Administration of growth hormone or IGF-I to pregnant rats on a reduced diet throughout pregnancy does not prevent fetal intrauterine growth retardation and elevated blood pressure in adult offspring

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Abstract

Increasing evidence from human epidemiological studies suggests that poor growth before birth is associated with postnatal growth retardation and the development of cardiovascular disease in adulthood. We have shown previously that nutritional deprivation in the pregnant rat leads to intrauterine growth retardation (IUGR), postnatal growth failure, changes in the endocrine parameters of the somatotrophic axis, and to increased blood pressure in later life. In the present study, we investigated whether administration of insulin-like growth factor-I (IGF-I) or bovine growth hormone (GH) during pregnancy could prevent IUGR and/or alter long-term outcome. Dams from day 1 of pregnancy throughout gestation received a diet of *ad libitum* available food or a restricted dietary intake of 30% of *ad libitum* fed dams. From day 10 of gestation, dams were treated for 10 days with three times daily subcutaneous injections of saline (100 µl), IGF-I (2 µg/g body weight) or GH (2 µg/g body weight). Maternal weight gain was significantly increased ($P < 0.001$) in *ad libitum* fed dams treated with GH, (98.9 ± 4.73 g) compared with the IGF-I (80.5 ± 2.17 g) and saline-treated (70.7 ± 2.65 g) groups. There was a small increase in maternal weight gain ($P < 0.06$) in 30% *ad libitum* fed dams following GH (16.3 ± 2.47 g) and IGF-I (15.8 ± 1.97 g) treatment compared with saline (9.2 ± 1.96 g). Whole spleen, kidney

and carcass weights were significantly ($P < 0.05$) increased in *ad libitum* fed and 30% *ad libitum* fed dams with GH treatment. Circulating IGF-I was significantly increased ($P < 0.001$) in *ad libitum* fed dams with both IGF-I (369.6 ± 32.33 ng/ml) and GH (457.9 ± 33.32 ng/ml) compared with saline treatment (211.7 ± 14.02 ng/ml), and with GH (223.4 ± 23.72 ng/ml) compared with saline treatment (112.0 ± 7.33 ng/ml) in 30% *ad libitum* fed dams. Circulating GH binding protein (GHBP) levels were significantly reduced ($P < 0.05$) in GH-treated (299.1 ± 51.54 ng/ml) compared with saline-treated (503.9 ± 62.43 ng/ml) *ad libitum* fed dams, but were not altered in 30% *ad libitum* fed dams. There was no significant effect of either IGF-I or GH treatment on fetal weight, placental weight, fetal organ weights or circulating IGF-I levels in both *ad libitum* fed and 30% *ad libitum* fed fetuses. Offspring of 30% *ad libitum* fed dams remained significantly growth retarded postnatally and showed elevated blood pressure in later life. The increased maternal weight gain following IGF-I or GH administration, without an effect on fetal and placental weights, suggests a modification in the mode of maternal nutrient repartitioning during mid to late pregnancy at the expense of the fetus.

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Introduction

Intrauterine growth retardation (IUGR) is a major cause of perinatal death and neonatal morbidity and mortality. Over the past decade a number of epidemiological studies have provided controversial evidence that certain major adult non-communicable diseases are associated with impaired fetal growth. Low birth size has been linked with

the subsequent development of hypertension (Gensser *et al.* 1988, Barker 1992), ischaemic heart disease (Barker *et al.* 1989) and non-insulin-dependent diabetes mellitus (NIDDM) (Hales *et al.* 1991). The mechanisms underlying the epidemiological observations remain to be elucidated. Barker (1992) proposed that the cardiovascular adaptations, which ultimately lead to the development of hypertension, originate in fetal life in response to a

sub-optimal intrauterine environment which can also affect fetal and placental growth.

There is increasing experimental evidence that the somatotrophic axis plays the dominant role in the regulation of fetal growth (Gluckman & Harding 1997). Despite adequate food rehabilitation, failure of catch-up growth and low circulating insulin-like growth factor (IGF)-I and hepatic IGF-I mRNA expression are observed following maternal undernutrition (Woodall *et al.* 1996a, 1998) and protein restriction (Muaku *et al.* 1996). In addition, elevated blood pressure in adult rat offspring has been observed as a consequence of maternal undernutrition (Langley & Jackson 1994, Woodall *et al.* 1996b).

Growth hormone (GH) is an important regulator of postnatal linear growth in mammals. GH may promote growth both directly and indirectly through its stimulation of IGF-I synthesis (Gargosky *et al.* 1991). The anabolic and re-partitioning effects of both GH and IGF-I have been demonstrated in non-pregnant rats (Philipps *et al.* 1988, Sillence & Etherton 1989). Several groups have studied the effects of administering IGF-I or GH to pregnant rats on the growth of their fetuses and have obtained conflicting results. While some studies have reported positive effects of GH on fetal growth when dams were treated throughout pregnancy (Kuhn-Sherlock *et al.* 1996), others have reported little or no effect of GH or IGF-I on fetal growth (Gargosky *et al.* 1991), or even a negative effect with a very high dose of GH (Chiang & Nicoll 1991) when treatment occurred during the second half of pregnancy. In another study, treatment of anti-rat GH during the second half of pregnancy increased the catabolic state of the mother and increased fetal body weight (Palmer *et al.* 1996).

