Endocrine regulation and extended follow up of longitudinal growth in intrauterine growth-retarded rats

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Abstract

Bilateral uterine artery ligation in late gestation was performed in pregnant dams in order to determine the effects of intrauterine growth retardation (IUGR) on long-term postnatal somatic growth and the GH neuro-endocrine axis in the adult female and male rat. Body weight (BW), nose–anus length (NAL) and tail length (TL) were recorded at regular intervals in both the IUGR and control (CON) offspring until the age of 93 days. Spontaneous 6-h GH secretory profiles and serum IGF-I were determined around the age of 100 days in both the IUGR and the CON group.

No catch-up growth in BW, NAL or TL was observed in young adult male IUGR rats. Female IUGR rats did catch up in NAL beyond the age of 57 days and in TL before weaning, but did not catch up at any time in BW. Spontaneous 6-h GH secretory profiles in female and male

IUGR rats at a mean age of 100 ± 4 days were similar to their controls at a mean age of 101 ± 4 days. Overall median 6-h rat GH plasma concentrations, rat GH peak amplitude, number of rat GH peaks and sum of peak area were not significantly different. Median serum IGF-I levels in young adult female and male IUGR rats showed no difference when compared with their respective controls.

These results demonstrate that IUGR, after bilateral uterine artery ligation in late gestation, leads to incomplete BW catch-up growth in young adult rats of both sexes with physiological GH/IGF-I secretion, suggesting intra-uterine modulation of tissue responsiveness to GH and IGF-I. Female IUGR rats do catch up in NAL and TL, developing disturbed body proportions.

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Introduction

Intrauterine growth retardation (IUGR) in children is generally known to be a common cause of persistent short stature, as approximately 8–12% of the children born small-for-gestational age do not achieve complete catch-up growth in adulthood (Karlberg & Albertsson-Wikland 1995, Leger *et al.* 1997, Albertsson-Wikland *et al.* 1998). The exact mechanism of incomplete catch-up growth in IUGR children is not fully understood. The primary hormonal regulators of postnatal growth are growth hormone (GH) and insulin-like growth factor-I (IGF-I), whereby the growth capacity of peripheral tissues appears to depend on the response to GH, hepatic IGF-I and locally synthesized IGF-I (D'Ercole *et al.* 1984, Cohick & Clemmons 1993). Subnormal GH secretion and reduced IGF-I levels are described in a subset of short IUGR children (Thieriot-Prevost *et al.* 1988, De Waal *et al.* 1994, Albertsson-Wikland *et al.* 1998); however, GH resistance may also play a role in the inability to grow properly after IUGR (Gluckman & Harding 1997, Chatelain *et al.* 1998).

Several animal models have been designed in order to reveal a catch-up growth momentum and the possible hormonal regulation of this mechanism by GH and IGF-I. Analysis of the pulsatile GH pattern in rats has shown either increased GH secretion linked to the catch-up growth mechanism after fasting (Mosier *et al.* 1985) and after cortisone injections (Mosier & Jansons 1985) or reduced plasma GH concentrations after temporary early life dietary restriction (Harel & Tannenbaum 1995). The decreased IGF-I levels due to nutritional restriction in growth-retarded rats normalize during nutritional rehabilitation and subsequent catch-up growth (Thissen *et al.* 1994). A comparable rapid normalization of IGF-I levels is seen during catch up after thyroxine replacement therapy in hypothyroidism (Mosier *et al.* 1977) and during recovery after cortisone treatment (Mosier *et al.* 1976).

The timing and the duration of the growth-retarding insult seem to be crucial for the occurrence of catch-up growth in the rat. Limited nutritional intake during different phases of pregnancy and during lactation in the rat results in different degrees of growth retardation in

the offspring (Williams *et al.* 1974a,b, Garofano *et al.* 1998). However, in general, accelerated growth with complete catch up can be shown after a sufficient period of long-term follow up in growth-retarded rats (Williams *et al.* 1974b, Woodall *et al.* 1996b).

