# Octreotide (somatostatin analog) Treatment Reduces Endothelial Cell Dysfunction in Patients With Diabetes Mellitus

Andreas Clemens, Martina S. Klevesath, Marion Hofmann, Friedrich Raulf, Mechthild Henkels, Jean Amiral, Markus J. Seibel, Jürgen Zimmermann, Reinhard Ziegler, Peter Wahl, and Peter P. Nawroth

**Octreotide is a long-acting somatostatin analog that has been shown to have various effects in diabetes. This study was performed to evaluate whether octreotide affects the vascular complications of diabetes mellitus. Albuminuria and serum thrombomodulin were used as markers of vascular and renal dysfunction. We studied the effect of octreotide in 27 patients with insulin-dependent diabetes mellitus (IDDM). They received 200 μg octreotide per day over a period of 6 months. As a marker of endothelial cell damage, we measured the serum thrombomodulin level. We also measured urinary albumin excretion, hemoglobin $A_{1c}$ ($HbA_{1c}$), insulin-like growth factor-1 (IGF-1), and other parameters. IGF-1 decreased from 123 ng/mL before treatment to 114 ng/mL after 6 months of octreotide treatment ($P = .009$), while no significant change was observed in the unblinded control group (from 103 ng/mL to 102 ng/mL after 6 months of treatment). Urinary albumin excretion in patients with macroalbuminuria declined from 1,124 mg/L before octreotide treatment to 556 mg/L after 6 months of treatment ($P < .05$), whereas no change was observed in the control group. There was also a reduction of the plasma thrombomodulin level from 61.8 ng/mL to 46.1 ng/mL ($P < .07$) after 6 months of treatment. Furthermore, $HbA_{1c}$ decreased from 8.75% ± 1.27% to 8.12% ± 1.23% ($P < .07$) after octreotide treatment.**

***Copyright © 1999 by W.B. Saunders Company***

THE LONG-ACTING somatostatin analog octreotide is a synthetic cyclic peptide consisting of eight amino acids. It has a higher potency and a longer half-life than the natural hormone.[1-3] The pharmacological effects of octreotide include the inhibition of numerous hormones (thyrotropin, insulin, glucagon, and all gastrointestinal hormones), of exocrine secretion (gastric acid and pancreatic enzyme), of small-bowel absorption,[2,4] and of growth hormone (GH).[3,5-7] Somatostatin is also believed to participate in inflammatory diseases of the gastrointestinal tract, as well as in rheumatoid arthritis.[8,9]

The potential for long-acting somatostatin analogs in the treatment of diabetes mellitus became apparent after the demonstration that constant intravenous infusion of native somatostatin reduces elevated serum GH levels.[10,11] The hypothesis that GH has a causal role in diabetic complications was first proposed in 1970.[12] Other studies have supported the hypothesis that GH may participate in accelerating the development of late diabetic complications.[13-16] Recent studies in human and experimental diabetes suggest that the role of GH in angiopathy may be mediated via insulin-like growth factor-1 (IGF-1).[5,13,16-18]

Octreotide (Sandostatin; Sandoz, Nürnberg, Germany) has now been tested in several studies of the metabolic, hormonal, and functional abnormalities in diabetic humans and streptozotocin-diabetic rats. It has been shown to reduce the insulin requirement by 30% to 50% in insulin-dependent diabetes mellitus (IDDM)[15,19-21] and to reduce circulating levels of GH and IGF-1 by approximately 50%.[17,22,23] It also has been shown that low-dose octreotide therapy in IDDM subjects leads to significantly increased insulin sensitivity.[20,24]

Elevated GH concentrations have been found in patients with poorly and moderately controlled IDDM and are associated with renal hyperfiltration. It has been shown that therapy with octreotide decreases the glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) in patients with IDDM and renal hyperfiltration.[4] It was speculated that octreotide reduces the GRF and ERPF by suppression of GH and IGF-1. Furthermore, it has been shown that an initial renal IGF-1 accumulation is a prerequisite for early diabetic kidney hypertrophy.[18,23]

Recently, receptors for octreotide have been demonstrated in the vascular system.[8,9,25] The occupancy of these receptors by octreotide resulted in decreased plasma extravasation and enhanced vascular permeability, which is a hallmark of diabetic nephropathy. Hence, the hypothesis arised that octreotide also has an independent GH effect on the late complications of diabetes. It remains unclear as to whether the effects of octreotide on diabetic complications[16,18,22,26] and renal hyperfiltration,[4,27-29] demonstrated previously are explained just by a reduction of GH levels or also by an independent GH effect via vascular or renal octreotide receptors. In an open unblinded study, we examined the effect of octreotide in diabetic patients without elevated IGF-1 levels, and determined not only the extent of albuminuria but also the thrombomodulin plasma level as a marker of endothelial cell damage.

