S.E.M. of six pools of two pituitaries (12 pups per group) of GH mRNA/18S rRNA ratios. Significant differences between either of the diet-restricted groups and the C group (*) or between the two diet-restricted groups (#) were determined by a multiple-comparisons test.

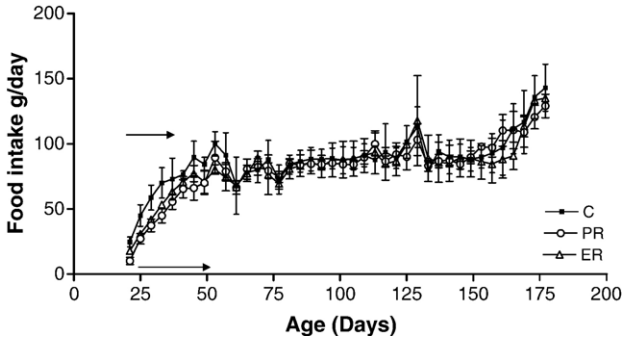

Fig. 3. Food intake from weaning to 180 days of offspring whose dams were fed a C, a PR or an ER diet during lactation. Values represent the mean±S.E.M. of 12 pups per group. The lower arrow shows that the differences between the C group and the RP group are significant ($P<.01$) from weaning to 53 days; the upper arrow, that the differences between the C group and the ER group are significant ($P<.01$) from weaning to 33 days. Significant differences were determined by a multiple-comparisons test.

3.2. Evaluation of adult rats whose dams were malnourished during lactation

Offspring of PR dams consumed less food from weaning until the 53rd day ($P<.01$) as compared with the C group, normalizing thereafter. Offspring of ER dams presented a similar eating behavior but normalized their ingestion earlier, at Day 33. At 90 and 180 days, food intake was not changed in both groups whose dams were malnourished during lactation (Fig. 3).

Body weight and length of the PR and ER adult animals are depicted in Fig. 1. The adult offspring of PR dams had lower body weight (90 days, −7%; 180 days, −10%; $P<.05$) as compared with the control animals. In contrast, the offspring of ER dams were 12% heavier than the offspring of control dams at 3 and 6 months ($F_{2,207}=54.23$; $P<.0001$). At 90 days, offspring of PR dams showed a lower body length (−5%; $P<.05$) and those of ER dams had a higher body length (+5%; $P<.05$), but there was no difference at 180 days.

As shown in Fig. 2, pituitary GH mRNA expression was significantly higher in ER offspring at 90 and 180 days (+19% and +22%, respectively; $P<.05$) and significantly lower in PR offspring at the same ages (−19% and −17%, respectively; $P<.05$).

4. Discussion

The present study confirms our hypothesis that malnutrition and the associated thyroid dysfunction, previously shown by our group in this experimental model of maternal malnutrition during lactation [9,11,13,15], could cause important changes in neonatal GH production and program its future regulation through adulthood.

The few studies that examined the relationship between birth weight and function of the GH–IGF axis in human adults produced inconsistent results, probably due to the complexity of the axis and the pulsatile secretion of GH [4,29–31]. Furthermore, the GH or IGF-I secretion is influenced by numerous factors, including adult body composition, physical fitness and nutritional status, and there is marked intra-individual variation [32]. Thus, rat models of neonatal malnutrition can overcome some of these pitfalls, such as the individual variation, and may better evaluate the pituitary production of GH, since we can obtain the gland.

During gestation, maternal caloric restriction was associated with a decrease in GH action in offspring at weaning [33], which agrees with our finding of a slower growth pattern during lactation and a lower GH mRNA expression. In our model, at weaning, both malnourished groups behaved in the same way for all the parameters analyzed. However, after weaning, the offspring's growth pattern and GH mRNA expression were dependent on the type of maternal malnutrition inflicted during lactation. The ER animals presented a faster growth pattern since the 80th day, with higher GH mRNA expression, whereas the PR animals behaved in the opposite direction for these values; these changes are similar to those described before, on the same experimental model, for body weight [9].

