# Inhibitory effects of octreotide on renal and glomerular growth in early experimental diabetes in mice

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## Abstract

It was recently discovered that the streptozotocin (STZ)-diabetic mouse model is characterised by GH hypersecretion in contrast to the STZ-diabetic rat, the former thus mimicking the changes in GH in human type 1 diabetes. Inhibition of circulating and renal IGF-I by long-acting somatostatin analogues reduces renal and glomerular growth and urinary albumin excretion in diabetic rats.

The aim of the present study was to examine renal and glomerular growth in early experimental diabetes in mice along with changes in the GH/IGF-I axis following treatment with the somatostatin analogue octreotide. Balb/C(a) mice were randomised into non-diabetic controls, placebo-treated and octreotide-treated diabetic (50 μg/day) mice and examined 7 and 14 days after induction of diabetes.

There was no effect of octreotide treatment on body weight, glycaemic control or food intake. However, octreotide treatment significantly inhibited renal and glomerular growth by the end of the study period when compared with placebo treatment. In addition, octreotide prevented an increase in kidney IGF-I by day 7. GH hypersecretion was observed in the diabetic groups but octreotide treatment reduced GH levels compared with placebo treatment by day 14. No significant differences in serum or kidney IGF-binding protein-3 levels were observed between placebo- and octreotride-treated diabetic mice.

In conclusion, this new diabetic mouse model mimicking human type 1 diabetes is characterised by GH hypersecretion and the somatostatin analogue octreotide is able to prevent renal and glomerular growth, probably mediated through changes in circulating GH and local kidney IGF-I levels.

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## Introduction

During the last decade a significant role for growth hormone (GH) and insulin-like growth factor I (IGF-I) has been demonstrated in the development and progression of renal and glomerular growth and increase in urinary albumin excretion (UAE) in experimental diabetes (for reviews see Flyvbjerg 1990, Flyvbjerg *et al.* 1998). In the same time-period, inhibition of the GH/IGF-I axis by somatostatin analogues has demonstrated inhibition of renal and glomerular growth and reduction of the increase in UAE in diabetic rats (for reviews see Grønbæk & Ørskov 1995, Flyvbjerg 2000, Grønbæk *et al.* 2000).

It was recently demonstrated that the streptozotocin (STZ)-diabetic mouse model is characterised by GH hypersecretion, thus mimicking human type 1 diabetes better than the rat model usually characterised by GH hyposecretion (Flyvbjerg *et al.* 1999). In addition, the mouse model is characterised by elevated kidney IGF-I levels in long-term experimental diabetes (Flyvbjerg *et al.* 1999) which has also been observed in the non-obese diabetic (NOD) mouse (Segev *et al.* 1997).

The aim of the present study was to investigate the effect of the somatostatin analogue octreotide on renal and glomerular growth in early STZ-induced experimental diabetes in mice, along with changes in the circulating levels of GH, IGF-I and IGF-binding proteins (IGFBPs) and kidney IGF-I and IGFBPs.

## Materials and Methods

Adult female Balb/C(a) mice (Møllegaards Avlslab., Eiby, Denmark) with body weights between 15 and 19 g were randomised by weight into non-diabetic control mice ($n=13$), diabetic placebo-treated ($n=36$) and diabetic

 

