**Adult growth hormone treatment reduces hypertension and obesity induced by an adverse prenatal environment**

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**Abstract**

The discovery of a link between an adverse *in utero* environment and the propensity to develop metabolic and cardiovascular disease in adult life is one of the most important advances in epidemiological research of recent years. Increasing experimental evidence suggests that alterations in the fetal environment may have long-term consequences for the development of metabolic disorders in adult life. This process has been termed 'fetal programming' and we have shown that undernutrition of the mother during gestation leads to development of the metabolic syndrome X during adult life. Striking metabolic similarities exist between syndrome X and untreated GH deficiency (GHD). In the present study we have investigated the effects of GH treatment on blood pressure and metabolic parameters. Virgin Wistar rats (age 75±5 days, n=20 per group) were time-mated and randomly assigned to receive food either *ad libitum* (AD) or 30% of AD intake (UN) throughout pregnancy. At weaning, male offspring were assigned to one of two diets (control or hypercaloric (30% fat)). Systolic blood pressure was measured at day 100 and following twice daily treatment with recombinant bovine GH for 21 days. GH treatment increased body weights in all treated animals but significantly reduced retroperitoneal and gonadal fat pad weights. Following GH treatment, systolic blood pressure was markedly decreased in all UN offspring. Saline-treated animals showed no change in systolic blood pressure over the treatment period. GH treatment increased heart-to-body weight ratio in all GH-treated animals. Our data demonstrated that GH treatment reduces hypertension and improves cardiovascular function in animals exposed to adverse environmental conditions during fetal or postnatal life. *Journal of Endocrinology* (2002) 175, 615–623

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**Introduction**

There is now increasing evidence that metabolic and cardiovascular disorders which manifest in adult life have their roots before birth. This concept of fetal programming is based on epidemiological and experimental observations of close associations between an adverse intrauterine environment and the later onset of adult metabolic and cardiovascular disorders (Barker 1995, Jackson *et al.* 1996, Reynolds & Phillips 1998, Godfrey & Barker 2000).

We have developed an animal model of fetal programming by maternal undernutrition throughout gestation, generating a nutrient-deprived intrauterine environment that results in fetal growth retardation and postnatal growth failure, and leads to changes in allometric growth patterns and endocrine parameters of the somatotropic axis (Woodall *et al.* 1996). Our model closely resembles the clinical and metabolic abnormalities seen in humans born at low birth weight and, furthermore, displays the phenotype of syndrome X (Reaven 1993, Smith *et al.* 1999). Offspring develop profound hyperphagia, obesity, hypertension, hyperinsulinaemia and hyperleptinaemia during adult life and postnatal hypercaloric nutrition amplifies the metabolic and cardiovascular abnormalities induced by fetal programming (Vickers *et al.* 2000).

Although the notion that growth hormone (GH) is critical for normal growth, maintenance of skeletal muscle mass and metabolic homeostasis is well accepted, increasing attention has recently been directed towards the specific influences of GH on cardiac structure and function (Cittadini *et al.* 1996). There is now increasing evidence implicating GH in the cascade of events that regulates heart development and cardiac function (Sacca *et al.* 1994, Sacca & Fazio 1996). For example, recent studies have shown improvements in diastolic blood pressure following GH therapy in hypopituitary adults (Johannsson *et al.* 1997, Bengtsson *et al.* 1999, Monson *et al.* 2000) and in GH-deficient (GHD) children (Sas *et al.* 2000). Although the precise mechanisms remain unknown, they may relate to changes in peripheral vascular resistance as proposed by others (Sacca *et al.* 1994, Bengtsson *et al.* 1999). This may be a direct result of reduced abdominal obesity via the lipolytic action of GH (Caidahl *et al.* 1994) or mediated by the action of insulin-like growth factor-I (IGF-I) on the...
vascular wall (Reaven et al. 1996) and increased nitric oxide synthesis (Bengtsson et al. 1999).

Striking similarities exist between syndrome X and untreated GHD in adults (Bengtsson 1993, Johannsson et al. 1997). Common to both these syndromes are abdominal/visceral obesity, insulin resistance and hypertension. The present study has investigated the response in blood pressure and metabolic parameters to GH treatment in adult life in offspring which experienced reduced substrate supply during fetal development alone or in combination with postnatal hypercaloric nutrition. The aim of the present study was to establish whether GH therapy can alleviate hypertension caused by an adverse fetal environment and/or postnatal diet-induced obesity.