## SUBJECTS AND METHODS

### *Subjects*

Only male and female IDDM patients aged 20 to 65 years were included. Female patients who were not surgically sterilized or postmenopausal were required to use appropriate contraception and every precaution to ensure that pregnancy did not occur during the course of treatment. Furthermore, all patients had to be able to determine their blood glucose level and inject insulin and octreotide themselves.

*From the Department of Medicine I, University of Heidelberg, Heidelberg, Germany; Novartis Pharma Research, Basel, Switzerland; Novartis, Nürnberg, Germany; and SERBIO Research Laboratory, Gennevilliers, France.*

*Submitted July 31, 1998; accepted April 26, 1999.*

*Supported by a grant from the Deutsche Forshungsgesellschaft (DFG) (Na-138); during the tenure of a Schilling professorship (P.P.N.), a grant from the faculty of the University of Heidelberg to M.H.; and a grant from Novartis Pharma.*

*Current address: P.P.N., Section of Vascular Medicine, Department of Internal Medicine IV, University of Tübingen, Tübingen, Germany.*

*Address reprint requests to Andreas Clemens, MD, Medizinische Universitätsklinik Heidelberg, Innere Medizin I, Bergheimer Straße 58, 69115 Heidelberg, Germany.*

*Copyright © 1999 by W.B. Saunders Company*

*0026-0495/99/4810-0008$10.00/0*

Patients of both sexes were recruited from our diabetic clinic and matched into this open, non–placebo-controlled study for sex, age, and duration of diabetes. Due to the detectable side effects of octreotide (such as diarrhea), we were unable to design a double-blind study. Octreotide and control patients were recruited simultaneously. The characteristics of the patients (octreotide-treated and control group) are detailed in Table 1.

Exclusion criteria were untreated hypertension over a maximum of 160/100 mm Hg, serum creatinin greater than 1.4 mg/dL, diabetic ketoacidosis, frequent hypoglycemia, any acute illness or chronic disease other than diabetes mellitus, and a history of alcohol or drug abuse. Elevated IGF-1 levels above the normal range (144 to 360 ng/mL) were also exclusion criteria.

The study was approved by the ethics committee of the University of Heidelberg. After informed consent was obtained, diabetic patients began receiving the subcutaneous octreotide medication.

### *Protocol*

The starting dose of octreotide was 50 μg two times per day subcutaneously for 4 weeks. After 4 weeks, the dose was increased to 100 μg octreotide twice daily for the last 5 months. Pancreatic enzymes (pancreatin 250 mg) were given three times daily to prevent octreotide side effects. The patients performed blood glucose measurements themselves four times per day. Four patients had an early termination of treatment after 2 to 3 months because of gastrointestinal side effects of octreotide (diarrhea). There was one early termination because of hypoglycemia.

The patients were evaluated by a physician every 4 weeks during the observation period. Diabetic nephropathy, thrombomodulin (as a marker of cell damage), hemoglobin $A_{1c}$ ([$HbA_{1c}$] as a marker of metabolic control), and IGF-1 (as an expected mediator of octreotide action) were evaluated at 0, 3, and 6 months. All patients provided a full medical history at the starting point. Diabetic retinopathy, neuropathy, and nephropathy were graded as follows.

- Nephropathy: Nephrological status was graded by the urinary albumin concentration (UAC) determined with a turbidimetric technique (BNA; Behring, Marburg, Germany). Based on the mean UAC in at least three consecutive urine collections, patients were classified as without albuminuria (UAC $<$ 20 mg/L), microalbuminuria (UAC $\leq$ 200 mg/L), or macroalbuminuria (UAC $>$ 200 mg/L).
- Retinopathy: The ocular fundus was examined by an experienced ophthalmologist after dilation of the pupils and was classified as normal or presence of microaneurysm (I°), exudates (II°), or neovascularization, retinal hemorrhage, and intravitreal hemorrhage (II° to IV°).
- Neuropathy: Clinical examination for neuropathy included patellar- and achilles-tendon reflexes and vibration and temperature perceptions, classified as neuropathic or normal.

