

Vitreous Levels of IGF-I, IGF Binding Protein 1, and IGF Binding Protein 3 in Proliferative Diabetic Retinopathy

A case-control study

ROSA BURGOS, PHD
CARLOS MATEO, PHD
ANA CANTÓN, PHD

CRISTINA HERNÁNDEZ, MD
JORGE MESA, MD
RAFAEL SIMÓ, MD

OBJECTIVE — To evaluate vitreous levels of IGF-I and its binding proteins IGFBP-1 and IGFBP-3 in patients with proliferative diabetic retinopathy (PDR). Because intravitreal proteins are elevated in patients with PDR due to the disruption of the blood-retinal barrier, we have corrected vitreal IGF-I and IGFBPs by total vitreal proteins to avoid this confounding factor.

RESEARCH DESIGN AND METHODS — We compared 21 diabetic patients with proliferative retinopathy (group A) and 13 nondiabetic patients (group B) in whom a vitrectomy was performed. Both groups were matched by age, serum IGF-I, IGFBP-1, and IGFBP-3 levels. Serum and vitreous levels of IGF-I, IGFBP-1, and IGFBP-3 were measured by immunological methods. Vitreal proteins were assessed by turbidimetric method.

RESULTS — Vitreal levels of IGF-I were elevated in group A (median 1.35 ng/ml [range 0.3–8.7]) in comparison with group B (median 0.25 ng/ml [range 0–1.38]), $P < 0.001$. After adjusting by vitreal proteins [ratio IGF-I (ng/ml)/protein (mg/ml)], the differences remain significant ($P < 0.005$). Vitreal levels of IGFBP-1 and IGFBP-3 were also elevated in diabetic patients (IGFBP-1: group A, median 1.6 ng/ml [range 0.6–20.7]; group B, median 0.4 ng/ml [range 0.3–1.9], $P < 0.001$. IGFBP-3: group A, median 102.6 ng/ml [range 53.9–350.8]; group B, median 29.0 ng/ml [range 3.2–87.8], $P < 0.001$). However, when the ratio IGFBP/protein was considered, the differences were not significant.

CONCLUSIONS — Intraocular synthesis contributes to elevated vitreous concentrations of IGF-I found in PDR. By contrast, unspecific increase of intravitreal proteins is the main factor explaining the elevated vitreous levels of IGFBP-1 and IGFBP-3 found in diabetic patients.

Diabetes Care 23:80–83, 2000

IGF-I is a polypeptide structurally homologous to insulin that regulates the proliferation and differentiation of several cell types (1,2). In vivo, IGF-I acts in a paracrine/autocrine manner to mediate many of the physiological actions of growth hormone, and its activity in extracellular fluids is regulated by insulin-like growth factor binding proteins (IGFBPs) that prolong

IGF-I half-life in the circulation and serve as a reservoir of IGFs. Moreover, IGFBPs have been shown to modulate IGF action on target cells (3,4).

Several clinical studies have suggested the role of growth hormone/IGF-I in the development of diabetic retinopathy. Growth hormone-deficient dwarfs with diabetes and diabetic patients with hemo-

chromatosis and infiltrative disease of the pituitary gland have little evidence of retinopathy (5,6). Conversely, proliferative diabetic retinopathy could improve after hypophysectomy (7–10). IGF-I has been involved in the impairment of diabetic retinopathy observed in puberty and pregnancy, two physiological conditions associated with serum IGF-I increasing (11–13). Furthermore, IGF-I has been implicated in the worsening of preexisting diabetic retinopathy observed after improved glycemic control by intensive insulin therapy (14–16). Interestingly, a transient elevation of serum IGF-I levels has been observed at the time of retinal vessel formation in a prospective 2-year follow-up study (17). Nevertheless, regional IGF-I concentrations in the retina may be more important than systemic levels. Vitreous fluid obtained from diabetic patients with proliferative diabetic retinopathy (PDR) submitted to a vitrectomy is a unique material to explore indirectly the synthesis of growth factors in the retina, and elevated vitreal levels of both IGF-I and IGFBPs have been reported (17–27). These proteins could be enhanced in vitreous fluid as a consequence of the increased synthesis by the retina. By contrast, the increase of vitreous levels of IGF-I and IGFBPs could be due to the disruption of the blood-retinal barrier, reflecting the leakage of proteins that occurs in diabetic microangiopathy. In fact, we have observed that intravitreal protein levels are elevated threefold in diabetic patients with PDR in comparison with control subjects (28). Therefore, the results of previous studies that have found higher intravitreal IGF-I and IGFBP concentrations could be due to this unspecific increase of proteins.