Unilateral and bilateral uterine artery ligation of the pregnant rat in late gestation is an animal model which appears to mimic third trimester IUGR in the human (Bussey *et al.* 1985, Ogata *et al.* 1986, 1990). The *in utero*-deficient nutrient flow gives rise to varying degrees of growth retardation in the rat fetus (Vileisis & D'Ercole 1986, Unterman *et al.* 1993) and no catch up in body weight gain is observed (Ogata *et al.* 1985, Jansson & Lambert 1999). However, information concerning long-term postnatal growth and possible changes in the GH/IGF-I axis related to the inability to catch up in this animal model is still lacking.

The aim of this study was to determine the long-term effects of IUGR induced by bilateral uterine artery ligation in the last trimester of gestation on postnatal growth and to evaluate the somatotrophic axis in the male and female IUGR rat in young adulthood.

Materials and Methods

Animals and experimental procedure

Timed pregnant Wistar rats (Harlan CPB, Austerlitz, The Netherlands) weighing between 250 and 300 g were housed individually in 42 × 25 cm cages containing fine wood chips as bedding and with free access to tap water and normal rat chow (AM-II; 24.8% protein, 6.6% fat, 1% vitamin, 4.5% minerals and 3.6% crude fibre; Hope Farms BV, The Netherlands) and allowed to acclimate for 24 h before surgery. The room was maintained at constant humidity and temperature (23 ± 1 °C) with a 12-h light:12-h darkness cycle (lights on between 0600 and 1800 h).

On day 17 of pregnancy a laparotomy was performed. General anaesthesia was induced by an intramuscular injection of ketamine (Aescoket®; Aesculaap BV, Boxtel, The Netherlands)/xylazine (Rompun®; Apharmo, Duiven, The Netherlands) (3:1 solution, 0.1 ml/100 g body weight (BW)) after ultrashort ether anaesthesia. A modified Wigglesworth method (Wigglesworth 1964, Ogata *et al.* 1985) was performed under aseptic conditions, the uterus exposed and the total number of fetuses in each horn counted. Both uterine arteries were ligated by a 5-0 Vicryl (Johnson and Johnson, Amersfoort, The Netherlands) suture. The uterus was returned to the abdominal cavity and the incision closed. Sham-operated pregnant rats underwent an identical procedure without ligation and their pups served as controls. All mothers recovered quickly after surgery. The pregnancy was allowed to continue to term with *ad libitum* feeding and water. Following spontaneous vaginal delivery around day

22, newborn pups in the IUGR group were considered growth retarded when initial BW (iBW), measured within 24 h of birth, was below -2 s.d. of the mean iBW of the sham-operated control (CON) pups. Mean iBW of the female and male CON pups was 6.4 ± 0.6 g.

Because of the limited number of live-born pups in the IUGR group the litter size was kept at three to four pups per lactating mother, regardless of sex. In the sham-operated CON group, litters consisted of six pups per lactating mother. The lactating mothers were given a normal diet and water *ad libitum*. Pups were weaned at day 25 and were housed in either triplets (females) or pairs (males) and allowed free access to normal rat chow and tap water.

Longitudinal growth in all animals was followed up from day 2 to day 93 of age. BW, nose-anus length (NAL) and tail length (TL) were recorded at days 2, 4, 10, 15, 22, 29, 35, 51, 57, 64, 71, 78, 86 and 93 in 16 female and 14 male rats in the IUGR and in 17 female and 16 male rats in the CON group. BW was measured on a digital scale (Mettler P1200) with an accuracy of 0.1 g. NAL, from tip of nose to anus and TL, from anus to tip of tail were measured along a standard ruler (cm). Longitudinal growth with regard to BW in both sexes was followed up only in a small subset of two female IUGR and six CON rats, as well as two male IUGR and four CON rats until 18 months.