octreotide-treated ($n=35$) mice. They were housed four to six per cage in a room with 12 h light:12 h darkness (lights on 0600 to 1800 h) cycle, a temperature of 21 ± 2 °C and humidity at 55 ± 2%. The animals had free access to standard chow (Altromin No.1324, Lage, Germany) and tap water throughout the experiment. Diabetes was induced by a single i.v. injection of STZ (250 mg/kg; Upjohn Company, Kalamazoo, MI, USA). Blood glucose was measured in tail vein blood by Haemoglucotest 1–44 and Reflolux II reflectance meter (Boehringer Mannheim, Mannheim, Germany). Urine was examined for ketone bodies by Neostix 4 (Ames Ltd, Slough, Bucks, UK). Food intake was measured on a group basis of three to four mice per cage by days 0, 7 and 14 along with body weights and blood glucose levels. A number of mice in the diabetic groups were excluded because of remission of diabetes or ketonuria which reduced the number of animals in the placebo-treated group to 12 and 10 and in the octreotide-treated group to 7 and 15 by days 7 and 14 respectively. Octreotide (250 μg/ml; Sandostatin, Sandoz, Basle, Switzerland) was dissolved in acetate buffer (50 mmol/l, pH=5·5) and mice were injected twice daily subcutaneously with the injection solution (50 μg/day). Controls and placebo-treated diabetic mice were injected with a similar volume vehicle (acetate buffer) subcutaneously twice daily. All principles of laboratory animal care and the current version of the Danish Law on Animal Experiments were followed.

At days 7 and 14, animals were anaesthetised with sodium barbital (10 mg/kg i.p.) and exactly 5 min later blood was drawn from the retro-orbital venous plexus for determination of serum GH, IGF-I and IGFBPs followed by excision of the kidneys. As barbital anaesthesia is a well-known stimulator of GH secretion lasting for up to 90 min (Takahashi *et al.* 1971), GH levels measured in the present study were stimulated values. A two millimetre thick horizontally cut slice from the middle of the right kidney (including the papilla) was fixed in 4% paraformaldehyde for morphological measurements (see below).

### *Immunoassays*

Serum GH was measured by radioimmunoassay (RIA) using a specific polyclonal rabbit rat GH antibody and rat GH as standard. Semilog linearity of mouse serum and rat GH (in the standard) was found at multiple dilutions, indicating antigen similarity between mouse GH and rat GH. The materials, including $^{125}$I-rat GH, were obtained from Amersham International plc (Amersham, Bucks, UK).

Serum IGF-I was measured after extraction with acid–ethanol (30 μl serum and 750 μl acid–ethanol). The mixture was incubated for 2 h at room temperature, centrifuged and 25 μl of the supernatant was diluted 1:200 before analysis. Serum IGF-I was measured by RIA using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Capistrano, CA, USA) and recombinant human IGF-I as standard (Amersham International plc). Monoiodinated IGF-I ($^{125}$I-(Tyr$^{31}$)-IGF-I) was obtained from Novo-Nordisk A/S (Bagsværd, Denmark). Intra- and interassay coefficient of variations for the GH and IGF-I assays were less than 5% and 10% for both assays.

### *Western ligand blotting (WLB) for determination of serum IGFBP-3*

SDS-PAGE and WLB were performed according to the method of Hossenlopp *et al.* (1986) as previously described (Flyvbjerg *et al.* 1992*c*). Serum (2 μl) was subjected to SDS-PAGE (10% polyacrylamide) under non-reducing conditions. The electrophoresed proteins were transferred by electroelution onto nitrocellulose paper (Schleicher & Schuell, Munich, Germany) and membranes were incubated overnight at 4 °C with approximately 500 000 c.p.m. $^{125}$I-IGF-I (specific activity 2000 Ci/mmol) in 10 ml TBS buffer (10 mmol/l Tris–HCl) containing 1% bovine serum albumin and 0·1% Tween (pH 7·4). Membranes were washed with TBS and, after drying overnight, the nitrocellulose sheets were autoradiographed with Kodak X-AR film and exposed to Du Pont–New England Nuclear enhancing screens (Sigma Chemical Co., St Louis, MO, USA) at − 80 °C for 3–7 days. Specificity of the IGFBP bands was ensured by competitive co-incubation with unlabelled IGF-I purchased from Bachem, Bubendorf, Switzerland. On WLB (with $^{125}$I-IGF-I as ligand) IGFBP-3 appears as a 38–42 kDa doublet band corresponding to the intact acid-stable IGF-binding subunit of IGFBP-3. A 30 kDa band representing IGFBP-1 and/or IGFBP-2 was observed followed by a 24 kDa band probably representing IGFBP-4. WLBs were quantified by densitometry using a Shimadzu CS-9001 PC dual wavelength flying spot scanner (Shimadau Corporation, Kyoto, Japan).