In the present study, vitreal levels of IGF-I, IGFBP-1, and IGFBP-3 were determined in diabetic patients with PDR and compared with a nondiabetic control group matched by serum IGF-I, IGFBP-1, and IGFBP-3. Furthermore, the results were adjusted by total vitreal proteins. This design permits one to analyze more accurately the results obtained in diabetic patients.

From the Diabetes Research Unit (R.B., A.C., C.H., J.M., R.S.) and the Ophthalmology Department (C.M.), Hospital General Universitari Vall d'Hebron, Barcelona, Spain.

Address correspondence and reprint requests to Dr. R. Simó, Diabetes Research Unit, Endocrinology Division, Hospital General Vall d'Hebron, Pg. Vall d'Hebron 119-129, 08035 Barcelona, Spain.

Abbreviations: CV, coefficient of variation; IGFBP, insulin-like growth factor binding protein; PDR, proliferative diabetic retinopathy.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—Age and serum levels of IGF-I, IGFBP-1, and IGFBP-3 in diabetic patients with PDR (group A) and control subjects (group B)

	Group A	Group B	P
n	21	13	—
Age (years)	62.2 ± 14.8	63.6 ± 13.5	0.72
Serum IGF-I (ng/ml)	136.7 (66.2–302.3)	123.3 (43–212)	0.88
Serum IGFBP-1 (ng/ml)	3.6 (0.7–17.2)	2.3 (0.6–12.5)	0.18
Serum IGFBP-3 (ng/ml)	3,212 (1,530–4,840)	3,141 (764–5,126)	0.80

Data are means ± SD or medians (range). Statistical analysis was performed with the Mann-Whitney U test.

RESEARCH DESIGN AND METHODS

Subjects

We included in the study 21 diabetic patients (6 type 1 and 15 type 2) with proliferative diabetic retinopathy in whom a classic three-port pars plana vitrectomy was performed (group A), and 13 nondiabetic patients (group B) with other conditions requiring vitrectomy (epiretinal or subretinal membrane). In all cases, a recent vitreous hemorrhage was excluded (less than 2 months). Both groups were matched by sex, age, and levels of serum IGF-I, IGFBP-1, and IGFBP-3 (Table 1).

Undiluted vitreous samples were obtained at the onset of vitrectomy by aspiration into a syringe attached to the vitreous cutter, transferred to a sterile tub, placed immediately on ice, and centrifuged at 16,000g for 5 min at 4°C. The samples were frozen at –80°C until assayed. A venous blood sample was collected simultaneously with the vitrectomy, then centrifuged at 3,000g for 15 min to obtain serum, aliquoted, and stored at –80°C until assayed.

The protocol was approved by the hospital ethical committee and all patients were fully informed before they gave their consent.

IGF-I method assay

IGF-I was measured by radioimmunoassay in which IGFBPs are dissociated by acidification and the addition of an excess of IGF-II (Mediagnost, Tübingen, Germany). The IGF-I antibodies have an extremely low cross-reactivity with IGF-II, and excess IGF-II does not disturb the interaction of the first antibody with IGF-I. This radioimmunoassay is validated to detect IGF-I in organic fluids other than serum. The minimum value detected was 0.02 ng/ml (coefficient of variation [CV] intra-assay 3.8%, CV interassay 6.1%).