Materials and Methods

Animal model

Virgin Wistar rats (age 75 ± 5 days, n=15 per group) were time-mated using a rat oestrus cycle monitor to assess the stage of oestrus of the animals prior to introducing the male. After confirmation of mating, rats were housed individually in standard rat cages containing wood shavings as bedding and free access to water. All rats were kept in the same room with a constant temperature maintained at 25 °C and a 12-h light:12-h darkness cycle. Animals were assigned to one of two nutritional groups: group 1, standard diet available ad libitum (AD) throughout pregnancy and group 2, undernutrition (30% of ad libitum intake (UN)) of a standard diet throughout gestation. Food intake and maternal weights were recorded daily until birth. After birth, pups were weighed and litter size was recorded. Pups from UN mothers were cross-fostered onto dams which received AD feeding throughout pregnancy. Litter size was adjusted to eight pups per litter to assure adequate and standardised nutrition until weaning. After weaning, male offspring from the two groups of dams, (a) AD offspring and (b) offspring from UN mothers, were divided into two balanced postnatal nutritional groups to be fed either a standard diet (total digestible energy 2959 kcal/kg, protein 19-4%, fat 5%, fat/energy ratio 15-21%, protein/energy ratio 26-23) or a hypercaloric diet (total digestible energy 4846 kcal/kg, protein 31-8%, fat 30%, fat/energy ratio 55-72%, protein/energy ratio 26-25%). The mineral and vitamin content in the two diets were identical and in accordance with the requirements for standard rat diets. The fat content of the hypercaloric diet is typical of that seen in many Western diets. Weights and food intake of all offspring were measured daily for the first 2 weeks then every second day. At day 100, systolic blood pressure (SBP) measurements were recorded using tail cuff plethysmography. Rats were then weight-matched and received either recombinant bovine GH (batch no. PR003, a gift from Dr W Baumbach, American Cyanamid Co., Princeton, NJ, USA) at a dose of 10 µg/g per day or saline by twice daily subcutaneous injection (0800 h and 1700 h). On the day prior to death, a repeated SBP was recorded. Rats were then fasted overnight and killed by halothane anaesthesia followed by decapitation. Blood was collected into heparinised vacutainers and stored on ice until centrifugation and removal of supernatant for analysis. All animal work was approved by the Animal Ethics Committee of the University of Auckland.

Blood pressure measurements

SBP was recorded by tail cuff plethysmography (blood pressure analyser IITC; Life Science, Woodland Hills, CA, USA) as described previously (Vickers et al. 2001b). Rats were restrained in a clear plastic tube in a pre-warmed room (25–28 °C). After the rats had acclimatised (10–15 min) the cuff was placed on the tail and inflated to 240 mmHg. Pulses were recorded during deflation at a rate of 3 mmHg/s and reappearance of a pulse was used to determine SBP. A minimum of three clear SBP recordings were taken per animal and the coefficient of variation for repeated measurements was <5%.

Endocrine analyses

IGF-I in rat blood plasma was measured using an IGF-binding protein-blocked radioimmunoassay (RIA) described previously (Vickers et al. 2001b). The half maximally effective dose (ED−50) was 0-1 ng/tube and the intra- and interassay coefficients of variation were <5% and <10% respectively. Rat insulin was measured by an in-house RIA as described previously (Lewis et al. 1999). The ED−50 was 0-5 ng/ml and the intra-assay coefficient of variation was <5% (all samples measured within a single assay). Leptin in rat plasma was measured by in-house RIA as described previously (Vickers et al. 2000). The ED−50 was 0-37 ng/ml and the intra-assay coefficient of variation was <5% (all samples measured within a single assay).

Blood biochemistry

Fasting plasma glucose concentrations from samples taken at the time of death were measured using a YSI Glucose Analyzer (Model 2300; Yellow Springs Instrument Co., Yellow Springs, OH, USA). All other plasma analytes were measured by a BM/Hitachi 737 analyser by Auckland Healthcare Laboratory Services. Blood haematocrit was measured immediately after death on trunk blood collected into capillary tubes.