### *Estimation of glomerular volume*

By the end of the experimental period seven animals from each experimental group were randomly selected for morphological analysis. Sections (2 μm thick) of whole kidney profiles were cut on a rotation microtome and stained with PAS and haematoxylin. The mean glomerular tuft volume ($V_G$) was determined from the mean glomerular cross-sectional area ($A_G$). The areas were estimated with a two-dimensional version of the nucleator (Gundersen 1988) (CAST, Olympus, Denmark) by light microscopy from an average area of 40–80 glomerular profiles (tuft omitting the proximal tubular tissue within the Bowmann capsule). $V_G$ was calculated as $V_G=\beta/k\times(A_G)^{3/2}$ where $\beta=1{\cdot}38$ is the shape coefficient for spheres (the idealised shape of glomeruli) and $k=1{\cdot}1$ is a size distribution coefficient.

**Table 1** Metabolic parameters in control animals (C), placebo-treated (DP) and octreotide-treated (DO) diabetic mice during the study period. Values are means ± S.E.M.

| | Body weight (g) | Blood glucose (mmol/L) | Food intake (g/24 h) |
|---|---|---|---|
| **Group and day** | | | |
| C, day 0 | 16·9 ± 0·2 | 4·8 ± 0·3 | 5·9 ± 0·7 |
| C, day 7 | 18·9 ± 0·5[a] | 5·0 ± 0·2[a] | 4·9 ± 0·2 |
| DP, day 7 | 14·2 ± 0·4 | 20·1 ± 1·7 | 7·4 ± 0·7 |
| DO, day 7 | 14·5 ± 0·5 | 22·0 ± 1·8 | 7·2 ± 0·7 |
| C, day 14 | 20·3 ± 0·4[a] | 5·5 ± 0·4[a] | 4·7 ± 0·4 |
| DP, day 14 | 17·0 ± 0·2 | 13·0 ± 1·1 | 8·3 ± 0·3 |
| DO, day 14 | 17·7 ± 0·3 | 16·1 ± 1·1 | 10·2 ± 0·6 |

[a]$P<0·05$, C vs DP and DO by days 7 and 14 respectively.

*Statistical analysis*

All results are given as mean values ± S.E.M. Differences between groups were analysed by one-way ANOVA in combination with the Duncan test for multiple comparisons, or Kruskal–Wallis test for data not following a normal distribution followed by the Mann–Whitney test. Statistics were performed by the statistical package SPSS for Windows. $P<0·05$ was considered statistically significant in a two-tailed test.

## Results

Metabolic parameters, i.e. body weight, blood glucose and food intake are given in Table 1. Non-diabetic control mice had a significant body weight gain compared with the diabetic groups after 7 days and by the end of the study period. No differences in body weights were observed between octreotide- and placebo-treated diabetic mice during the study period. There was no significant difference in blood glucose levels between octreotide- and placebo-treated diabetic mice; however, both diabetic groups had significantly elevated blood glucose levels compared with the non-diabetic mice. Both diabetic groups were characterised by hyperphagia compared with the non-diabetic mice, and there was no difference in food intake between the two diabetic groups.

Kidney weight changes are given in Fig. 1 and mean glomerular volume in Fig. 2. After 7 days of diabetes there was no significant difference in total kidney weight between the experimental groups. However, a significant 16% increase in kidney weight was observed in placebo-treated diabetic mice compared with non-diabetic control mice by the end of the study period. By this time, octreotide treatment significantly inhibited renal growth compared with placebo-treated diabetic mice. Furthermore, a significant inhibition of glomerular growth was observed in the octreotride-treated diabetic mice compared with placebo-treated mice by the end of the study period. Placebo-treated diabetic mice demonstrated significant glomerular growth compared with the non-diabetic control mice.