Previous changes observed during lactation for body weight [9] and serum leptin [10] in the pups showed no difference between the two malnourished groups (PR and ER), as observed here, for body weight and GH mRNA expression. Since leptin stimulates GH [20,21], the lower GH mRNA expression at weaning may be consequent to the lower serum leptin at the middle of lactation [10]. However, we previously detected hyperleptinemia in both malnourished groups at weaning [10]; therefore, variations in serum leptin cannot be an explanation for the different patterns of GH mRNA expression in adulthood. On the other side, during lactation, thyroid hormones presented different serum level patterns [11]. The PR group showed marked changes in both serum T_3 and T_4 as compared with the ER group, especially in the beginning of lactation, when only the PR group showed higher T_3 and lower T_4 serum concentrations. However, GH mRNA expression was similar in both malnourished groups during lactation. Nevertheless, the different patterns of thyroid hormone secretion in the two malnourished groups during lactation may have a role in the differences in the GH and growth programming.

In our model, we did not evaluate fat/lean mass, and we cannot tell for sure whether the higher growth rate observed in the ER group was associated with obesity. It is interesting that the animals of both groups showed a resistance to the anorectic effect of leptin as adults [19] but a higher Ob–Rb leptin receptor expression at the pituitary [18] as observed in obesity [34]. It was suggested that higher Ob–Rb expression is associated with an impairment of leptin signaling transduction [35]. Thus, at least in the PR animals, the impairment of leptin action can contribute to their lower GH mRNA expression since leptin increases GH secretion [20,21].

Thus, the programming effect depends on the different kinds of nutritional restriction. Furthermore, there was a

relation between growth pattern and GH mRNA expression in this model, which is coherent with a normal GH action.

It is known that there is an age-induced decline in the activity of the GH–IGF-I axis [36,37]. In this study, we showed that at 180 days, rats did not have a further significant increase in their body length but still showed an increase in body weight. Also, in humans, the programming effect of low birth weight on the GH–IGF-I axis is more pronounced in children or young adults than in late middle-aged persons [38]. Despite these less-marked changes in 180-day-old animals, all the groups have a decrease in GH mRNA expression when compared with 90-day-old animals. However, the difference in GH mRNA expression among the groups remains the same in 90- and 180-day-old animals.

Since ER animals presented a higher nose–rump length, it is possible that they effectively had a higher GH action through the increase of IGF-I. There is an important association between IGF-I and coronary artery disease progression [39]. In contrast, the PR group had a lower nose–rump length and a lower expression of GH mRNA, with possible low serum IGF-I. Low GH and IGF-I are related to a high percentage of body fat and waist circumference, well-established risk factors for atherosclerotic disease [40]. Also, adult patients with GH deficiency have increased body fat [41,42], insulin resistance, hypertension and higher mortality from cardiovascular disease [43,44].

According to our findings, we hypothesize that ER rats have more chances of developing vascular disease and that PR rats have more chances of having a higher fat/lean body mass, which can also compromise their cardiovascular function. Thus, despite the fact that adult ER and PR animals had different behaviors in their GH mRNA expression, body length and body weight gain, GH changes potentially could contribute to a higher risk of cardiovascular disease.

In conclusion, maternal malnutrition during lactation programs the GH mRNA expression pattern of offspring in adulthood; these changes may be responsible for the distinct growth patterns observed.