We have demonstrated previously that maternal undernutrition in the rat, sufficient to induce IUGR, results in offspring which are 30% smaller in fetal and placental weight at day 22 of gestation and which do not demonstrate significant catch-up growth in the postnatal period (Woodall *et al.* 1996a). The current study extends these original studies and was designed to examine the response of dams, undernourished throughout pregnancy, to bovine GH and human IGF-I treatment in late pregnancy, together with the effects on fetal and placental growth. We tested the hypothesis that GH and/or IGF treatment of undernourished dams results in maternal repartitioning of absorbed nutrients in favour of the fetus, thereby enhancing fetal growth and/or preventing the subsequent hypertension which develops in the offspring in later life. A cohort of offspring from treated dams was therefore maintained to examine postnatal growth and to measure their blood pressure. A growth response was observed in the dams following IGF-I and GH treatment. However, offspring from undernourished dams remained growth retarded and exhibited elevated blood pressure later in life, despite GH or IGF-I treatment to the mother during the last half of gestation.

Materials and Methods

Animal model

Timed matings were performed in virgin Wistar rats aged between 70–100 days. A rat oestrous cycle monitor (Fine Science Tools Inc., North Vancouver, BC, Canada) was used to assess the stage of oestrus of the animals, prior to introducing the males. Day 1 of pregnancy was determined by the presence of spermatozoa in a vaginal smear. Following confirmation of mating, animals were housed individually in standard rat cages containing wood shavings as bedding, with free access to water. The room was maintained at a constant temperature (25 °C) and with a 12-h light-darkness cycle.

The experimental approach to induce IUGR in this study has been documented elsewhere (Woodall *et al.* 1996a). In brief, dams were randomly assigned to one of two feeding regimes either *ad libitum* fed or restricted fed. Control dams were fed pelleted rat chow available *ad libitum* throughout pregnancy. Mean food intake ranged from 25–34 g per day. The restricted diet group (30% *ad libitum* fed) were fed 30% of the food intake of the *ad libitum* fed group throughout pregnancy, commencing on day 1. The animal protocol was approved by the Animal Ethics Committee of the University of Auckland.

Hormone treatments

On day 9 of pregnancy, dams from the *ad libitum* fed and restricted fed groups were randomly assigned on the basis of weight to one of three treatment groups. Dams were given three times daily (0800, 1600 and 2200 h) subcutaneous (s.c.) injections of saline ($n=9$ per nutritional group), bovine (b) GH ($n=8$ per nutritional group) (2 µg/g body weight (BW)/day) or recombinant human IGF-I ($n=9$ per nutritional group) (rhIGF-I 2 µg/g BW/day) from day 10 through to day 20 of gestation. These doses of IGF-I and bGH were based on those previously reported to increase maternal weight (Kuhn-Sherlock *et al.* 1996). Recombinant bGH (batch No. PR.003, a gift from Dr W Baumbach, American Cyanamid Co. Princeton, NJ, USA) was weighed out and stored at –20 °C. It was dissolved in carbonated buffered saline (pH 9.4) immediately prior to use. Recombinant hIGF-I (batch No. 56820AS1, Pharmacia, Uppsala, Sweden) was supplied in suspension at 2 mg/ml and was diluted in 0.9% saline prior to injection.

The body weight of each dam was recorded daily. Dams were killed by decapitation under halothane anaesthesia 2 h after the last hormone injection. Fetuses were weighed and body length was measured before decapitation. Blood was collected from the cervical blood vessels on ice and plasma separated by centrifugation at 4 °C for 20 min at 3000 g and stored at –20 °C until analysed. Blood collected from the fetuses within each litter was pooled and

analysed as one sample. Tissue samples collected were rinsed in saline, weighed and immediately placed in liquid nitrogen and subsequently stored at -80°C .

An additional cohort of animals (3 saline-, 3 IGF-I- and 3 bGH-treated *ad libitum* fed litters and 2 saline-, 2 IGF-I- and 2 bGH-treated 30% *ad libitum* fed litters) were maintained to measure postnatal weight and blood pressure following maternal administration of IGF-I and GH during pregnancy. At birth (postnatal day 1), the size of each litter was reduced to 8 pups per dam in order to improve and standardize postnatal nutritional conditions. Litters of the restricted fed group were immediately fostered on to *ad libitum* fed dams which had given birth within 24 h. No pups were rejected by the foster dams. Litters from the *ad libitum* fed groups remained with their birth mothers.

IGF-I RIA

Plasma IGF-I was measured by radioimmunoassay using a rabbit polyclonal antiserum to rh-metIGF-I (878/4) after extraction of serum by acid ethanol cryoprecipitation. This extraction system has been extensively validated for the rat against extraction by gel chromatography under acid conditions (Breier *et al.* 1991). Assays were saturated with IGF-II (50 ng/sample) to prevent potential interference from residual IGF-binding proteins (Breier *et al.* 1993). Samples were analysed in a single assay in duplicate. Intra-assay variation was less than 10%.

Plasma GH binding proteins

Total plasma GH binding protein (GHBP) was measured using a radioimmunoassay described in detail previously (Barnard *et al.* 1994). The GHBP radioimmunoassay was based on the mouse GHBP assay developed by Cramer *et al.* (1992). The rat GHBP assay uses a monoclonal antibody (MAb 4.3) raised against the synthetic peptide sequence of the rat GHBP as described by Sadeghi *et al.* (1990).