Changes in kidney IGF-I and IGFBPs are given in Fig. 3. A significant increase in kidney IGF-I levels was observed in placebo-treated diabetic mice compared with the non-diabetic mice 7 days after diabetes induction and,

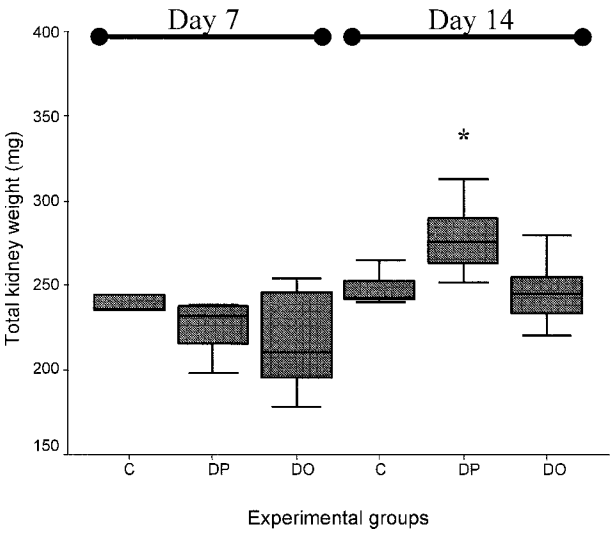


**Figure 1** Box and whiskers plot of total kidney weight changes by days 7 and 14 in non-diabetic controls (C), diabetic placebo-treated (DP) and diabetic octreotide-treated (DO) mice. The boxes represent the interquartile range and the whiskers represent highest and lowest values. *$P<0·05$, DP vs DO and C by day 14.

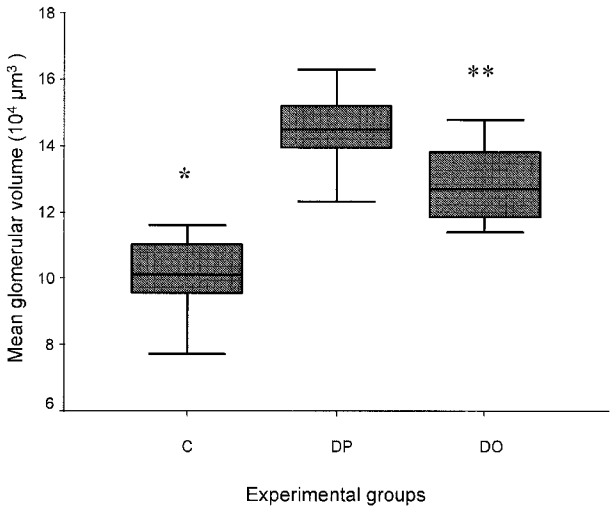


**Figure 2** Box and whiskers plot of mean glomerular volume differences by the end of the study period in non-diabetic controls (C), diabetic placebo-treated (DP) and diabetic octreotide-treated (DO) mice. The boxes represent the interquartile range and the whiskers represent highest and lowest values. *$P<0·05$, C vs DP and DC by day 14. **$P<0·05$, DO vs DP by day 14.