**Table 1. Characteristics of Octreotide-Treated Patients at the Time of Study Entry and the Control Group Without Octreotide Treatment**

| Characteristic | Treated | Untreated |
|---|---|---|
| Patients with IDDM (n) | 27 | 27 |
| Age (yr) | 49 ± 13.85 | 39.67 ± 15.04 |
| Male/female ratio | 15/12 | 17/10 |
| Duration of diabetes (yr) | 22.41 ± 11.19 | 15.04 ± 8.39 |
| Patients with macroangiopathy (n) | 6 | 10 |
| Patients with diabetic retinopathy, n (I°/II°/III°) | 7/1/13 | 9/3/6 |
| Patients with diabetic nephropathy, n (microalbuminuria/macroalbuminuria) | 7/11 | 12/13 |
| UAC (mg/L) at the beginning of study, n (microalbuminuria/macroalbuminuria) | 51.83/1,124 | 49.2/1,090 |
| Patients with diabetic neuropathy (n) | 18 | 20 |
| Thrombomodulin (ng/mL) | 61.8 | 60.5 |

### *Angiologic Studies*

Patients were classified as having macrovascular disease (ie, coronary heart disease or peripheral arterio-occlusive disease) based on objective findings (Doppler sonography, stress electrocardiogram [ECG], and/or angiography, including coronary angiography) or, alternatively, on clinical symptoms or a history of clinical events (increase in troponin T and acute ECG alteration).

### *Measurement Procedures*

*Serum creatinine.* The serum creatinine concentration was determined with the Jaffé method (kinetic) without deproteinization (Boehringer, Mannheim, Germany).

*HbA.* $HbA_{1c}$ levels were measured by high-performance liquid chromatography using the Diamant from Bio-Rad (Heidelberg, Germany). The normal range is 3.5% to 6.1%.

*IGF-1.* IGF-1 levels were measured with a commercially available IGF-1 radioimmunoassay (Biochem Immunosystems, Freiburg, Germany). The normal range is 144 to 360 ng/mL.

*Thrombomodulin.* Blood was collected into tubes containing 0.138 mol/L trisodium citrate at a final vol ratio of 9:1. The plasma was separated by centrifugation at 1,000 × *g* for 15 minutes at room temperature, and thrombomodulin was determined by a two-site enzyme-linked immunosorbent assay ([ELISA] Asserachrom Thrombomodulin ELISA kit; Diagnostica Stago, Asnières, France). This ELISA works with specific monoclonal antibodies according to the sandwich method. It was performed with precoated plates, according to the manufacturer's instructions, and measured at 492 nm with an automated ELISA plate reader (Titertek Multiscan Plus MKII; INC/Flow, Meckenheim, Germany) as described previously.[30,31] The intraassay coefficient of variation for this method was 6% and the interassay variation 16%. The normal range of serum thrombomodulin in an age-matched population without diabetes, infectious disease, hypertension, or known vascular disease was 36.0 ± 9.3 ng/mL.

### *Statistical Analysis*

Statistical analyses were performed using SAS Release 6.12 (SAS Institute, Cary, NC). For descriptive purposes, the mean ± SD or SEM is presented. The two-tailed Student *t* test was used to determine the significance of differences between the octreotide-treated group and control group. A paired *t* test was performed to analyze changes in the characteristics within groups during the study period. Two-tailed *P* values less than .05 used to indicate statistical significance.

## RESULTS

During the pilot phase of this study, we observed frequent side effects of octreotide treatment in diabetic patients, such as diarrhea. Therefore, a double-blind study was impossible to perform, and patients treated with octreotide and control patients were recruited simultaneously in the open unblinded study. To decrease gastrointestinal side effects in octreotide-treated patients, pancreatin (250 mg three times daily) was given.