Blood pressure measurements

Systolic blood pressure was measured in a total of 100 conscious male and female offspring from 14 litters (8 *ad libitum* fed group and 6 30% *ad libitum* fed group) by using tail cuff plethysmography (Blood Pressure Analyzer IITC, Life Science, Woodland Hills, CA, USA) as previously validated (Bunag 1973). Rats were placed in a clear perspex restraint tube, maintained in a darkened room at $28\text{--}29^{\circ}\text{C}$. After allowing 15 min for each animal to acclimatise in the tube, an 18 mm cuff or 11 mm cuff was placed around the tail of male and female rats respectively and inflated to 240 mmHg. Pulses were recorded during deflation at a rate of 3 mmHg/s. Training for indirect tail-cuff blood pressure determination began

when the rats were 15 weeks old and blood pressure measurements were recorded at 18 and 50 weeks of age. Blood pressure was determined in triplicate for each animal, and average systolic pressure recorded as the mean of 3 readings. We were not able to obtain reliable diastolic pressure measurements.

Statistical analyses

All statistical analyses were carried out using the Sigma Stat Statistical package (Jandel Scientific, San Rafael, CA, USA). To determine the respective influences of gestational diet and hormonal therapy, differences were analysed using two-way ANOVA. To determine the influence of hormonal therapy within gestational diet, one-way ANOVA was used followed by a *post-hoc* Bonferroni *t*-test. Data are shown as means $\pm$ S.E.M. and *P* values of less than 0.05 were considered significant.

Results

Maternal weight

Nutritional restriction throughout gestation resulted in a significant reduction ($P<0.01$) in body weight from conception until day 16 in the saline-treated 30% *ad libitum* fed group (Fig. 1). While not significant ($P=0.1$), there was a trend towards an increase in body weight in the hIGF-I- and bGH-treated 30% *ad libitum* fed dams, apparent from as early as day 12 of gestation, 2 days after the start of hormone treatment (Fig. 1). In the *ad libitum* fed group, there was a significant increase ($P<0.05$) in body weight in the GH-treated dams in the latter stages of gestation (Fig. 1). Body weight gain from day 10–20 of gestation was increased ($P<0.05$) in 30% *ad libitum* fed dams treated with hIGF-I or bGH (saline: 9.2 ± 1.96 g; IGF-I: 15.8 ± 1.97 g; bGH: 16.4 ± 2.47 g). *Ad libitum* fed dams treated with bGH significantly increased their body weight gain ($P<0.05$) compared with saline-treated dams, while hIGF-I treatment did not significantly increase body weight gain ($P=0.1$) (saline: 70.7 ± 2.65 g; IGF-I: 80.6 ± 2.17 g; bGH: 98.9 ± 4.73 g). There was a marked difference in the amount of body weight gained between *ad libitum* fed and 30% *ad libitum* fed dams ($P<0.001$) during the treatment period (Fig. 1).

Litter size at the time of sacrifice was not significantly different ($P>0.1$) between *ad libitum* fed and 30% *ad libitum* fed groups (Table 1). No differences were observed in gestation length (term=23 days) or in pup viability with maternal hormonal administration. Maternal organ weights were significantly reduced ($P<0.05$) in 30% *ad libitum* fed group compared with *ad libitum* fed dams; however, organ weight ratios (calculated by dividing wet organ weight by body weight) were not altered with undernutrition (Table 1). GH treatment significantly

Figure 1 Maternal body weight of *ad libitum* fed and 30% *ad libitum* fed dams from conception until day 20 of gestation following 3 times daily s.c. injection with saline (●), or 2 µg/g BW/day hIGF-I (■) or bGH (▼) from days 10–20 of gestation. Values are means ± S.E.M. #*P*<0.05 compared with saline treatment, **P*<0.01 compared with *ad libitum* fed dams; two-way ANOVA followed by Bonferroni test.

increased whole spleen and carcass weights in *ad libitum* fed dams, and kidney and carcass weights in 30% *ad libitum* fed dams compared with saline-treated animals. Organ weight ratios were unchanged with hormone treatments.

Fetal weight

Mean fetal weights and placental weights remained unchanged (*P*>0.1) following either maternal IGF-I or

bGH treatment (Table 2). Body weights of 30% *ad libitum* fed fetuses were significantly smaller than *ad libitum* fed fetuses (Table 2). Whole organ weights were significantly reduced (*P*<0.001) in 30% *ad libitum* fed fetuses (data not shown). Liver and kidney organ weight ratios were significantly reduced (*P*<0.001) in 30% *ad libitum* fed fetuses and nose-rump length was also reduced (*P*<0.001) (Table 2). However, neither organ weights nor body length were affected by hIGF-I or bGH treatment (Table 2) in either group.