Statistical analyses

Statistical analyses were carried out using SigmaStat (Jandel Scientific, San Rafael, CA, USA) and StatView (SAS Institute Inc.) statistical packages. Differences
between groups were determined by two-way (pre-GH treatment) or three-way (post-GH treatment) factorial ANOVA followed by Bonferroni post-hoc analysis and data are shown as means ± S.E.M. Statistical significance was assumed at the $P<0.05$ level. The ANOVA effects are defined as follows: offspring of UN mothers ‘programming effect’; postnatal hypercaloric nutrition ‘diet effect’; GH treatment ‘GH treatment effect’.

**Results**

**Maternal and fetal weights**

There was a marked reduction in maternal body weights in the UN group until day 15 of gestation. From day 15 of gestation, the UN dams gained weight and had achieved pre-mating weights by the time of parturition. Gestation was increased by 1 day in the UN dams. Litter size was not significantly different between the two groups (AD 11·7 ± 1·93, UN 11·2 ± 2·03). Maternal undernutrition resulted in fetal growth retardation reflected by significantly decreased body weight at parturition in offspring from UN dams (AD 6·08 ± 0·03 g, UN 4·04 ± 0·02 g, $P<0.0001$). Body lengths (nose–anus) were markedly reduced in UN offspring at birth (AD 44·4 ± 0·11 mm, UN 38·8 ± 0·16 mm). From parturition until weaning at day 22, neonatal weights remained significantly lower in UN offspring ($P<0.001$).

**Postnatal growth prior to and following GH treatment**

Hypercaloric nutrition resulted in a significant increase in body weight in both AD and UN animals. Total body weights for each diet remained significantly lower in UN animals compared with AD animals for the remainder of the study (Fig. 1). UN animals fed hypercaloric nutrition showed apparent catch-up growth and by postnatal day 100 had reached the same weight as AD animals fed the control diet. Body lengths (nose–anus) were significantly shorter in UN animals compared with AD animals on both diets. GH treatment resulted in marked body weight gain and increased body length in all treated animals (Table 1). There was no significant difference in weight gain response to GH treatment between AD and UN animals (Fig. 2).

**Systolic blood pressure**

Prior to the onset of GH treatment, UN offspring had significantly ($P<0.001$) elevated SBP compared with AD offspring. SBP was further significantly ($P<0.001$) elevated by postnatal exposure to hypercaloric nutrition (AD control 114·3 ± 3·23 mmHg, UN control 136·3 ± 3·52 mmHg, AD hypercaloric 143·6 ± 3·37 mmHg, UN hypercaloric 150·0 ± 3·15 mmHg). Treatment with GH for 21 days significantly reduced SBP ($P<0.05$) with the reduction in SBP significantly ($P<0.005$) more pronounced in hypertensive UN animals on both control and hypercaloric nutrition (Fig. 3). The GH-induced fall in SBP occurred independently of diet. SBP in normotensive AD animals fed the control diet was unaltered by GH treatment.

**Food intake**

Caloric intake (calories consumed/g body weight per day) was significantly increased ($P<0.001$) in AD and UN animals fed the hypercaloric diet. UN animals were hyperphagic ($P<0.0001$) on both diets compared with AD animals. During the GH-treatment period caloric intake remained significantly ($P<0.0001$) increased in AD and UN animals fed hypercaloric nutrition (Fig. 4). Treatment with GH resulted in an overall significant ($P<0.0005$) increase in caloric intake in all treated animals although the GH-induced increase in caloric intake was less marked in UN animals compared with AD animals (programming+GH interaction $P<0.05$). However, in animals fed hypercaloric nutrition, the effect of GH treatment on food intake was significantly reduced in AD animals and was absent in UN animals (diet+GH interaction $P<0.05$).

**Plasma hormone concentrations following GH treatment**

IGF-I plasma concentrations were significantly increased following treatment with GH (Fig. 5) and were not different between AD and UN offspring nor affected by postnatal hypercaloric nutrition. Maternal undernutrition...
resulted in offspring with significantly elevated fasting plasma insulin concentrations that were further amplified by hypercaloric nutrition. Treatment with GH significantly increased plasma insulin levels in all treated animals (P<0·05). A significant diet+GH interaction indicated that GH increased insulin concentrations to a greater extent in hypercalorically fed animals than in those fed the control diet (Fig. 5). Plasma leptin concentrations were significantly elevated in UN offspring and were amplified in AD and UN offspring fed hypercalorically (Fig. 5). GH treatment had no effect on plasma leptin concentrations.