IGFBP-1 method assay

IGFBP-1 was measured by immunoenzymometric assay, which uses a monoclonal anti-

body specific to human IGFBP-1 precoated in microwell plates, and another monoclonal antibody specific for IGFBP-1 conjugated with horseradish peroxidase (Medix Biochemica, Kauniainen, Finland). The minimum value detectable was 0.3 ng/ml (CV intra-assay 2.7%, CV interassay 6.2%).

IGFBP-3 method assay

IGFBP-3 was measured by radioimmunoassay (Nichols Institute Diagnostics, Wijchen, the Netherlands). The lowest measurable concentration was 0.25 ng/ml (CV intra-assay 5.6%, CV interassay 5.8%).

Protein assay

Vitreous proteins were measured by a previously validated microturbidimetric method with an autoanalyzer (Hitachi 917; Boehringer Mannheim, Mannheim, Germany). This method, based in the benzotriazinium chloride reaction, is a highly specific method for detection of proteins and has a higher sensibility and reproducibility than the classic method of Lowry. The lowest level of proteins detected is 0.02 mg/ml. The coefficients of variation intra-assay and interassay were 2.9 and 3.7%, respectively.

Statistical analysis

IGF-I, IGFBPs, and total intravitreal proteins were displayed as a median and range, because of their skewed distribution. A nonparametric test (Mann-Whitney U test) was used to compare serum and vitreous levels of IGF-I and IGFBPs. The Spearman rank test was performed to explore the correlation between total vitreal proteins and intravitreal IGF-I, and it has been graphically represented by means of a Pearson's correlation test after a log transformation. Levels of statistical significance were set at $P < 0.05$.

RESULTS — Vitreal levels of IGF-I were elevated in group A in comparison with group B (Table 1). Total vitreal proteins were also elevated in diabetic patients in comparison with control subjects (group A, median 2.99 mg/ml [range 0.62–9.29]; group B, median 0.82 mg/ml [range 0.28–2.63], $P < 0.005$). A positive correlation was observed between vitreous IGF-I and total vitreous proteins in group A (Fig. 1), but it was not detected in group B ($r = 0.44$; $P = 0.13$). When a correction considering vitreal proteins was performed (ratio IGF-I/total vitreous proteins), the differences between group A and group B were not so evident, but remained significant (Table 2). In addition, intravitreal levels of IGF-I were higher in the subgroup of diabetic patients who had similar intravitreal proteins than in control subjects (Table 3).

Vitreous levels of IGFBP-1 and IGFBP-3 were also elevated in group A in comparison with group B. However, after correcting for total vitreal proteins, the differences were not significant (Table 2).

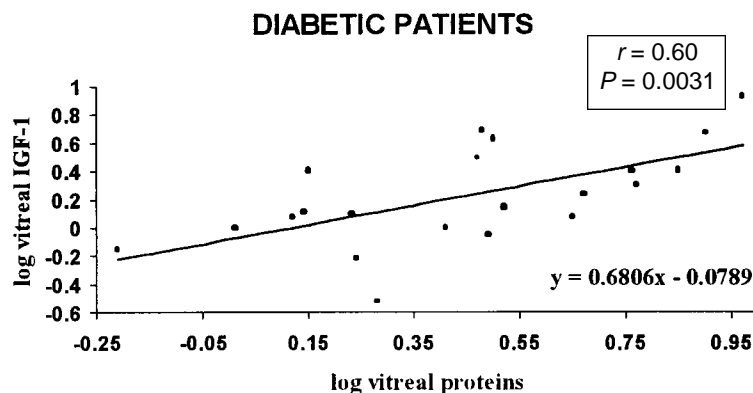


Figure 1—Correlation between total vitreal proteins and vitreal IGF-I in diabetic patients (group A). A logarithmic transformation was performed because of the skewed distribution of both parameters.