Table 1 Litter size, maternal organ weights (g), organ weight ratios (% body weight), carcass weights (g) and carcass weight ratio (% body weight) of *ad libitum* fed and 30% *ad libitum* fed dams at day 20 of gestation following 10 days treatment with saline, IGF or bGH. Values are expressed as means ± S.E.M. Numbers of dams treated in each group are in parentheses

	Ad libitum fed			30% Ad libitum fed		
	Saline (9)	IGF-I (9)	bGH (8)	Saline (9)	IGF-I (9)	bGH (8)
Litter size	12.8 ± 0.89	11.8 ± 1.02	12.9 ± 0.89	13.8 ± 0.83	14.0 ± 0.44	10.0 ± 1.23
Liver	15.5 ± 0.45	16.2 ± 0.53	16.9 ± 0.60	8.9 ± 0.24*	8.8 ± 0.35*	8.4 ± 0.18*
Weight ratio	4.5 ± 0.12	4.4 ± 0.11	4.5 ± 0.11	3.9 ± 0.11	3.7 ± 0.09	3.5 ± 0.10
Heart	1.0 ± 0.03	1.1 ± 0.05	1.1 ± 0.04	0.7 ± 0.24*	0.7 ± 0.02*	0.7 ± 0.02*
Weight ratio	2.9 ± 0.08	2.9 ± 0.07	2.9 ± 0.08	3.2 ± 0.09	3.1 ± 0.09	2.9 ± 0.08
Spleen	0.9 ± 0.04	0.9 ± 0.05	1.1 ± 0.05 [#]	0.4 ± 0.02*	0.5 ± 0.03*	0.5 ± 0.02*
Weight ratio	2.5 ± 0.07	2.7 ± 0.08	2.9 ± 0.09	1.9 ± 0.05	1.9 ± 0.06	1.9 ± 0.05
Kidney	2.1 ± 0.07	2.2 ± 0.07	2.3 ± 0.08	1.4 ± 0.03*	1.5 ± 0.05*	1.6 ± 0.02 ^{**}
Weight ratio	5.9 ± 0.27	6.2 ± 0.31	5.9 ± 0.28	6.3 ± 0.25	6.4 ± 0.32	6.7 ± 0.34
Carcass	210.9 ± 3.42	225.3 ± 4.43	233.6 ± 6.93 [#]	152.4 ± 4.13*	158.9 ± 3.52*	169.9 ± 4.8 ^{**}
Weight ratio	60.7 ± 1.02	62.2 ± 1.13	61.7 ± 1.34	66.6 ± 1.21	66.6 ± 1.24	71.2 ± 1.35

[#]*P*<0.05 compared with saline treatment. **P*<0.05 compared with *ad libitum* fed dams; two-way ANOVA followed by Bonferroni test.

Table 2 Fetal and placental weights (g), organ weight ratios (% body weight) and nose–rump length (cm) of fetuses from *ad libitum* fed and 30% *ad libitum* fed dams at day 20 of gestation following 10 days treatment with saline, IGF or bGH. Values are expressed as means $\pm$ S.E.M. Numbers of dams treated in each group are in parentheses

	<i>Ad libitum</i> fed			30% <i>Ad libitum</i> fed		
	Saline (9)	IGF-I (9)	bGH (8)	Saline (9)	IGF-I (9)	bGH (8)
Fetal weight (g)	2.3 $\pm$ 0.02	2.2 $\pm$ 0.05	2.3 $\pm$ 0.03	1.8 $\pm$ 0.06*	1.6 $\pm$ 0.05*	1.7 $\pm$ 0.07*
Placental wt (g)	0.5 $\pm$ 0.01	0.5 $\pm$ 0.02	0.5 $\pm$ 0.02	0.4 $\pm$ 0.01*	0.3 $\pm$ 0.02*	0.4 $\pm$ 0.02*
Liver (%)	7.8 $\pm$ 0.21	7.9 $\pm$ 0.23	7.5 $\pm$ 0.22	6.6 $\pm$ 0.22*	6.5 $\pm$ 0.31*	6.9 $\pm$ 0.29
Heart (%)	0.5 $\pm$ 0.05	0.5 $\pm$ 0.06	0.4 $\pm$ 0.06	0.5 $\pm$ 0.06	0.4 $\pm$ 0.06	0.4 $\pm$ 0.03
Lung (%)	3.3 $\pm$ 0.14	3.1 $\pm$ 0.12	3.2 $\pm$ 0.10	2.9 $\pm$ 0.14	2.4 $\pm$ 0.15	2.9 $\pm$ 0.16
Kidney (%)	0.7 $\pm$ 0.05	0.5 $\pm$ 0.04	0.7 $\pm$ 0.12	0.5 $\pm$ 0.06*	0.4 $\pm$ 0.05	0.5 $\pm$ 0.04
Nose–rump (cm)	2.7 $\pm$ 0.31	2.7 $\pm$ 0.04	2.7 $\pm$ 0.04	2.5 $\pm$ 0.04*	2.4 $\pm$ 0.33*	2.4 $\pm$ 0.04*

* $P < 0.05$ compared with *ad libitum* fed fetuses by two-way ANOVA followed by Bonferroni test.

Plasma IGF-I and GH binding proteins

Circulating IGF-I levels were significantly elevated in maternal *ad libitum* fed dams treated with hIGF-I and bGH ($P < 0.001$) and in 30% *ad libitum* fed dams treated with bGH ($P < 0.001$) compared with saline-treated dams (Table 3). Plasma IGF-I levels were significantly reduced ($P < 0.05$) in 30% *ad libitum* fed dams compared with *ad libitum* fed dams. Plasma IGF-I levels were similar between *ad libitum* fed and 30% *ad libitum* fed fetuses and were unchanged ($P > 0.1$) with hIGF-I or bGH treatment (data not shown). GHBP concentrations were significantly reduced ($P < 0.05$) following bGH treatment in *ad libitum* fed dams compared with saline-treated dams but were unaltered with hormone treatment in 30% *ad libitum* fed group (Table 3). GHBP concentrations were not significantly altered by undernutrition.