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References

[1] Barker DJ. The fetal and infant origins of disease. Eur J Clin Invest 1995;25:457–63.
[2] Jaquet D, Deghmoun S, Chevenne D, Collin D, Czernichow P, Levy-Marchal C. Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth. Diabetologia 2005;48:849–55.
[3] Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin North Am 2004;33:283–303.
[4] Flanagan DEH, Moore VM, Godsland IF, Cockington RA, Robinson JS, Phillips DI. Reduced foetal growth and growth hormone secretion in adult life. Clin Endocrinol 1999;50:735–40.
[5] Albertsson-Wikland K, Boguszewski M, Karlberg J. Children born small-for-gestational age: postnatal growth and hormonal status. Horm Res 1998;49(Suppl 2):7–13.
[6] Boguszewski MCS, Rosberg S, Albertsson-Wikland K. Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. J Clin Endocrinol Metab 1995;80:2599.
[7] Boguszewski CL, Jansson C, Boguszewski MCS, Rosberg S, Carlsson B, Albertsson-Wikland K, et al. Increased proportion of circulating non-22-kilodalton growth hormone isoforms in short children: a possible mechanism for growth failure. J Clin Endocrinol Metab 1997;82:2944–9.
[8] Ackland FM, Stanhope R, Eyre C, Hamill G, Jones J, Preece MA. Physiological growth hormone secretion in children with short stature and intra-uterine growth retardation. Horm Res 1988;30:241–5.
[9] Passos MCF, Ramos CF, Moura EG. Short and long term effects of malnutrition in rats during lactation on the body weight of offspring. Nutr Res 2000;20:1603–12.
[10] Teixeira CV, Passos MCF, Ramos CF, Dutra SCP, Moura EG. Leptin serum concentration in rats whose mothers were submitted to malnutrition during lactation. J Nutr Biochem 2002;13:493–8.
[11] Passos MCF, Ramos CF, Dutra SCP, Mouço T, Moura EG. Long-term effects of malnutrition during lactation on the thyroid function of offspring. Horm Metab Res 2002;34:40–3.
[12] Dutra SCP, Passos CF, Lisboa PC, Santos R, Cabanelas AP, Pazos-Moura CC, et al. Liver deiodinase activity is increased in adult rats whose mothers were submitted to malnutrition during lactation. Horm Metab Res 2003;35:268–70.
[13] Lisboa PC, Passos MCF, Dutra SCP, Santos RS, Bonomo IT, Cabanelas AP, et al. Increased 5′ -iodothyronine deiodinase activity is a maternal adaptative mechanism in response to protein restriction during lactation. J Endocrinol 2003;177:261–7.
[14] Passos MCF, Ramos CF, Dutra SCP, Moura EG. Transfer of iodine through the milk in protein-restricted lactating rats. J Nutr Biochem 2001;12:300–3.
[15] Passos MCF, Ramos CF, Mouço T, Moura EG. Increase of T_3 secreted through the milk in protein restricted lactating rats. Nutr Res 2001; 21:917–24.
[16] Volpato CB, Nunes MT. Role of thyroid hormone in the control of growth hormone gene expression. Braz J Med Biol Res 1994;27(5): 1269–72.
[17] Petersenn S, Rasch AC, Penshorn M, Beil FU, Schulte HM. Genomic structure and transcriptional regulation of the human growth hormone secretagogue receptor. Endocrinology 2001;142(6):2649–59.
[18] Vicente LL, Moura EG, Lisboa PC, Costa AMA, Amadeu T, Mandarim-de-Lacerda CA, et al. Malnutrition during lactation is associated with higher expression of leptin receptor in pituitary of the adult offspring. Nutrition 2004;20(10):924–8.
[19] Passos MCF, Vicente LL, Lisboa PC, Moura EG. Absence of anorectic effect to acute peripheral leptin treatment in adult animals whose mothers were malnourished during lactation. Horm Metab Res 2004;36(9):625–9.
[20] Tannenbaum S, Gurd W, Lapointe M. Leptin is a potent stimulator of spontaneous pulsatile growth hormone (GH) secretion and the GH response to GH-releasing hormone. Endocrinology 1998;139(9): 3871–5.
[21] Sone M, Osamura RY. Leptin and the pituitary. Pituitary 2001;4(1–2): 15–23.
[22] de Boer H, Blok GJ, Van der Veen EA. Clinical aspects of growth hormone deficiency in adults. Endocr Rev 1995;16:63–86.
[23] Colao A, Marzullo P, Di Somma C, Lombardi G. Growth hormone and the heart. Clin Endocrinol 2001;54:137–54.
[24] Bayne K. Revised guide for the care and use of laboratory animals available. Am Physiol Soc 1996;39:208–11.
[25] Reeves G, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76 rodent diet. J Nutr 1993;123:1939–51.