Cannulation was performed in both the IUGR and CON animals between days 93 and 102. Under general anaesthesia with ketamine (Aescoket®)/xylazine (Rompun®) in a solution of 3:1 (0.1–0.15 ml/100 g BW) injected intramuscularly, after ultrashort ether anaesthesia, an incision in the neck was made and a catheter (polyethylene PP 50 with a Silastic tip) was implanted in the right jugular external vein. The cannula was then tunnelled under the skin behind the right ear, exteriorized between the shoulder blades and led through a spring (length approximately 30 cm) which was fixed, subcutaneously, on the back of the animal. Patency of the cannula was secured by injecting a solution of polyvinylpyrrolidone (PVP), 0.9% NaCl and heparin (0.5 g PVP and 50 U heparin/ml 0.9% NaCl) in the catheter and repeated once the following day. After surgery the animals were placed, separately, in isolation test chambers and the spring, containing the cannula, was attached to a swivel so that the animal could move around freely. They were given free access to regular rat chow and tap water and left to recover for 4–6 days.

At a mean age of 100 ± 4 days in the IUGR and 101 ± 4 days in the CON group, the 6-h GH sampling in eight female and six male IUGR as well as in six female and six male CON rats was performed. On the morning of the experiment, after a cannula patency check, one basal blood sample of 350 µl was withdrawn and subsequently blood samples (50 µl) were withdrawn every 10 min for a period of 6 h (0800–1400 h). Blood samples were centrifuged and the plasma separated and stored at -20 °C for measurements of the different hormones. The protocol was

Table 1 Characteristics of progeny of rats from dams that underwent bilateral uterine artery ligation in late gestation and from control sham-operated dams. Values are expressed as means $\pm$ S.E.M. The number of animals in each group is in parentheses

	CON females	IUGR females	CON males	IUGR males
Body weight				
At birth (g)	6.5 $\pm$ 0.001 (17)	4.7 $\pm$ 0.1** (16)	6.6 $\pm$ 0.001 (16)	4.9 $\pm$ 0.001** (14)
At weaning (g)	88.0 $\pm$ 1.5 (17)	73.8 $\pm$ 2.7** (16)	92.2 $\pm$ 1.9 (16)	74.2 $\pm$ 2.4** (14)
At sampling (g)	253.2 $\pm$ 3.5 (6)	211.6 $\pm$ 6.9** (8)	433.5 $\pm$ 10.4 (6)	363.2 $\pm$ 7.4** (6)
At 510 days (g)	350.7 $\pm$ 9.6 (6)	299.5 $\pm$ 16.5* (2)	589.0 $\pm$ 13.8 (4)	515.0 $\pm$ 20.0* (2)

CON, progeny of sham-operated dams; IUGR, progeny of dams after bilateral uterine artery ligation in late gestation.

* $P < 0.01$, ** $P < 0.001$ compared with control group.

approved by the Animal Ethical Committee of the University 'Vrije Universiteit' at Amsterdam.

Hormone assays

Rat GH (rGH) was measured in heparinized plasma samples by radioimmunoassay using reagents supplied by NIDDK-NIH (Torrance, CA, USA). To assay the samples, 100 μ l 125 I-labelled rGH (5000–7000 c.p.m.) and rGH antibody (1:945 000 final concentration) were added to 10 μ l heparinized plasma and incubated overnight at room temperature. rGH standards (0–800 ng/ml) were prepared by doubling dilutions in human serum. To separate the antibody-bound fraction, a mixture of 100 μ l of a second antibody and 500 μ l 6% polyethyleneglycol was added to the tubes. After incubation at room temperature for 30 min, the tubes were centrifuged, the supernatant was decanted and the pellet was counted for radioactivity. Results are expressed in ng/ml in terms of the NIDDK standard RP-2, and represent the mean of duplicate determinations. The intra-assay coefficient of variation was 10.6–13.6% and the lowest detectable concentration of plasma rGH was 6 ng/ml.

Serum IGF-I was measured in the basal blood sample by a human radioimmunoassay suitable for the measurement of rat IGF-I (Medgenix Diagnostics, Belgium). Results are expressed in ng/ml.

Statistical analysis

rGH data from repetitive blood samples were analyzed using PC PULSAR program version 2.1 (Merriam & Wachter 1982). Peaks were assigned a value of 0.05 for baseline calculations. The mean rGH level after smoothing was subtracted from PC PULSAR-identified rGH peaks to obtain amplitudes. The cut off value for peak splitting was set at 2.7. The 'G' criteria for determining significant pulses were: G(1), 3.80; G(2), 2.60; G(3), 1.90; G(4), 1.50; G(5), 1.20. Data were analyzed in eight female

IUGR and six male IUGR rats as well as in six female CON and six male CON rats. Group differences in individual pulse parameters were analyzed using the Mann–Whitney U test (non-parametric test/SPSS).