Postnatal body weight and systolic blood pressure

A cohort of animals (8 *ad libitum* fed litters and 6 30% *ad libitum* fed litters) were maintained to measure postnatal growth development and blood pressure following administration of hIGF-I and bGH to the dams during gestation. IGF-I or bGH treatment to the mother had no effect ($P > 0.1$) on postnatal growth of the offspring. Body weights in the 30% *ad libitum* fed offspring remained significantly reduced compared with *ad libitum* fed offspring ($P < 0.01$) up to 18 weeks of age (Fig. 2). However, by 50 weeks of age, body weights were similar between

male *ad libitum* fed (702.7 $\pm$ 7.85 g) and 30% *ad libitum* fed offspring (689.4 $\pm$ 4.86 g, $P < 0.08$) and between female *ad libitum* fed (404.8 $\pm$ 14.4 g) and 30% *ad libitum* fed offspring (387.8 $\pm$ 9.50 g) ($P > 0.4$). Systolic blood pressure was measured on two separate occasions, at 18 and 50 weeks of age. hIGF-I or bGH treatment of the mother had no effect ($P > 0.1$) on the systolic blood pressure of the offspring. In addition, there were no differences ($P > 0.1$) in blood pressure between male and female offspring within *ad libitum* fed and 30% *ad libitum* fed groups so the systolic readings of males and females were pooled within each nutritional group. At 18 weeks of age, there was no difference in systolic blood pressure ($P > 0.2$) between *ad libitum* fed (119.7 $\pm$ 1.44 mmHg ($n=59$)) and 30% *ad libitum* fed (117.0 $\pm$ 1.84 mmHg ($n=34$)) rats. However, by 50 weeks of age, systolic blood pressure was significantly increased ($P < 0.03$) in the 30% *ad libitum* fed group (*ad libitum* fed: 113.7 $\pm$ 3.77 mmHg ($n=59$); 30% *ad libitum* fed: 123.7 $\pm$ 2.46 mmHg ($n=34$)).

Discussion

Previously reported changes associated with IUGR following maternal undernutrition (Woodall *et al.* 1996a) concur with the studies presented here. In the IUGR fetus and in neonatal offspring, body weight remained significantly reduced with no evidence of catch-up growth occurring until the offspring were mature adults.

Table 3 Plasma IGF-I and GHBP concentrations of *ad libitum* fed and 30% *ad libitum* fed dams at day 20 of gestation following 10 days treatment with saline, IGF-I or bGH. Values are expressed as means $\pm$ S.E.M. Numbers of dams treated in each group are in parentheses

	<i>Ad libitum</i> fed			30% <i>Ad libitum</i> fed		
	Saline (9)	IGF-I (9)	bGH (8)	Saline (9)	IGF-I (9)	bGH (8)
IGF-I (ng/ml)	211.7 $\pm$ 14.02	369.6 $\pm$ 32.33 [#]	457.9 $\pm$ 33.34 [#]	112.0 $\pm$ 7.35*	158.4 $\pm$ 13.73*	223.4 $\pm$ 23.74* [#]
GHBP (ng/ml)	503.9 $\pm$ 62.43	411.8 $\pm$ 39.62	299.1 $\pm$ 51.52 [#]	402.8 $\pm$ 18.22	423.4 $\pm$ 28.56	340.4 $\pm$ 23.36

[#] $P < 0.05$ compared with saline treatment. * $P < 0.05$ compared with *ad libitum* fed dams; two-way ANOVA followed by Bonferroni test.

(a) Males

(b) Females

Figure 2 Postnatal body weights of (a) male and (b) female offspring from *ad libitum* fed and 30% *ad libitum* fed dams treated from days 10–20 of gestation with 3 times daily s.c. injections of saline (○ – *ad libitum* fed litters, $n=24$; ● – 30% *ad libitum* fed litters, $n=16$), 2 $\mu\text{g/g}$ BW/day hIGF-I (□ – *ad libitum* fed litters, $n=24$; ■ – 30% *ad libitum* fed litters, $n=16$) or 2 $\mu\text{g/g}$ BW/day bGH (▽ – *ad libitum* fed litters, $n=16$; ▼ – 30% *ad libitum* fed litters, $n=16$). Values are means $\pm$ S.E.M. * $P<0.01$ compared with *ad libitum* fed offspring; two-way ANOVA followed by Bonferroni test.

In addition, these studies confirm our recent observation (Woodall *et al.* 1996b) that rats born with IUGR secondary to maternal undernutrition develop hypertension in adulthood. This study was designed to investigate whether hIGF-I or bGH treatment of rats

undernourished throughout pregnancy ameliorates growth retardation of their fetuses and/or affects blood pressure or growth rate later in life. IUGR was induced experimentally by undernourishing pregnant rats to 30% of the *ad libitum* diet throughout gestation and bGH or

hIGF-I was administered for the second half of pregnancy.