[26] Fischbeck KL, Rasmussen KM. Effect of repeated cycles on maternal nutritional status, lactational performance and litter growth in ad libitum-fed and chronically food-restricted rats. J Nutr 1987;117: 1967–75.
[27] Volpato CB, Nunes MT. Functional evidence for the presence of type II 5′-deiodinase in somatotropes and its adaptive role in hypothyroidism. Neuroendocrinology 2001;74(4):220–6.
[28] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.
[29] Fall C, Hindmarsh P, Dennison E, Kellingray S, Barker D, Cooper C. Programming of growth hormone secretion and bone mineral density in elderly men: a hypothesis. J Clin Endocrinol Metab 1998;83:135–9.
[30] Jernström H, Olsson H. Insulin-like growth factor-1 in relation to adult weight and birth weight in healthy nulliparous women. Int J Gynaecol Obstet 1998;62:11–8.
[31] Kajantie E, Fall CH, Seppala M, Koistinen R, Dunkel L, Yliharsila H, et al. Serum insulin-like growth factor (IGF)-I and IGF-binding protein-1 in elderly people: relationships with cardiovascular risk factors, body composition, size at birth, and childhood growth. J Clin Endocrinol Metab 2003;88:1059–65.
[32] Holt RI, Webb E, Pentecost C, Sonksen PH. Aging and physical fitness are more important than obesity in determining exercise-induced generation of GH. J Clin Endocrinol Metab 2001;86:5715–20.
[33] Woodall SM, Breier BH, Johnston BM, Gluckman PD. A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotropic axis and postnatal growth. J Endocrinol 1996;150:231–42.
[34] Lloyd RV, Jin L, Tsumanuma I, Vidal S, Kovacs K, Horvath E, et al. Leptin and leptin receptor in anterior pituitary function. Pituitary 2001;4(1–2):33–47.
[35] Huang XF, Zhang R. Upregulation of leptin receptor mRNA expression in obese mouse brain. NeuroReport 1997;8:1035–8.
[36] Savine R, Sonksen P. Growth hormone — hormone replacement for the somatopause? Horm Res 2000;53(Suppl 3):37–41.
[37] Ho KKY, Hoffman DM. Aging and growth hormone. Horm Res 1993;40:80–6.
[38] Holt RI, Syddall HE, Phillips DIW, Martyn CN, Gluckman PD, Breier H, et al. Serum insulin-like growth factor-I concentrations in late middle age: no association with birthweight in three UK cohorts. Acta Physiol Scand 2004;180:359–66.
[39] Ruotolo G, Båvenholm P, Brismar K, Eféndic S, Ericsson CG, de Faire U, et al. Serum insulin-like growth factor-I level is independently associated with coronary artery disease progression in young male survivors of myocardial infarction: beneficial effects of bezafibrate treatment. J Am Coll Cardiol 2000;35:647–54.
[40] Després JP, Lemieux I, Prud'homme D. Treatment of obesity: need to focus on high risk abdominally obese patients. BMJ 2001;322: 716–20.
[41] Attanasio AF, Howell S, Bates PC, Frewer P, Chipman J, Blum WF, et al. Body composition, IGF-I and IGFBP-3 concentrations as outcome measures in severely GH-deficient (GHD) patients after childhood GH treatment: a comparison with adult onset GH patients. J Clin Endocrinol Metab 2002;87:3368–72.
[42] Snel YEM, Brummer RJM, Doerga ME, Zelissen PM, Bakker CJ, Hendriks MJ, et al. Adipose tissue assessed by magnetic resonance imaging in growth hormone-deficient adults: the effect of growth hormone replacement and comparison with control subjects. Am J Clin Nutr 1995;61:1290–4.
[43] Johansson JO, Wiren L, Oscarsson J, Bengtsson BA, Johannsson G. Growth hormone (GH) replacement in GH-deficient adults: a crossover trial comparing the effect on metabolic control, well-being and compliance of three injections per week versus daily injections. Growth Horm IGF Res 2003;13:306–15.
[44] Sverrisdottir YB, Elam M, Caidahl K, Soderling AS, Herlitz H, Johannsson G. The effect of growth hormone (GH) replacement therapy on sympathetic nerve hyperactivity in hypopituitary adults: a double-blind, placebo-controlled, crossover, short-term trial followed by long-term open GH replacement in hypopituitary adults. J Hypertens 2003;21:1905–14.