Differences in growth parameters between groups were evaluated with a Student's *t*-test at separate time points and with MANOVA (BMDP5V, general autoregression and 1st order autoregression) to determine changes in time. Statistical significance was assigned to *P* values of < 0.05 .

Results

Overall survival rate

A total of 34 pregnant rats underwent laparotomy. Another seven pregnant rats were sham-operated. Overall survival rate of the pups was 41% (pups counted *in utero*: 359; pups live born: 148) in the IUGR group and 97% (pups counted *in utero*: 89; pups live born: 86) in the sham-operated group. Of the live-born pups in the IUGR group only 25% were growth retarded with iBW < 5.2 g within 24 h after birth. A small proportion of IUGR pups ($< 5\%$) died in the first week with iBW < 3.0 g.

Longitudinal somatic growth

iBW (BW within 24 h of birth) in the IUGR offspring was 26% decreased in the male and 28% in the female pups compared with their respective controls ($P < 0.001$). Percentage BW change from day 1 to weaning was not different in the IUGR offspring (93.6%) versus the CON (92.6%) juvenile animals. At the time of the 6-h GH sampling BWs in the severely growth-retarded IUGR offspring (iBW < -2 s.d. mean BW of controls) were significantly lower ($P < 0.01$) than BW in control rats of the same sex (Table 1).

Figure 1 BW from 2 to 93 days of age in (left-hand graph) female (●, $n=16$) and (right-hand graph) male (■, $n=14$) IUGR rats after bilateral uterine artery ligation in late gestation and in sham-operated female (○, $n=17$) and male (□, $n=16$) control (CON) rats. The insets show BW from day 2 to day 22. Data are presented as means $\pm$ S.E.M. ** $P<0.01$, *** $P<0.001$ compared with CON rats.

Females Figure 1 (left-hand graph) shows the significantly lower BW from day 2 to day 93 in the IUGR female rats. NAL in the female IUGR rats was significantly reduced ($P<0.05$) from day 2 to 57 compared with female controls, but catch up in NAL did occur thereafter (Fig. 2, left-hand graph). TL in this group was significantly reduced prior to day 22 ($P<0.01$). From day 22 until adulthood, TL in the female IUGR rats was not significantly different from the CON group (Fig. 3a).

Males Figure 1 (right-hand graph) shows the significantly lower BW from day 2 to day 93 in the IUGR male rats. NAL remained significantly reduced ($P<0.05$) in male IUGR rats from day 2 to day 93 (Fig. 2, right-hand graph). The same applied to TL, which was significantly reduced compared with that of the CON group throughout the study period (Fig. 3b).

The GH secretory pattern

Representative 6-h rGH profiles from female and male IUGR and CON rats are shown in Fig. 4. Table 2

summarizes the 6-h rGH profiles in both sexes in the IUGR and CON group.

Females All females in the IUGR group showed similar rGH secretion when compared with their CON counterparts. The median 6-h rGH was 21.9 (range: 9.5–30.1) vs 15.4 (range: 11.4–28.4) ng/ml and median baseline rGH was 14.0 (range: 8.7–22.4) vs 11.4 (range: 6.9–15.4) ng/ml. The median number of rGH peaks was 1.0 (range: 0.0–3.0) vs 2.0 (range: 0.0–3.0), the median sum of peak area was 1590.6 (range: 0.0–4103.2) vs 756.0 (range: 0.0–1981.0) ng/ml.min and the median rGH peak amplitude was 36.4 (range: 0.0–105.5) vs 32.9 (range: 0.0–56.5) ng/ml (Table 2).