Table 2—Vitreous concentrations of IGF-I, IGFBP-1, and IGFBP-3 in absolute terms and after correction by total vitreal proteins in patients with PDR (group A) and control subjects (group B)

	Group A	Group B	P
n	21	13	—
IGF-I (ng/ml)	1.35 (0.3–8.7)	0.25 (0.02–1.38)	<0.001
IGF-I/proteins (ng/mg)	0.44 (0.17–1.87)	0.25 (0.00–0.71)	<0.005
IGFBP-1 (ng/ml)	1.6 (0.6–20.7)	0.4 (0.3–1.9)	<0.001
IGFBP-1/proteins (ng/mg)	0.6 (0.1–4.8)	0.4 (0.1–0.9)	0.4
IGFBP-3 (ng/ml)	102.6 (53.9–350.8)	29.0 (3.2–87.8)	<0.001
IGFBP-3/proteins (ng/mg)	43.5 (15.9–88.5)	33.3 (6.8–50.8)	0.2

Data are medians (range). Statistical analysis was performed with the Mann-Whitney U test.

CONCLUSIONS — There is increasing experimental evidence regarding the role of IGF-I in diabetic retinopathy. Intravitreal injections of IGF-I in rabbits (29) and pigs (30) cause a breakdown of the blood-retinal barrier and neovascularization that progresses in a similar manner to PDR. IGF-I is expressed constitutively by human retinal endothelial cells (31,32) as well as by retinal pigment epithelium (33,34), and participates in each step for neovascularization (2). Interestingly, IGF-I has been involved in the degradation of basement membranes and extracellular matrix proteolysis, a capital event to initiate the angiogenesis (35–37). Furthermore, other angiogenic agents, such as the basic fibroblast growth factor and vascular endothelial growth factor, have an additive effect with IGF-I on endothelial cell growth (38).

Vitrectomy fluid samples obtained from diabetic patients with PDR are currently used to explore indirectly the synthesis of growth factors by the retina. Several authors have obtained elevated IGF-I levels in the vitreous of diabetic patients with PDR (19,23–27). However, some caveats should be kept in mind: First, serum levels of IGF-I could influence its vitreal concentration and it should be considered in the evaluation of the results. This problem has been solved in the present study because diabetic patients and control subjects were matched by serum IGF-I.

Second, the elevated IGF-I levels in the vitreous of diabetic patients with PDR could only reflect the increase of total vitreal proteins observed in these patients due to the disruption of the blood-retinal barrier. Our results underscore the importance of this concept because a positive correlation was found between intravitreal IGF-I and total vitreal proteins in diabetic patients with PDR. However, in the present study, vitreal levels of IGF-I in diabetic patients with PDR were higher than those in control subjects, not only in absolute terms, but also after adjusting for total vitreal proteins. Furthermore, intravitreal levels of IGF-I were higher in the subgroup of diabetic patients who had similar intravitreal protein than in control subjects. These findings suggest that intraocular synthesis of IGF-I also contributes to its intravitreal increase. Locally synthesized IGF-I could be more pathogenic for PDR development due to its ability to act as an autocrine-paracrine factor. In this regard, it has been demonstrated that microvascular endothelial cells and retinal pigment epithelial cells express IGF-I mRNA as well as receptors for IGF-I, and the autocrine/paracrine actions of IGF-I in PDR are supported by several studies (2,23,31–33). Nevertheless, it must be noted that the median of vitreous IGF-I concentrations obtained in our study was 5.4-fold higher in diabetic patients than in control subjects in absolute terms,

but the ratio decreased to 1.7 after correction by vitreal proteins. Therefore, the main contributing factor to vitreal IGF-I levels in diabetic patients with PDR seems to be serum diffusion.