The study patients were evaluated for 6 months. Their blood pressure did not change significantly during the observation time. The octreotide treatment was sufficient; compared with pretreatment values, IGF-1 was reduced from 123 ng/mL to 114

**Table 2. IGF-1, Daily Insulin Dose, and $HbA_{1c}$ in Patients With Octreotide Treatment and the Control Group at the Beginning of Study and After 3 and 6 Months**

| Parameter | Treated | | | Untreated | | |
|---|---|---|---|---|---|---|
| | 0 mo | 3 mo | 6 mo | 0 mo | 3 mo | 6 mo |
| IGF-1 (ng/mL)* | 123 ± 42 | ND | 114 ± 40‡ | 103 ± 32 | ND | 102 ± 50 |
| Daily insulin dose (IU/d) | 40 ± 13 | 35 ± 10 | 36 ± 12§ | 33 ± 8 | ND | 33 ± 8 |
| $HbA_{1c}$ (%)† | 8.75 ± 1.27 | 8.29 ± 1.02 | 8.12 ± 1.23‖ | 7.63 ± 1.27 | ND | 7.42 ± 1.28 |

Abbreviation: ND, not determined.
*Normal value, 102-257 μg/mL.
†Normal value, <6.2%.
‡$P = .009$ and §$P < .0001$ are statistically significant.
‖$P < .07$ reflects the trend for decrease.

ng/mL in the octreotide-treated group ($P = .009$). Furthermore, no significant change was found in the control group (Table 2).

The daily insulin dose in the octreotide-treated group declined from 40.03 ± 12.8 IU/d at the beginning of treatment to 35 ± 10.3 IU/d after 3 months ($P < .0001$), consistent with the increase in insulin sensitivity reported previously.[20] The daily insulin dose remained in this range after 6 months. $HbA_{1c}$ declined from 8.75% ± 1.27% to 8.12% ± 1.23% after 6 months ($P < .07$). In the control group, no change was observed for $HbA_{1c}$ or the daily insulin dose (Table 2). Thus, compared with the control group, octreotide treatment resulted not only in a reduction of the daily insulin dose but also in a reduction of $HbA_{1c}$.

Thrombomodulin has been described as a marker of endothelial cell damage[32] that (in contrast to von Willebrand factor) is not affected by platelet activation. Plasma thrombomodulin is increased in patients with diabetes in the presence of microvascular complications.[31,33,34] A decrease of thrombomodulin from 61.8 ng/mL at entry into the study to 46.1 ng/mL after 6 months ($P < .07$) was found in patients treated with octreotide. There was no change in plasma thrombomodulin in patients who were not treated with octreotide (Fig 1A).

Thrombomodulin has been shown to correlate with the degree of albuminuria in diabetic patients.[30,31,34] When the UAC was measured before and after treatment with octreotide, a significant decrease from 1,124 mg/L at the time of study entry