**Tissue data following GH treatment**

Retroperitoneal fat pad weight was significantly higher in UN offspring on both diets compared with AD offspring.

**Table 1** Body weight and tissue data following 21 days of treatment with GH. Data are means ± S.E.M. and were analysed by three-way factorial ANOVA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (BW) (g)</th>
<th>Heart (% BW)</th>
<th>Liver (% BW)</th>
<th>Kidneys (% BW)</th>
<th>Spleen (% BW)</th>
<th>Adrenals (% BW)</th>
<th>Nose–anus (mm)</th>
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<tr>
<td>Saline</td>
<td>453 ± 17</td>
<td>0·315 ± 0·012</td>
<td>2·911 ± 0·05</td>
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<td>0·015 ± 0·001</td>
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<td>GH</td>
<td>548 ± 16</td>
<td>0·354 ± 0·011</td>
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<td>0·017 ± 0·001</td>
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Effects

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Interactions

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<th>NS</th>
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<th>NS</th>
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<td>NS</td>
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<tr>
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<tr>
<td>Programming+dieth+GH</td>
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</table>

NS, not significant.
and was significantly amplified by hypercaloric nutrition. GH treatment significantly reduced fat mass in all treated animals and was more effective in reducing fat mass in AD and UN offspring fed hypercaloric nutrition postnatally. A highly significant programming+GH interaction indicated that GH treatment was more effective in reducing retroperitoneal fat pad mass in UN offspring on both diets compared with AD offspring (Fig. 6). Gonadal fat pad mass was significantly higher in UN offspring on both diets compared with AD offspring and was significantly amplified by hypercaloric nutrition. GH treatment reduced gonadal fat mass in all treated animals (Fig. 6). As observed with retroperitoneal fat, a diet+GH interaction was evident with GH treatment being more effective at reducing gonadal fat mass in AD and UN offspring fed hypercaloric nutrition.

UN offspring had significantly smaller hearts than AD offspring and heart size was not affected by hypercaloric nutrition. Treatment with GH significantly increased heart-to-body weight ratios in all treated animals (Table 1). UN offspring had significantly smaller kidneys than AD offspring and kidney-to-body ratios were reduced in hypercalorically fed animals. Treatment with GH had no effect on kidney size (Table 1). Adrenal size was not affected by fetal programming although hypercalorically fed animals had smaller adrenals relative to body weight than control fed animals. Treatment with GH significantly increased adrenal size in all GH-treated animals and adrenal size was reduced relative to body weight in hypercalorically fed animals (Table 1). Liver and spleen weights were not different between AD and UN animals. GH treatment significantly increased liver and spleen weights in all treated animals (Table 1).

**Blood biochemistry following GH treatment**

Fasting blood plasma glucose was not different between AD and UN offspring. Glucose concentrations were significantly increased by hypercaloric nutrition and amplified further following GH treatment (Table 2). Plasma urea concentrations were significantly decreased in
hypercalorically fed animals but there was no effect of programming or GH treatment. Creatinine levels were significantly increased with GH treatment. A programming+GH interaction was present with GH treatment, increasing creatinine more significantly in UN animals. Plasma creatinine was not affected by hypercaloric nutrition (Table 2).

Plasma albumin was significantly reduced in UN offspring compared with AD offspring and was increased following GH treatment in all groups. There was no effect of diet on plasma albumin concentrations (Table 2). Plasma potassium concentrations were not different between AD and UN offspring. GH treatment increased potassium in all treated groups and hypercaloric nutrition caused a reduction in plasma potassium. Plasma alanine aminotransferase (ALT) concentrations were significantly increased in hypercalorically fed animals but there was no effect of programming or GH treatment (Table 2). A

Table 2  Blood biochemistry data following 21 days of treatment with GH. Data are means ± s.e.m. and were analysed by three-way factorial ANOVA