IGFBPs regulate bioavailability of IGF. IGFBPs can extend the half-life of the IGFs and, in addition, are capable of modulating IGF activity by either enhancing or inhibiting ligand receptor interactions. These proteins are intimately involved in the mechanisms of IGF action (1,3). Like other authors (23–25,27), we have found elevated vitreal levels of IGFBP-1 and IGFBP-3 in patients with PDR. However, our results suggest that serum diffusion is the main contributing factor explaining this finding, thus reflecting the unspecific increase of proteins found in PDR. This finding is in agreement with the lack of evidence of the expression in vivo of IGFBPs in the retina. Finally, it could be speculated that the imbalance between vitreal IGF-I and IGFBP-1/IGFBP-3 increases the bioavailability of vitreal IGF-I quantitatively and qualitatively, thus promoting neovascularization. Further studies with free IGF-I immunoassays are required to prove this hypothesis.

In summary, our results suggest that although serum diffusion is the main factor accounting for the increase of IGF-I observed in diabetic patients with PDR, intraocular synthesis also contributes to this enhancement. By contrast, the elevated vitreous levels of IGFBP-1 and IGFBP-3 could be only the consequence of the unspecific leakage of serum proteins.

Acknowledgments — This work was supported by a grant FIS 98/1270 from the Fondo de Investigaciones Sanitarias, National Health Institute of Spain.

References

1. Jones JL, Clemmons DR: Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Rev* 16:3–34, 1995
2. Grant M, King GL: IGF-I and blood vessels: implications for microvascular and macrovascular disease. *Diabetes Rev* 3:113–128, 1995
3. Bach LA, Rechler MM: Insulin-like growth factor binding proteins. *Diabetes Rev* 3:38–61, 1995
4. Rechler M, Clemmons D: Regulatory actions of insulin-like growth factor-binding proteins. *Trends Endocrinol Metab* 9:176–183, 1998
5. Merimee TJ: Metabolic and clinical studies in growth hormone deficient dwarfs: a ten-year

Table 3—Vitreous concentration of total proteins and IGF-I in diabetic patients and control subjects matched by vitreal protein levels

	Diabetic patients	Control subjects	P
n	9	13	—
Vitreous proteins (mg/ml)	1.41 (0.61–2.58)	0.82 (0.28–2.63)	0.31
Vitreous IGF-I (ng/ml)	1.0 (0.3–2.6)	0.25 (0.02–1.38)	<0.001

Statistical analysis was performed with the Mann-Whitney U test.