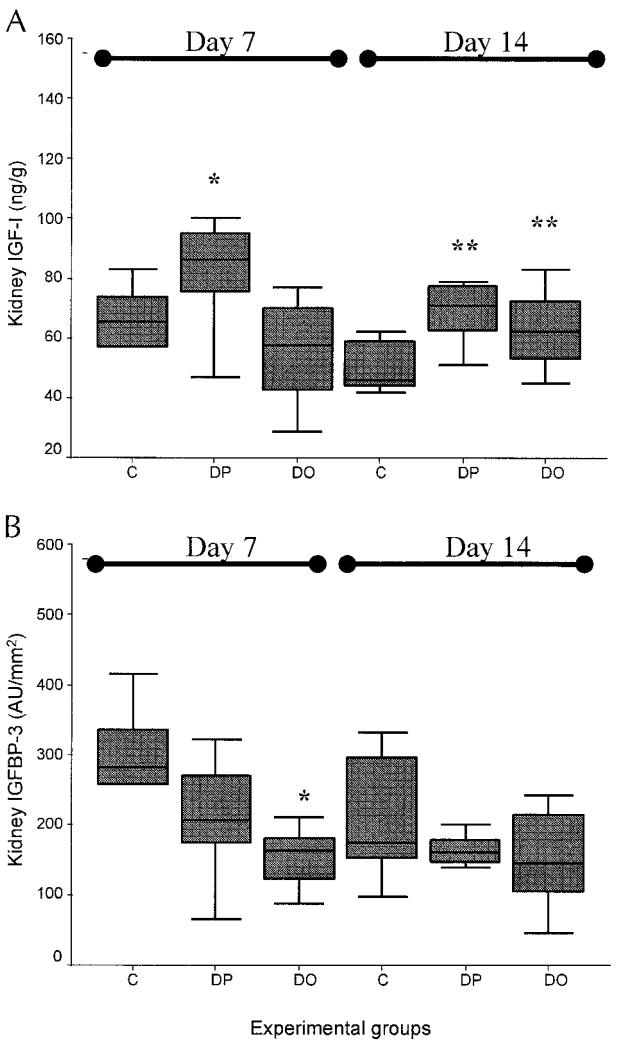


**Figure 3** Box and whiskers plot of kidney (A) IGF-I and (B) IGFBP-3 levels by days 7 and 14 in non-diabetic controls (C), diabetic placebo-treated (DP) and diabetic octreotide-treated (DO) mice. The boxes represent the interquartile range and the whiskers represent highest and lowest values. (A) *$P$<0·05, DP vs C and DO by day 7. **$P$<0·05, C vs DP and DO by day 14. (B) *$P$<0·05, DO vs C by day 7.

further, octreotide treatment completely abolished the increase in kidney IGF-I. By day 14, there was no difference in kidney IGF-I levels between octreotide- and placebo-treated diabetic mice and both groups had significantly elevated levels compared with the non-diabetic control mice. Kidney IGFBP-3 levels were significantly reduced in octreotride-treated diabetic mice compared with non-diabetic controls by day 7. There was no significant difference between placebo-treated diabetic mice when compared with non-diabetic controls and octreotride-treated diabetic mice respectively by day 7. By day 14, similar kidney IGFBP-3 levels were found in the

**Table 2** Changes in serum GH, IGF-I and IGFBP-3 levels in control animals (C), placebo-treated (DP) and octreotide-treated (DO) diabetic mice during the study period. Values are means ± S.E.M.

| | GH (μg/l) | IGF-I (μg/l) | IGFBP-3 (absorbancy units/mm$^2$) |
|---|---|---|---|
| **Group and day** | | | |
| C, day 7 | 22 ± 3[a] | 194 ± 15 | 294 ± 55[a] |
| DP, day 7 | 202 ± 61 | 118 ± 9 | 124 ± 31 |
| DO, day 7 | 73 ± 21 | 130 ± 7 | 159 ± 80 |
| C, day 14 | 18 ± 3[b] | 193 ± 18[b] | 574 ± 42 |
| DP, day 14 | 197 ± 94 | 116 ± 3 | 533 ± 22 |
| DO, day 14 | 85 ± 42[c] | 114 ± 4 | 568 ± 35 |

[a]$P$<0·05, C vs DP and DO by day 7; [b]$P$<0·05, C vs DP and DO by day 14; [c]$P$<0·05, DO vs DP by day 14.

experimental groups. There were no significant differences between the experimental groups with regard to the 30 kDa band or the 24 kDa band representing IGFBP-4 levels (data not shown).