Males When comparing male IUGR rats with male CON rats no significant difference in the rGH secretory pattern was found. Male IUGR rats showed a median 6-h rGH of 24.5 (range: 18.5–32.0) vs 23.2 (range: 14.5–28.9) ng/ml and a median baseline rGH of 9.7 (range: 8.1–12.7) vs 9.0 (range: 6.1–11.8) ng/ml. The median number of rGH peaks was 4.0 (range: 2.0–5.0) vs 3.0

Figure 2 NAL from 2 to 93 days of age in (left-hand graph) female (●, $n=16$) and (right-hand graph) male (■, $n=14$) IUGR rats after bilateral uterine artery ligation in late gestation and in sham-operated female (○, $n=17$) and male (□, $n=16$) control (CON) rats. Data are presented as means $\pm$ S.E.M.

(range: 3.0–7.0), the median sum of peak area was 1757.8 (range: 1549.8–2068.7) vs 1295.9 (range: 795.9–2963.0) ng/ml . min and the median rGH peak amplitude: 54.0 (range: 30.1–98.5) vs 31.8 (range: 23.0–67.3) ng/ml (Table 2).

Within their group, IUGR male rats showed a significantly higher median number of rGH peaks compared with IUGR female rats ($P<0.05$). Median baseline rGH was significantly lower in males compared with females in the IUGR group ($P<0.05$).

Serum IGF-I levels

Females The serum IGF-I levels, obtained in adult female rats at the start of the 6-h rGH sampling, did not differ significantly between the female IUGR and CON rats with a median level of 780 ng/ml (range: 640–1000) vs 700 ng/ml (range: 260–1200) respectively (Table 2).

Males The serum IGF-I levels, obtained in adult male rats at the start of the 6-h rGH sampling, were not significantly different in the male IUGR versus CON rats

with a median of 850 ng/ml (range: 560–1100) vs 940 ng/ml (range: 860–1200) respectively (Table 2).

Within the IUGR group, serum IGF-I levels did not differ significantly when female rats were compared with male rats, nor did serum IGF-I concentrations differ between female and male rats in the CON group.

Discussion

The results of the present study demonstrate that IUGR caused by third trimester bilateral uterine artery ligation leads to persistent failure to catch up in BW in the young adult offspring. This is accompanied by a normal adult GH secretory pattern and circulating IGF-I at physiological levels at the mean age of 100 days in the female and male IUGR rat.

Bilateral uterine artery ligation in the last week of gestation in the pregnant rat, followed by natural birth of the IUGR rats, is an animal model mimicking placental insufficiency in the human (Ogata *et al.* 1985). Third trimester placental insufficiency in humans gives rise to

Figure 3 TL in 2-, 22-, 51-, 64- and 93-day-old (a) female and (b) male rats born from dams after bilateral uterine artery ligation in late gestation (IUGR: 16 females and 14 males; solid bars) or born from sham-operated mothers (CON: 17 females and 16 males; open bars). Values are given as means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with CON rats.

Figure 4 Representative 6-h GH secretory profiles in an IUGR (●) and a sham-operated (control) female rat (○) (upper panel) as well as in an IUGR (■) and a sham-operated (control) male rat (□) (lower panel).

Table 2 Plasma 6-h rGH, baseline rGH, sum of peak area, number of rGH peaks and rGH peak amplitude as well as serum IGF-I in 100-day-old female and male rats with IUGR after bilateral uterine artery ligation and sham-operated controls (CON). Values are expressed as median (range) with the numbers in each group in parentheses

Group	GH (ng/ml)	Baseline GH (ng/ml)	Peak area GH (ng/ml · per min)	No. of GH peaks	GH peak amplitude (ng/ml)	IGF-I (ng/ml)
CON female (6)	15.4 (11.4–28.4)	11.4 (6.9–15.4)	756.0 (0.0–1981.0)	2.0 (0.0–3.0)	32.9 (0.0–56.5)	700 (260–1200)
IUGR female (8)	21.9 (9.5–30.1)	14.0 (8.7–22.4)	1590.6 (0.0–4103.2)	1.0 (0.0–3.0)	36.4 (0.0–105.5)	780 (640–1000)
CON male (6)	23.2 (14.5–28.9)	9.0 (6.1–11.8)	1295.9 (795.9–2963.0)	3.0* (3.0–7.0)	31.8 (23.0–67.3)	940 (860–1200)
IUGR male (6)	24.5 (18.5–32.0)	9.7* (8.1–12.7)	1757.8 (1549.8–2068.7)	4.0* (2.0–5.0)	54.0 (30.1–98.5)	850 (560–1100)

* $P < 0.05$, males compared with females within each group (Mann–Whitney U test (non-parametric test/SPSS)).

disproportionately growth-retarded newborns, who fail to catch up their longitudinal growth in 8–12% of young adults (Leger *et al.* 1997, Albertsson-Wikland *et al.* 1998).