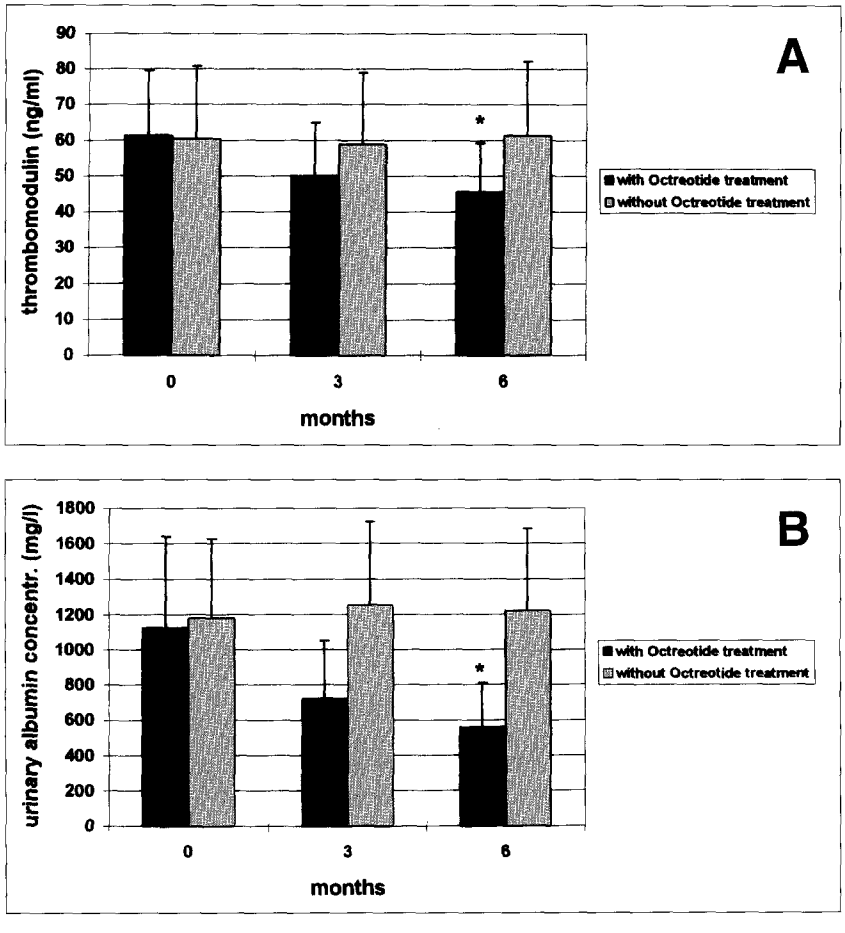


**Fig 1. (A) Reduction of thrombomodulin plasma levels in the octreotide-treated group compared with the control group. *$P < .07$ (reflects the trend for decrease). (B) Reduction of the UAC in the octreotide-treated group compared with the control group. *$P < .05$ (statistically significant).**

to 710 mg/L at 3 months and 556 mg/L at 6 months ($P < .05$) was observed. No significant change in albuminuria was observed in the control group (Fig 1B).

The effect of octreotide was greater in patients with more severe complications at the time of study entry. The decrease in Δthrombomodulin (0 *v* 6 months) was −6.7 in patients without albuminuria, +5.8 in patients with microalbuminuria, and −16.6 in patients with macroalbuminuria ($P < .05$, microalbuminuria *v* macroalbuminuria). Simular results were obtained with respect to ΔUAC (0 *v* 6 months). The decrease was largest in patients with macroalbuminuria ($\Delta = -553.3, P < .009$) and smallest in patients with normoalbuminuria ($\Delta = -1.5, P < .05$) (Fig 2).

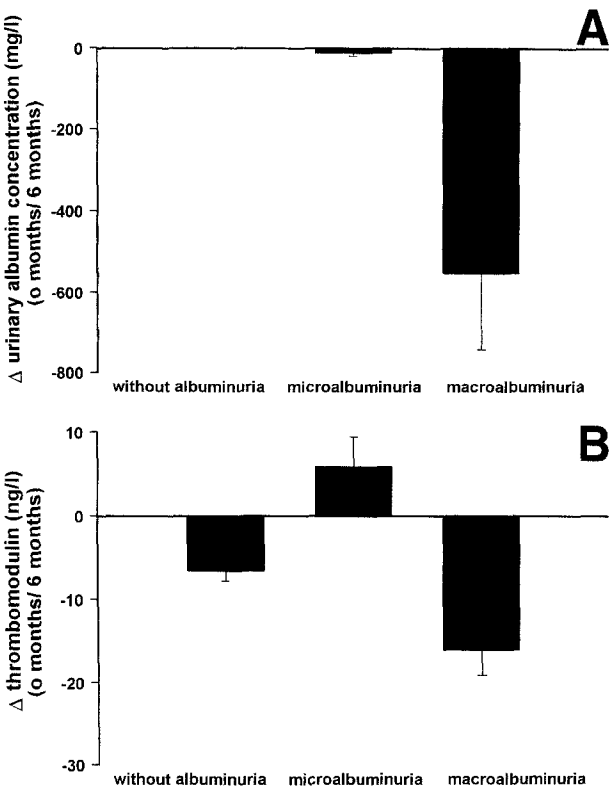


**Fig 2. Reduction of (A) thrombomodulin plasma levels and (B) UAC in patients without albuminuria or with microalbuminuria or macroalbuminuria. Error bars indicate the SEM.**

## DISCUSSION

Octreotide has been studied in diabetic patients primarily with respect to its GH-inhibitory function. Several studies have recently shown vascular effects of octreotide,[5,8] pointing potentially to direct effects of octreotide on vascular cells that are unrelated to GH. Binding sites for somatostatin have been described on cultured human retinal cells,[5] synovial venous, but not arterial, endothelial cells,[8] and vessels in inflamed gut.[9] Recent immunohistochemical studies have shown that $sst_2$ is expressed on endothelial cells in normal and injured (increased) rat vessels.[25] In our study, we observed a decrease in plasma thrombomodulin after 6 months of octreotide treatment. This corresponds well to a significant decrease in urinary macroalbuminuria. These data are compatible with the hypothesis of an endothelial cell–protective effect of octreotide, since thrombomodulin plasma levels reflect endothelial cell damage. Future studies are needed to show whether this effect of octreotide is also observed in a larger study.