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<tr>
<th></th>
<th>Glucose (mmol/l)</th>
<th>Urea (mmol/l)</th>
<th>Creatinine (mmol/l)</th>
<th>Albumin (g/l)</th>
<th>Potassium (mmol/l)</th>
<th>ALT (mmol/l)</th>
<th>Sodium (mmol/l)</th>
<th>Haematocrit (%)</th>
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<tr>
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<td>27.5±3.1</td>
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<td>43.8±0.3</td>
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Effects
- Programming effect: NS NS P<0.05 P<0.005 NS NS P<0.05 NS
- GH effect: P<0.05 NS P<0.0001 P<0.0005 P<0.05 NS P<0.05 P<0.0001
- Diet effect: P<0.05 P<0.0005 NS NS P<0.005 NS NS NS NS

Interactions
- Programming+diet: NS NS NS NS NS NS NS NS NS
- Programming+GH: NS NS NS P<0.05 NS NS P<0.05 NS NS
- Diet+GH: NS NS NS NS NS NS NS NS NS
- Programming+diet+GH: NS NS NS NS NS NS NS NS NS

NS, not significant.
programming + GH interaction indicated that GH reduced ALT levels in UN offspring on both diets but not in AD offspring. Plasma sodium was significantly lower in UN offspring and was increased in animals fed hypercaloric nutrition. GH increased plasma sodium levels in all treated groups. Blood haematocrit was significantly reduced in all GH-treated animals but was not affected by programming or hypercaloric diet.

Discussion

We have previously shown that an adverse prenatal environment induced by maternal undernutrition can result in hyperinsulinaemia, hyperleptinaemia, hyperphagia, hypertension and development of obesity in offspring during postnatal life (Vickers et al. 2000, 2001a). Furthermore, the pathogenesis is amplified by postnatal hypercaloric nutrition (Vickers et al. 2000). We have recently shown that IGF-I treatment can alleviate the hypertension induced by an adverse prenatal environment and/or diet-induced obesity (Vickers et al. 2001b). Since IGF-I is regulated by GH, we have investigated in the present study whether metabolic and cardiovascular disorders in this paradigm can be alleviated by GH therapy. Our results demonstrate that GH treatment can normalise blood pressure in animals that are hypertensive as a result of adverse prenatal influences or diet-induced obesity. The decrease in blood pressure is paralleled by a marked reduction in fat mass. However, treatment with GH resulted in a further increase in hyperinsulinaemia. Importantly, this study suggests a divergence between hypertension and hyperinsulinaemia; blood pressure was normalised in the setting of GH-exacerbated hyperinsulinaemia. These results suggest that GH treatment during adult life may attenuate hypertension indirectly via amelioration of obesity or more directly via GH-induced increases in IGF-I acting on the vasculature.

Central to both syndrome X and GHD are abdominal/visceral obesity, insulin resistance and hypertension. The increased cardiovascular morbidity and mortality demonstrated in GHD adults suggests a close association between the two syndromes. However, until recently, GH and IGF-I were considered essential only to the control of linear growth and glucose homeostasis, and for the maintenance of skeletal muscle mass. However, a large body of evidence from animal and human studies has shown that the heart is a target for the GH–IGF-I axis (Sacca et al. 1994). In particular, studies on GHD adults have suggested that GH is essential for the maintenance of a normal cardiac structure and function, since these patients exhibit left ventricular hypertrophy and striking inotropic impairment, which is reversed by GH treatment (Merola et al. 1993, Cittadini et al. 1994). In the present study, GH treatment caused a significant reduction in blood pressure in animals that were hypertensive as a result of adverse prenatal influences. Importantly, SBP in normotensive animals was unaffected by GH treatment. GH treatment also resulted in a significant increase in relative heart size in all treated animals as reported by others (Cittadini et al. 1996, Isgaard et al. 1997). While these findings agree with those of others for a role for GH in improving cardiac function, this is the first report of GH acting as a potent antihypertensive agent via reduction of SBP.

Because early postnatal growth is GH and IGF-I dependent, failure of catch-up growth in children following exposure to an adverse prenatal environment has been attributed to alterations in somatotrophic axis regulation. However, while some studies report GHD and decreased plasma IGF-I concentrations (Woodall et al. 1996, Albertsson-Wikland et al. 1998), others report resistance to GH, IGF-I and/or insulin in the presence of normal GH, IGF-I and insulin profiles (Gluckman & Harding 1997, Chatelain et al. 1998). Furthermore, it has been shown that GH secretion, either spontaneous or evoked, is blunted in obesity (Scacchi et al. 1999). It may be possible that an adverse prenatal environment leads to alterations in GH action which, amplified by chronic postnatal hypercaloric nutrition, may lead to a secondary GHD. However, in our study this would seem unlikely as there was no reduction in plasma IGF-I concentrations as a result of programming or hypercaloric nutrition. This fits with work by ourselves (Woodall et al. 1996) and others showing that postnatal alterations in GH secretion and IGF-I concentrations as a result of prenatal events are normalised at a young age (Muaku et al. 1996).