Treatment with bGH at 2 µg/g body weight per day significantly increased maternal weight gain during the treatment period in both *ad libitum* fed and 30% *ad libitum* fed dams, while saline-treated 30% *ad libitum* fed dams exhibited pronounced weight loss until day 15 of gestation, as reported previously (Woodall *et al.* 1996a). Plasma IGF-I levels were also significantly elevated in both *ad libitum* fed and undernourished dams following bGH treatment. These results contrast with the findings of Gargosky *et al.* (1991) who reported that infusion of GH at a similar dose to that used in our study during the second half of pregnancy did not significantly increase IGF-I levels or maternal weight. In the present study, GH was injected rather than infused and this is reported to enhance body weight gain compared with administration by infusion (Clark *et al.* 1995). In this regard, our increases in maternal weight are consistent with those of Kuhn-Sherlock *et al.* (1996), who injected 2 µg/g/day bGH in divided doses three times daily throughout pregnancy and observed increases in both maternal and fetal weights. In the present study, however, maternal bGH injection had no effect on fetal or placental weight in either *ad libitum* fed or undernourished litters.

Administration of IGF-I during the second half of pregnancy, when IGF-I concentrations were otherwise depressed (Woodall *et al.* 1996a), significantly increased circulating IGF-I levels of *ad libitum* fed dams. Plasma IGF-I levels were also elevated, but not significantly, in undernourished dams. Maternal body weight gain was increased with IGF-I treatment in the undernourished dams but not in *ad libitum* fed dams. This may be explained by changes in IGF binding protein (IGFBP) concentrations in dams reported previously (Woodall *et al.* 1996a), whereby circulating IGFBP-1 and IGFBP-2 were elevated in undernourished dams during pregnancy, thereby increasing the capacity of circulating IGF-I to promote growth. Plasma IGF-I levels were previously shown to be significantly reduced in undernourished dams compared with *ad libitum* fed dams during pregnancy (Woodall *et al.* 1996a). The observed increase in plasma IGF-I levels in the undernourished dams following IGF-I or bGH demonstrates that, under conditions of prolonged under-nutrition, hormone therapy can improve maternal weight. However, neither fetal nor placental weights were affected by IGF-I treatment, consistent with an action of IGF-I in the re-partitioning of nutrients to maternal tissues, as previously observed in normally nourished dams treated with IGF-I either in late pregnancy (Gargosky *et al.* 1991) or throughout pregnancy (Kuhn-Sherlock *et al.* 1996).

GHBP levels were down-regulated in *ad libitum* fed dams following bGH administration, while remaining unchanged in the undernourished dams. The GHBP levels reported in this study were rather low for pregnant rats, as circulating GHBP levels normally increase during

pregnancy (Barnard & Waters 1997). A 5-fold increase in GHBP levels has previously been observed in pregnant rats compared with non-pregnant rats (R Barnard, S M Woodall & B H Breier, unpublished observations), which was similar to that reported in the dwarf rat (Gargosky *et al.* 1995). Plasma GHBP levels in the saline-treated undernourished dams were similar to those measured in the saline-treated *ad libitum* fed rats. Similar serum GHBP levels were observed in pregnant dwarf rats with an isolated GH deficiency (Gargosky *et al.* 1995). The effect on GHBP concentrations following GH and IGF-I treatment to pregnant rats has not been reported to date, but in male rats other studies have reported conflicting effects of hormone treatment on circulating GHBP levels. Barnard *et al.* (1994) observed no effect on serum GHBP levels following one week or four weeks of twice daily injections of GH and one week of continuous IGF-I infusion to 12-week-old male dwarf rats. Maiter *et al.* (1992) also reported that serum GHBP in hypophysectomised rats was not affected following one week of repeated GH injections. In contrast, Carmignac *et al.* (1992) observed an up-regulation of serum GHBP in male rats following one week of GH infusion. In the present study, whilst we observed no significant effect of IGF-I treatment, GH administration significantly down-regulated GHBP levels in *ad libitum* fed dams, while having no effect in the undernourished dams. These data suggest a nutritional interaction of GH action on the regulation of plasma GHBP concentrations during pregnancy.

When administered to dams in the present study, neither IGF-I nor bGH affected fetal or placental weight in either *ad libitum* fed or undernourished rats. While it is possible that the doses used here may have been too low, this seems unlikely, given that maternal total body and carcass weights were increased with bGH or IGF-I treatment. Instead, our results suggest that the placenta may be relatively unresponsive to maternal supplementation with growth promoting hormones normally produced by the mother. Treatment of pregnant rats with human placental lactogen has resulted in significantly enhanced fetal and placental growth (Collins *et al.* 1988), but maternal supplementation with GH (Gargosky *et al.* 1991, Spencer *et al.* 1994, Kelley *et al.* 1995) or IGF-I (Gluckman *et al.* 1992, Kuhn-Sherlock *et al.* 1996) has resulted in little or no change in fetal or placental weight. In pregnant sheep, although maternal GH therapy in late gestation was shown to increase placental diffusion capacity, neither fetal nor placental growth was increased (Harding *et al.* 1997). It was suggested that the anabolic effect of GH supplementation to the mother predominated, with a resulting limitation of fetal substrate supply that prevented any increase in fetal growth. When restricted maternal diet results in poor maternal nutrient supply, maternal GH treatment clearly favours the mother at the expense of the fetus, as shown by Chiang & Nicoll (1991). They reported a reduction in fetal and placental weights following ovine GH (1 mg/day)

treatment to dams on a restricted diet, and when a very high dose of bGH (5 mg/day) was given there was almost complete resorption of the fetuses and placentae by day 20 of gestation.