The degree of growth retardation can determine the ability to catch up. In humans, prematurity and severity of the growth retardation are poor prognostic factors for normal height attainment at 4 years (Fancourt *et al.* 1976) and at 2 years of age (Leger *et al.* 1997, Luo *et al.* 1998). The initially reported 15–40% failure of catch-up growth in IUGR children (Fitzhardinge & Inwood 1989, Albertsson-Wikland *et al.* 1993, Hokken-Koelega *et al.* 1995) is reduced to 8–12% if followed up into adulthood (Karlberg & Albertsson-Wikland 1995, Leger *et al.* 1997, Albertsson-Wikland *et al.* 1998). A comparable situation is seen in animal studies. Mildly growth-retarded rats (birth weight between -1 and -2 s.d. below mean birth weight of the controls) after bilateral uterine artery ligation, with adequate postnatal nutritional intake, had already caught up in BW by the age of 14 days (Cha *et al.* 1987). Severely growth-retarded rats (birth weight below -2 s.d. of mean birth weight of the controls) in the same study did not catch up, but were only followed up for 14 days. Severe growth retardation, due to chronic maternal undernutrition, is followed by complete catch up in BW between 13 and 30 weeks of age in both female and male rats (Woodall *et al.* 1996b). These observations led to the hypothesis that the more severe the degree of growth retardation, the longer time it will take to catch up growth.

This study was therefore designed to follow up only the severely growth-retarded animals, defined as rats with a birth weight below -2 s.d. of mean birth weight of controls of either sex, for a prolonged period of time until they were 18 months of age. Interestingly, while the rate of BW gain *in utero* was clearly diminished, the rate of BW gain in the IUGR rats after postnatal day 10 was similar to the control animals, suggesting some initial catch-up growth. However, during the extended follow-up period

we did not observe complete catch up in BW gain. There are several possible explanations for this failure to catch up.

A reduced ability of the IUGR pups to suckle could be responsible for the initial lack of catch up in BW. However, in our animal model, pups were nursed by one lactating mother with a maximum of four pups per mother, whereas 12–14 pups in this strain of rats is a normal litter size. Sufficient food intake is also demonstrated by equal growth rates in the IUGR and control pups during lactation in this study, as confirmed by others (Zeman 1970, Muaku *et al.* 1996), suggesting that IUGR pups receive an equivalent amount of milk, in proportion to their body size. In the young adult rats in our study, nutrient-induced insufficient catch-up growth is unlikely. Both serum IGF-I concentrations and GH secretion are not different from control rats and reflect adequate and comparable nutritional status (Thissen *et al.* 1994).

A reduced thyroid hormone level, known to impair the growth-promoting effect of GH (Glasscock *et al.* 1991), can cause growth failure in these IUGR rats. Serum levels of thyroid hormone at the age of 25 days are not different in our IUGR versus control rats (data not shown). Also in favour of normal and effective serum levels of thyroid hormone is the fact that female IUGR rats in our study show complete catch-up growth in TL, thought to be more dependent on thyroid hormone in the presence of both thyroid hormone and GH (Glasscock *et al.* 1991). However, catch-up growth in TL does not occur in the male IUGR rat and we do not have a good explanation for this difference between the sexes.

Failure to catch up growth in IUGR rats could be linked to changes in the GH/IGF-I axis. Reduced spontaneous GH secretion rate in combination with low serum IGF-I levels in IUGR children (De Waal *et al.* 1994, Albertsson-Wikland *et al.* 1998) are thought to contribute to persistent short stature in adulthood. Data on the GH secretory pattern in short young adults after IUGR have not been reported.