Furthermore, it remains unknown as to whether the decrease in UAC and plasma thrombomodulin indicates a protection from late diabetic complications. In a previous study in diabetic rats, neither octreotide nor normalization of the metabolism with insulin affected albuminuria.[16,18] However, insulin and octreotide were only given for 3 weeks, whereas in the study, an increase in the effect of octreotide was found after 3 months, indicating the need for long-term therapy. Thrombomodulin plasma levels are dependent on renal function. Since octreotide treatment affects the GFR and ERPF,[4,16,18,27,28] it remains unknown as to whether the decrease in plasma thrombomodulin and UAC is due to the octreotide effect on renal function or a direct endothelial cell–protective effect of octreotide. IGF-1 decreased from normal to subnormal levels after octreotide treatment.[23] Therefore, it remains unknown as to whether the octreotide effect is indirect via suppression of IGF-1 or direct via binding to endothelial cells. Nevertheless, this open study shows the first evidence of an effect of octreotide on the UAC and a marker of endothelial cell damage (plasma thrombomodulin) in diabetic patients with normal IGF-1 levels.

## REFERENCES

1. Marbach P, Briner U, Lemaire M, et al: From somatostatin to Sandostatin: Pharmacodynamics and pharmacokinetics. Metabolism 41:7-10, 1992

2. Meier R, Dierdorf R, Gyr K: Somatostatin-Analog (Octreotide) im klinischen Einsatz: Neuere und potentielle Indikationen. Schweiz Med Wochenschr 122:957-968, 1992

3. Lamberts SW, Van der Lely AJ, De Hederer WW, et al: Drug therapy octreotide. N Engl J Med 25:246-254, 1996

4. Jacobs ML, Derkx FHM, Stijnen T, et al: Effect of long-acting somatostatin analog (somatulin) on renal hyperfiltration in patients with IDDM. Diabetes Care 20:632-636, 1997

5. Grant MB, Caballero S, Millard WJ: Inhibition of IGF-1 and b-FGF stimulated growth of human retinal endothelial cells by the somatostatin analogue, octreotide: A potential treatment for ocular neovascularization. Regul Pept 48:267-278, 1993

6. Krassowski J, Rogala H, Jeske W, et al: The attenuated inhibitory action of octreotide, a somatostatin analogue, on GHRH-induced growth hormone response in type 1 diabetes mellitus. Horm Metab Res 28:207-208, 1996

7. Lunetta M, Di-Mauro M, Le-Moli R: Lack of pharmacological effect of subcutaneous octreotide in an insulin-dependent diabetic patient: Reversal after mixing with aprotinin. J Endocrinol Invest 20:331-334, 1997

8. Reubi JC, Waser B, Markusse HM, et al: Vascular somatostatin receptors in synovium from patients with rheumatoid arthritis. Eur J Pharmacol 271:371-378a, 1994

9. Reubi JC, Mazzuchelli L, Laissue J: Intestinal vessels express high density somatostatin receptors in human infammatory bowel disease. Gastroenterology 196:951-959, 1994

10. Ørskov H, Flyvbjerg A, Frystyk J, et al: Octreotide and diabetes: Theoretical and experimental aspects. Metabolism 41:66-71, 1992

11. Fisker S, Kaal A, Montini M, et al: Growth hormone binding protein and growth hormone availability in acromegalic patients with long-acting octreotide (Sandostatin-LAR). Eur J Endocrinol 136:61-66, 1997

12. Lundbæk K, Christensen NJ, Jensen VA: Diabetes, diabetic angiopathy, and growth hormone. Lancet 2:131-133, 1970

13. Shumak SL, Grossman LD, Chew E, et al: Growth hormone suppression and nonproliferative diabetic retinopathy: A preliminary feasibility study. Clin Invest Med 13:287-292, 1990