We observed no postnatal effects in offspring of dams treated with hIGF-I or bGH during pregnancy. As reported previously (Woodall *et al.* 1996a), offspring from undernourished dams showed persistent growth failure and remained markedly smaller than offspring of *ad libitum* fed dams until adulthood. Catch-up growth did not occur until between 18 and 50 weeks of age, and maternal treatment with IGF-I or bGH did not alter this. We first measured blood pressure at 18 weeks, before any catch-up growth was apparent, and at this age systolic pressures were similar in all groups. Neither maternal dietary restriction nor maternal hormone treatment during pregnancy had any effect on systolic pressure at 18 weeks. In an earlier study, using the same model of maternal undernutrition, catch-up growth occurred between 13 and 30 weeks of age and was associated with significantly raised systolic pressure when measured on several occasions between 30 and 56 weeks (Woodall *et al.* 1996b). In the present study, by the time the second systolic pressure measurements were made at 50 weeks, prenatal exposure to maternal undernutrition was again associated with adult hypertension, and maternal treatment with IGF-I or bGH could not prevent this. Our findings are in general agreement with the extensive studies of Langley-Evans. In his laboratory, rats are fed a protein-deficient diet throughout pregnancy and elevated systolic pressures are seen in the offspring from weaning (Langley-Evans *et al.* 1996) through to young adulthood at 21 weeks (Langley & Jackson 1994). Even though the onset of hypertension is delayed until adulthood when maternal diet is restricted to 30% of *ad libitum* fed animals, our observations, nevertheless, add to the experimental evidence in support of the 'Barker Hypothesis' (Barker 1992).

Because a causal association between IUGR and the subsequent development of cardiovascular disease in adulthood has been suggested (Barker 1992), our study was designed to determine whether maternal treatment with GH and IGF-I during pregnancy could enhance intra-uterine growth or prevent the development of hypertension in offspring during adult life. Given that we saw no indication of any beneficial effect of maternal hormone treatment on fetal growth or placental function, the lack of any long-term influence on postnatal growth or adult blood pressure is, perhaps, not unexpected. The potent anabolic effects of both GH and IGF-I are well established, but neither can cross the placenta (Fohlenhag *et al.* 1994), and it is clear from this study that, if treatment with either hormone is only given maternally, then it is only the mother who benefits from any anabolic or anticatabolic effects. Maternal weight gain probably occurs at the expense of the fetus, particularly when either hormone is given during maternal undernutrition.

In conclusion, maternal treatment with IGF-I or GH to rats from days 10–20 of pregnancy in undernourished rats raised plasma IGF-I concentrations and increased maternal weight, but did not improve fetal or placental growth. Thus, a partitioning of nutrients was observed, particularly with bGH treatment, in favour of increased maternal body weight rather than alterations in placental or fetal weights. Postnatally, the offspring of undernourished dams remained growth retarded until adulthood and neither hormone treatment altered the development of adult onset hypertension, which results from prenatal exposure to maternal undernutrition. It remains to be investigated whether continuous and/or combined maternal treatment with IGF-I and GH throughout pregnancy may be more successful in enhancing fetal or placental growth and improving long-term outcome in the offspring.

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References

- Barker DJP 1992 The fetal origins of adult hypertension. *Journal of Hypertension* **10** S39–S44.
- Barker DJP, Osmond C, Golding J, Kuh D & Wadsworth ME 1989 Growth *in utero*, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *British Medical Journal* **298** 564–567.
- Barnard R & Waters MJ 1997 The serum growth hormone binding protein: pregnant with possibilities. *Journal of Endocrinology* **153** 1–14.
- Barnard R, Mulcahy J, Garciaaragon J, Wyse B, Owens PC, Rowlinson SW, Talamantes F, Baumbach WR & Waters MJ 1994 Serum growth hormone binding protein and hepatic GH binding sites in the Lewis dwarf rat: effects of IGF-I and GH. *Growth Regulation* **4** 147–154.
- Breier BH, Gallaher BW & Gluckman PD 1991 Radioimmunoassay for insulin-like growth factor-I: solutions to some potential problems and pitfalls. *Journal of Endocrinology* **128** 347–357.
- Breier BH, Milsom SR, Blum WF, Schwander J, Gallaher BW & Gluckman PD 1993 Insulin-like growth factors and their binding proteins in plasma and milk after growth hormone-stimulated galactopoiesis in normally lactating women. *Acta Endocrinologica* **129** 427–435.
- Bunag RD 1973 Validation in awake rats of a tail-cuff method for measuring systolic pressure. *Journal of Applied Physiology* **34** 279–282.
- Carmignac DF, Wells T, Carlsson LM, Clark RG & Robinson IC 1992 Growth hormone (GH)-binding protein in normal and GH-deficient dwarf rats. *Journal of Endocrinology* **135** 447–457.
- Chiang MH & Nicoll CS 1991 Administration of growth hormone to pregnant rats on a reduced diet inhibits growth of their fetuses. *Endocrinology* **129** 2491–2495.