In rats, as in the human, the central endocrine regulator of somatic growth is GH, whose episodic secretion from the pituitary is regulated by both stimulatory (e.g. GH-releasing factor) and inhibitory (e.g. somatostatin) hypothalamic hormones. During pubertal development of the rat, rGH amplitude increases in both sexes and after the age of 54 days the typical pattern of low basal rGH secretion, combined with high-amplitude, low-frequency rGH pulses in the male versus the many small irregular peaks on a higher baseline rGH secretion in female rats is evident (Jansson *et al.* 1985, Gabriel *et al.* 1992).

Results from our study show that, in both female and male young adult IUGR rats, no differences were seen in the rGH secretory pattern when compared with controls at a mean age of 100 days. If the amount of spontaneously secreted rGH in the control rats is thought to be sufficient to induce normal growth, the measured concentrations of rGH in the IUGR rats in this study could be too low to permit catch-up growth to occur. Evidence for the necessity of increased peak rGH concentrations to induce catch-up growth comes from studies in adult rats after a short period of fasting (Mosier *et al.* 1985) or following glucocorticoid-induced growth inhibition (Mosier & Jansons 1985). The absence of increased GH secretion in our experimental rats may be responsible for the lack of normalization of growth.

In the IUGR human and rat fetus, serum IGF-I is reduced with a concomitant increase in serum IGF-binding protein-1 (IGFBP-1), both correlating with fetal BW (Vileisis & D'Ercole 1986, Unterman *et al.* 1993, Verhaeghe *et al.* 1993). In IUGR infants with failure to catch up growth, serum IGF-I levels are reported to be reduced in the first year of life (Thieriot-Prevost *et al.* 1988). Several other investigators have since confirmed the finding of decreased serum IGF-I levels (De Waal *et al.* 1994, Albertsson-Wikland *et al.* 1998) in relation to insufficient catch-up growth in prepubertal IUGR children. However, normal serum levels of IGF-I in prepubertal IUGR children are also reported (Rochiccioli *et al.* 1989, Leger *et al.* 1996). Data on serum IGF-I concentrations in short IUGR adults are lacking.

In the present study, serum IGF-I levels at the age of 14–15 weeks in both female and male persistently growth-retarded rats were not different. The meaning of this is unclear. With the knowledge of decreased serum IGF-I levels in the IUGR rat around birth (Vileisis & D'Ercole 1986, Unterman *et al.* 1993), the measured IGF-I concentrations in young adult rats in our study suggest a relative increase of circulating IGF-I to physiological levels. Despite this relative increase of IGF-I no complete catch-up growth was observed. The finding of physiological IGF-I levels without concomitant catch-up growth in adulthood, preceded by reduced IGF-I levels until day 9 postnatally, is also reported in another rat model in which IUGR is due to gestational nutritional restriction (Woodall *et al.* 1996a). Thus this relative increase of circulating

IGF-I fails to induce complete catch-up growth, independent of whether IUGR is caused by maternal nutritional restriction during pregnancy or an interruption of the utero-placental-fetal circulation in late gestation.

In this respect one can only speculate on possible mechanisms responsible for this failure to catch up growth. Serum IGFBP-1 and IGFBP-2 concentrations might be persistently increased with consequently diminished bioavailability of IGF-I (Drop *et al.* 1992). However, in young adult IUGR rats without catch-up growth after gestational nutritional restriction IGFBP-1 and -2 levels are not increased (Muaku *et al.* 1996, Woodall *et al.* 1996a). Furthermore, the IGF-I receptor, which modulates the physiological action of IGF-I (Leroith *et al.* 1995), could be substantially damaged or reduced in number due to undernutrition *in utero*. Another mechanism might also be a resetting of the IGF-I receptor to a lower level of response in several tissues, resulting in a relative IGF-I resistance. In general, information on the role of the IGF-I receptor in relation to catch-up growth is lacking. Future research should focus on the IGFBPs and the function of the IGF-I receptor at tissue level in these IUGR animal models.

In conclusion, our study shows that the long-term negative effect of severe IUGR after bilateral uterine artery ligation on postnatal growth in the rat is accompanied by a physiological GH/IGF-I axis. This experimental model can therefore be used to investigate pathophysiology of failure to catch up longitudinal growth in IUGR children with a normal GH/IGF-I axis.

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