Changes in serum GH, IGF-I and IGFBP-3 levels are given in Table 2. By day 7, barbital-stimulated GH levels were significantly increased in both diabetic groups compared with non-diabetic mice. A trend towards a reduction in GH levels was observed in octreotride-treated diabetic mice compared with placebo-treated mice by day 7 ($P$=0·12). However, by day 14, octreotide treatment significantly inhibited GH secretion compared with placebo treatment, though both diabetic groups had significantly elevated GH levels compared with the non-diabetic controls. By day 7, there was no significant difference in serum IGF-I levels between the experimental groups; however, by day 14, both diabetic groups had significantly reduced IGF-I levels compared with the non-diabetic control mice with no difference between octreotide and placebo treatment. By day 7, comparable reductions in IGFBP-3 levels were observed in both diabetic groups compared with non-diabetic controls. However, by the end of the study period no significant difference in IGFBP-3 levels was observed between the three experimental groups. A significant increase in IGFBP-3 was observed during the study period in non-diabetic mice. There were no significant differences between the experimental groups with regard to the 30 kDa band representing IGFBP-1 and/or IGFBP-2, or IGFBP-4 levels (data not shown).

## Discussion

The main finding of the present study was that the somatostatin analogue octreotide is able to inhibit renal and glomerular growth in this diabetic mouse model characterised by GH hypersecretion. The effect seems to be mediated partly through inhibition of GH hypersecretion and partly through inhibition of local kidney IGF-I

levels. The latter effect has been demonstrated previously in the STZ-diabetic rat model as discussed below.

In experimental diabetes in rats, GH levels are suppressed with loss of pulsatility (Robinson *et al.* 1987, Tannenbaum 1981). In contrast, human diabetes is characterised by GH hypersecretion positively correlated to the diabetic aberration (Yde 1969, Hansen & Johansen 1970, Hansen 1972). In concert with this we observed elevated barbital-stimulated GH levels in the present study, which have been demonstrated previously in the same model (Flyvbjerg *et al.* 1999) and also in NOD mice (Landau *et al.* 2000). It is therefore suggested that the diabetic mouse models, with respect to changes in GH, mimic human type 1 diabetes better than diabetic rats. With regard to the other components of the GH/IGF-I axis, there is agreement on the fact that similar changes are seen in human and experimental diabetes in rats (Flyvbjerg 1997, 2000). Accordingly, similar changes have been reported for the other elements in the axis with decreased circulating levels of GH-binding protein (GHBP), reduced levels of IGF-I and IGFBP-3 and increased IGFBP-1 (Flyvbjerg 1997, 2000).

In the present study, octreotide treatment inhibited GH hypersecretion along with inhibition of renal and glomerular growth. GH-deficient diabetic dwarf rats with low levels of circulating GH (and IGF-I) have similarly reduced renal and glomerular growth and less increase in UAE compared with GH-normal diabetic rats (Flyvbjerg *et al.* 1992*b*, Grønbæk *et al.* 1997). Further, using a specific GH receptor (GHR) antagonist, renal and glomerular growth and increase in UAE were inhibited without a difference in the GH or IGF-I levels (Flyvbjerg *et al.* 1999). This may suggest that GH *per se* is involved in diabetic renal and glomerular growth.

This has further been emphasised by the fact that transgenic diabetic mice overexpressing GHR antagonist demonstrate less glomerular hypertrophy and damage along with inhibition of increase in urinary protein excretion (Chen *et al.* 1995, 1996, Esposito *et al.* 1996). In a combined short- and long-term experimental diabetes study in rats, no change in kidney GHR mRNA was observed; however, cortical GHBP mRNA and peptide levels were increased and this may theoretically enhance GH availability to the GHR and thus be involved in glomerular growth (Landau *et al.* 1998). In addition, overexpression of GH in transgenic mice was followed by significant glomerular damage and increased urinary protein excretion even more pronounced than in animals overexpressing IGF-I (Doi *et al.* 1988, 1990).