- Clark RG, Mortensen D & Carlsson LMS 1995 Insulin-like growth factor-I and growth hormone have distinct and overlapping anabolic effects in GH-deficient rats. *Endocrine* **3** 297–304.
- Collins JWJ, Finley SL, Merrick D & Ogata ES 1988 Human placental lactogen administration in the pregnant rat: acceleration of fetal growth. *Pediatric Research* **24** 663–667.
- Cramer SD, Barnard R, Engbers C, Ogren L & Talamantes F 1992 Expression of the growth hormone receptor and growth hormone-binding protein during pregnancy in the mouse. *Endocrinology* **131** 876–882.
- Fohlenhag KI, Sandstrom IM, Malmlof K, Skottner AI & Nyberg FJ 1994 Human growth hormone does not cross the placenta of the pregnant rat. *Growth Regulation* **4** 181–187.
- Gargosky SE, Owens JA, Walton PE, Owens PC, Wallace JC & Ballard FJ 1991 Administration of insulin-like growth factor-I, but not growth hormone, increases maternal weight gain in late pregnancy without affecting fetal or placental growth. *Journal of Endocrinology* **130** 395–400.
- Gargosky SE, Breier BH, Borski RJ, Mulcahy J & Barnard R 1995 Upregulation of GH receptor and GH binding protein during pregnancy in the GH deficient rat. *Endocrine* **3** 813–817.
- Gennser G, Rymark P & Isberg PE 1988 Low birth weight and risk of high blood pressure in adulthood. *British Medical Journal* **296** 1498–1500.
- Gluckman PD & Harding JE 1997 The physiology and pathophysiology of intrauterine growth retardation. *Hormone Research* **48** 11–16.
- Gluckman PD, Morel PCH, Ambler GR, Breier BH & McCutcheon SN 1992 Elevating maternal insulin-like growth factor-I in mice and rats alters the pattern of fetal growth by removing maternal constraint. *Journal of Endocrinology* **134** R1–R3.
- Hales CN, Barker DJP, Clark PM, Cox LJ, Fall C, Osmond C & Winter PD 1991 Fetal and infant growth and impaired glucose tolerance at age 64. *British Medical Journal* **303** 1019–1022.
- Harding JE, Evans PC & Gluckman PD 1997 Maternal growth hormone treatment increases placental diffusion capacity but not fetal or placental growth in sheep. *Endocrinology* **138** 5352–5358.
- Kelley RL, Jungst SB, Spencer TE, Owsley WF, Rahe CH & Mulvaney DR 1995 Maternal treatment with somatotropin alters embryonic development and early postnatal growth of pigs. *Domestic Animal Endocrinology* **12** 83–94.
- Kuhn-Sherlock B, Back P, Blair HT, McCutcheon SN, Breier BH & Gluckman PD 1996 The effect of bGH and rhIGF-I treatment on maternal and fetal growth in non-virgin, pregnant rats. *Proceedings of the Endocrine Society of Australia* **39** 141 (Abstract).
- Langley SC & Jackson AA 1994 Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clinical Science* **86** 217–222.
- Langley-Evans SC, Gardner DS & Jackson AA 1996 Association of disproportionate growth of fetal rats in late gestation with raised systolic blood pressure in later life. *Journal of Reproduction and Fertility* **106** 307–312.
- Maiter D, Walker JL, Adam E, Moats-Staats B, Mulumba N, Ketelslegers J & Underwood LE 1992 Differential regulation by growth hormone (GH) of insulin-like growth factor-I and GH receptor/binding protein gene expression in rat liver. *Endocrinology* **130** 3257–3264.
- Muaku SM, Beauloye V, Thissen JP, Underwood LE, Fossion C, Gerard G, Ketelslegers JM & Maiter D 1996 Long-term effects of gestational protein malnutrition on postnatal growth, insulin-like growth factor (IGF)-I, and IGF-binding proteins in rat progeny. *Pediatric Research* **39** 649–655.
- Palmer RM, Thom A & Flint DJ 1996 Repartitioning of maternal muscle protein towards the foetus induced by a polyclonal antiserum to rat GH. *Journal of Endocrinology* **151** 395–400.
- Philipps AF, Persson B, Hall K, Lake M, Skottner A, Sanengen T & Sara VR 1988 The effects of biosynthetic insulin-like growth factor-I supplementation on somatic growth, maturation, and erythropoiesis on the neonatal rat. *Pediatric Research* **23** 298–305.
- Sadeghi H, Wang BS, Lumanglas AL, Logan JS & Baumbach WR 1990 Identification of the origin of the growth hormone-binding protein in rat serum. *Molecular Endocrinology* **4** 1799–1805.
- Sillence MN & Etherton TD 1989 Changes in serum concentrations of ACTH, corticosterone and in growth following morning or evening injection of female rats with porcine growth hormone. *Journal of Endocrinology* **123** 113–119.
- Spencer GS, Robinson GM, Berry CJ & Dobbie PM 1994 Alteration of maternal growth hormone levels during pregnancy influences both fetal and postnatal growth in rats. *Biology of the Neonate* **66** 112–118.
- Woodall SM, Breier BH, Johnston BM & Gluckman PD 1996a A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth. *Journal of Endocrinology* **150** 231–242.
- Woodall SM, Johnston BM, Breier BH & Gluckman PD 1996b Chronic maternal undernutrition in the rat leads to delayed postnatal growth and elevated blood pressure of offspring. *Pediatric Research* **40** 438–443.
- Woodall SM, Bassett NS, Gluckman PD & Breier BH 1998 Consequences of maternal undernutrition for fetal and postnatal hepatic insulin-like growth factor-I, growth hormone receptor and growth hormone binding protein gene regulation in the rat. *Journal of Molecular Endocrinology* **20** 313–326.

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