Replacement of GH in GHD adults results in a marked reduction of central obesity and significant reduction in total cholesterol but little change in other risk factors, in particular insulin resistance and dyslipidaemia (Hew et al. 1998). The marked effect of GH therapy on body composition has been a consistent observation in many studies (de Boer et al. 1995) and our observation of markedly reduced fat mass fits with the lipolytic effects of GH. Furthermore, our observations of marked reductions in body fat mass following GH therapy and the corresponding fall in SBP also concur with the well-established association between abdominal obesity and elevated blood pressure. However, as in GHD patients treated with GH, we observed a marked worsening of the diet-induced hyperinsulinaemia. Importantly, our study indicates a disassociation between insulin resistance and hypertension with GH treatment. Despite the marked worsening of the hyperinsulinaemia in UN offspring, there was a highly significant fall in SBP.

UN offspring were hyperphagic compared with AD animals on both diets prior to the onset of GH treatment. While rising plasma insulin levels are normally associated with decreasing appetite (Schwartz et al. 1992), the hyperinsulinaemia seen in the UN animals is likely to reflect insulin resistance or reduced insulin action, as seen in children born with intrauterine growth retardation (Hofman et al. 1997). Although GH treatment resulted in
an overall increase in food intake, there was a lower response in the UN offspring, particularly in those animals fed hypercaloric nutrition. Although the effect of GH on food intake has been observed previously (Byatt et al. 1993), the mechanism underlying the differential response in appetite stimulation in UN animals warrants further investigation.

The fall in blood hematocrit and increased plasma sodium concentration in GH-treated animals concur with previous observations by others (Patel et al. 1978, Feld & Hirschberg 1996). An increment in plasma volume after GH administration has been attributed to the well-known sodium-retaining effects of GH, possibly mediated by a direct effect on the renal tubule. Alternatively, the GH–IGF-I axis may also be an important link in mediating a structurally adaptive growth response in the blood vessel wall (Wickman et al. 1997).

The precise mechanisms underlying the induction of adult hypertension and obesity by an adverse fetal environment induced by maternal undernutrition are not fully understood. Nephron endowment at birth is inversely related to the risk of developing essential hypertension in later life (Mackenzie & Brenner 1995, Mackenzie et al. 1996). We have observed significant reductions in glomeruli number in UN offspring (authors’ unpublished observations) and this supports recent work by others showing that an adverse fetal environment can give rise to a reduction in nephron number (Hinchliffe et al. 1992, Merlet-Benichou et al. 1997). It is possible that the decrease in renal fat mass following GH treatment in the present study directly lowered blood pressure via a reduction in medullary compression as proposed by Hall et al. (1998). More recently, an adipose-derived renin–angiotensin system has been described with angiotensinogen overproduction by adipose tissue resulting in elevated angiotensin II levels in the progression to obesity-related hypertension. We therefore speculate that GH treatment may indirectly lower blood pressure by ablation of fat mass concomitant with a down-regulation of the paracrine renin–angiotensin system. It is also tempting to speculate that GH treatment may lower blood pressure via IGF-I mediated down-regulation of the angiotensin II type 1 receptor as proposed in previous reports (Leri et al. 1999, Nilsson et al. 2000, Vickers et al. 2001b).

This study adds to the growing body of evidence supporting a beneficial role for GH in improving cardiovascular function and, in particular, in those adults who are hypertensive and obese as a consequence of adverse prenatal or postnatal environmental conditions. The mechanisms underlying the normalisation of blood pressure after GH treatment are still to be elucidated. A possible explanation which requires further investigation may relate to the profound vasodilatory effect of insulin and IGF-I, which has been observed in vitro. Interestingly, this response has been shown to remain intact in patients with type 2 diabetes (Izhar et al. 2000).

References

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