In previous studies, somatostatin analogue treatment has been able to reduce renal and glomerular growth in short- and long-term experimental diabetes in rats (Flyvbjerg *et al.* 1989, 1992*a*, Grønbæk *et al.* 1995*a*,*b*, 1996, 1998). In the diabetic rat models, only a transient early increase in kidney IGF-I levels is observed (Flyvbjerg *et al.* 1989, Grønbæk *et al.* 1996). Further, the kidney IGF-I increase seems to be important as postponement of octreotide treatment to 3–9 days after the induction of diabetes is followed by less significant inhibition of renal and glomerular growth (Grønbæk *et al.* 1995*b*). In the present study, octreotide treatment significantly reduced kidney IGF-I levels after 7 days, but not by the end of the study, and elevated IGF-I levels were observed in both diabetic groups compared with controls by day 14. A sustained increase in kidney IGF-I has been demonstrated recently in STZ-diabetic mice and NOD mice (Segev *et al.* 1997, Flyvbjerg *et al.* 1999). In general, kidney IGF-I levels appear to be increased in this experimental diabetic mouse model compared with only a transient but important increase in the STZ-diabetic rat model.

A transient increase in IGFBPs has been observed in early experimental diabetes in rats (Flyvbjerg *et al.* 1992*c*). IGFBP mRNA expression has previously been examined in short- and long-term experimental diabetes and a pronounced and sustained increase in cortical IGFBP-1 mRNA was observed while medulla IGFBP-1 mRNA was reduced. In contrast, medulla IGFBP-5 mRNA was increased and cortical IGFBP-5 mRNA was decreased (Landau *et al.* 1995). It has been suggested that the IGFBP-1 most likely resulting from increased IGFBP-1 mRNA expression may be involved in trapping IGF-I from the circulation and thus increases IGF-I availability to the IGF-I receptor (De Vroede *et al.* 1986, Flyvbjerg 2000). However, in *in vitro* studies, IGFBP-1 has also demonstrated inhibition of cellular IGF-I binding and action (Ritvos *et al.* 1988). The effect of octreotide treatment on IGFBPs has only been examined briefly. The somatostatin analogue lanreotide inhibited circulating levels of IGFBP-3 and a 30 kDa IGFBP band (containing IGFBP-1 and IGFBP-2) in early experimental diabetes in rats (Grønbæk *et al.* 1993). In a recent study, octreotide inhibited the increased kidney IGFBP-1 mRNA expression and peptide in early experimental diabetes (Raz *et al.* 1998). In the present study, reduced serum IGFBP-3 was observed after 7 days but not after 14 days of diabetes compared with controls and with no effect of octreotide treatment. In a recent study, serum IGFBP-3 was reduced after 4 weeks of diabetes and with no change following GHR antagonist treatment (Flyvbjerg *et al.* 1999). The discrepancy in serum IGFBP-3 in the two studies may be caused by a difference in the duration of the diabetes. Kidney IGFBP-3 levels were significantly reduced in octreotride-treated diabetic mice by day 7 compared with non-diabetic controls; however, by day 14, similar kidney IGFBP-3 levels were found in the experimental groups. No differences in serum or kidney 30 kDa IGFBPs or IGFBP-4 were observed. It may be speculated that the reduction in IGFBP-3 may, in part, be involved in impairment of renal and glomerular growth.

In conclusion, we have confirmed previous studies that the experimental diabetic mouse model is characterised by GH hypersecretion and thus mimics human type 1

diabetes better than the STZ experimental diabetic rat model with regard to the GH/IGF-I axis. Further, as a novel finding we have demonstrated octreotide treatment to be effective in reducing renal and glomerular growth along with a reduction in GH hypersecretion and increased local kidney IGF-I levels. Similar effects were observed just recently in the NOD mouse using a novel somatostatin analogue (Landau *et al.* 2001). This may provide further evidence for a significant role for GH and IGF-I in diabetic renal growth and morphological changes in experimental diabetes in mice as has previously been demonstrated in diabetic rats.

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