14. Mallet B, Vialettes B, Haroche S, et al: Stabilization of severe proliferative diabetic retinopathy by long-term treatment with SMS 201-995. Diabete Metab 18:438-444, 1992

15. Harris AG, Gutniak M, Efendic S: Somatostatin analogue therapy of diabetes mellitus, in Bailey CJ, Flatt PR (eds): New Antidiabetic Drugs. 1990, pp 279-288

16. Grønbæk H, Nielsen B, Østerby R, et al: Effect of octreotide and insulin on manifest renal and glomerular hypertrophy and urinary albumin excretion in long-term experimental diabetes in rats. Diabetologia 38:135-144, 1995

17. Hyer SL, Sharp PS, Brooks RA, et al: Continuous subcutaneous octreotide infusion markedly suppresses IGF-I levels whilst only partially suppressing GH secretion in diabetics with retinopathy. Acta Endocrinol (Copenh) 120:187-194, 1989

18. Grønbæk H, Nielsen B, Frystyk J, et al: Effect of octreotide on experimental diabetic renal and glomerular growth: Importance of early intervention. J Endocrinol 147:95-102, 1995

19. Navascues I, Gil J, Pascau C, et al: Effect of a long-acting somatostatin derivate SMS 201-995 (Sandostatin) on glucose homeostasis in type I diabetes mellitus. Horm Res 29:92-94, 1988

20. Ørskov L, Møller N, Bak JF, et al: Effects of the somatostatin analog, octreotide, on glucose metabolism and insulin sensitivity in insulin-dependent diabetes mellitus. Metabolism 45:211-217, 1996

21. Ezzat S, Melmed S: Endocrine applications of the somatostatin analogue octreotide (Sandostatin). Metabolism 41:34-38, 1992

22. Nørgaard K, Snorgaard O, Jensen T, et al: Effects of octreotide on lipoproteins and endothelial function in type 1 (insulin-dependent) diabetic patients. Diabet Med 7:909-913, 1990

23. Raz I, Rubinger D, Popovtzer M, et al: Octreotide prevents the early increase in renal insulin-like growth factor binding protein 1 in streptozotocin diabetic rats. Diabetes 47:924-930, 1998

24. Hirose T, Arita N, Otsuki M, et al: A patient with acromegaly who showed remarkable improvement of hyperglycemia after treatment with octreotide. Intern Med 36:345-350, 1997

25. Chen JC, Hsiang YN, Buchan AM: Somatostatin receptor expression in rat iliac arteries after balloon injury. J Invest Surg 10:17-23, 1997

26. Flyvbjerg A, Marshall SM, Frystyk J, et al: Octreotide administration in diabetic rats: Effects on renal hypertrophy and urinary albumin excretion. Kidney Int 41:805-812, 1992

27. Igarashi K, Nakazawa I, Tani N, et al: Effect of a somatostatin analogue (SMS 201-995) on renal function and urinary protein excretion in diabetic rats. J Diabetes Complications 5:181-183, 1991

28. Serri O, Beauregard H, Brazeau P, et al: Somatostatin analogue, octreotide, reduces increased glomerular filtration rate and kidney size in insulin-dependent diabetes. JAMA 265:888-892, 1991

29. Petersen MM, Christensen SE, Christiansen JS, et al: Acute effect of a somatostatin analogue on kidney function in type 1 diabetic patients. Diabet Med 7:304-309, 1990

30. Hergesell O, Andrassy K, Geberth S, et al: Plasma thrombomodulin levels are dependent on renal function. Thromb Res 72:455-458, 1993

31. Gabath SJ, Keller C, Kempe HP, et al: Plasma thrombomodulin: A marker of vascular complications in diabetes. Vasa 25:233-241, 1996

32. Ishii M, Uchiyama H, Kazama M: Soluble thrombomodulin antigen in conditioned medium is increased by damage of endothelial cells. Thromb Haemost 65:618-623, 1991

33. Tanaka A, Ishii H, Hiraishi S, et al: Increased thrombomodulin values in plasma of diabetic men with microangiopathy. Clin Chem 37:269-272, 1991

34. Iwashima Y, Sato T, Watanabe K, et al: Elevation of plasma thrombomodulin levels in diabetic patients with early diabetic nephropathy. Diabetes 39:983-987, 1990