- follow-up. *N Engl J Med* 298:1217-1222, 1978
6. Passa R, Rousselie F, Gauville C, Canivet J: Retinopathy and plasma growth hormone levels in idiopathic hemochromatosis with diabetes. *Diabetes* 26:113-120, 1977
 7. Kohner EM, Dollery CT, Fraser TR, Bulpitt CJ: Effect of pituitary ablation on diabetic retinopathy studied by fluorescence angiography. *Diabetes* 19:703-714, 1970
 8. Ray BS, Pazianos AG, Greenberg E, Peretz WL, McLean JM: Pituitary ablation for diabetic retinopathy. *JAMA* 203:101-106, 1968
 9. Kohner EM, Hamilton AM, Joplin GF, Fraser TR: Florid diabetic retinopathy and its response to treatment by photocoagulation or pituitary ablation. *Diabetes* 25:1054-1060, 1976
 10. Sharp FS, Fallon TJ, Brazier OJ: Long-term follow-up of patients who underwent yttrium-90 pituitary implantation for treatment of proliferative diabetic retinopathy. *Diabetologia* 30:199-207, 1987
 11. Bhaumick B, Danilkewich AD, Bala RM: Insulin-like growth factors (IGF) I and II in diabetic pregnancy: suppression of normal pregnancy-induced rise of IGF-I. *Diabetologia* 29:792-797, 1986
 12. Rogers DG, Sherman LD, Gabbay KH: Effect of puberty on insulinlike growth factor I and HbA_{1c} in type 1 diabetes. *Diabetes Care* 14:1031-1035, 1991
 13. Klein BEK, Moss SE, Klein R: Is menarche associated with diabetic retinopathy? *Diabetes Care* 13:1034-1038, 1990
 14. Hyer SL, Sharp PS, Brooks RA, Burrin JM, Kohner EM: Progression of diabetic retinopathy and changes in serum insulin-like growth factor-1 (IGF-1) during continuous subcutaneous insulin infusion. *Horm Metab Res* 21:18-22, 1989
 15. Diabetes Control and Complications Trial: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986, 1993
 16. Chantelau E: Evidence that upregulation of serum IGF-1 concentration can trigger acceleration of diabetic retinopathy. *Br J Ophthalmol* 82:725-730, 1998
 17. Hyer SL, Sharp PS, Brooks RA, Burrin JM, Kohner EM: A two-year follow-up study of serum insulinlike growth factor-1 in diabetics with retinopathy. *Metabolism* 38:586-589, 1989
 18. Merimee TJ, Zapf J, Froesch ER: Insulin-like growth factors: studies in diabetics with and without retinopathy. *N Engl J Med* 309:527-530, 1983
 19. Grant M, Russell B, Fitzgerald C, Merimee TJ: Insulin-like growth factors in vitreous: studies in control and diabetic subjects with neovascularization. *Diabetes* 35:416-420, 1986
 20. Dills DG, Moss SE, Klein R, Klein BEK: Is insulin-like growth factor-I associated with diabetic retinopathy? *Diabetes* 39:191-195, 1990
 21. Arnold DR, Moshayedi P, Schoen TJ, Jones BE, Chader GJ, Waldbillig RJ: Distribution of IGF-I and -II, IGF binding proteins (IGFBPs) and IGFBP mRNA in ocular fluid and tissues: potential sites of synthesis of IGFBPs in aqueous and vitreous. *Exp Eye Res* 56:555-565, 1993
 22. Schoen TJ, Beebe DC, Clemmons DR, Chader GJ, Waldbillig RJ: Local synthesis and developmental regulation of avian vitreal insulin-like growth factor binding proteins: a model for independent regulation in extravascular and vascular compartments. *Endocrinology* 131:2846-2854, 1992
 23. Meyer-Schwickerath R, Pfeiffer A, Blum WF, Freyberger H, Klein M, Lösche C, Röhlmann R, Schatz H: Vitreous levels of the insulin-like growth factors I and II, and the insulin-like growth factor binding proteins 2 and 3, increase in neovascular eye disease: studies in nondiabetic and diabetic subjects. *J Clin Invest* 92:2620-2625, 1993
 24. Pfeiffer A, Spranger J, Meyer-Schwickerath R, Schatz H: Growth factor alterations in advanced diabetic retinopathy: a possible role of blood retina barrier breakdown. *Diabetes* 46 (Suppl. 2):S26-S30, 1997
 25. Waldbillig RJ, Jones BE, Schoen TJ, Moshayedi P, Heidersbach S, Bitar MS, van Kuijk FJ, de Juan E, Kador P, Chader GJ: Vitreal insulin-like growth factor binding proteins (IGFBPs) are increased in human and animal diabetics. *Curr Eye Res* 13:539-546, 1994
 26. Boulton M, Gregor Z, McLeod D, Charteris D, Jarvis-Evans J, Moriarty P, Khaliq A, Foreman D, Allamby D, Bardsley B: Intravitreal growth factors in proliferative diabetic retinopathy: correlation with neovascular activity and glycaemic management. *Br J Ophthalmol* 81:228-233, 1997
 27. Hopkins KD, Russell-Jones DL, Chignell AH, Sönksen PH: Insulin-like growth factor binding protein-1 levels in diabetic proliferative retinopathy. *Horm Metab Res* 25:331-332, 1993
 28. Burgos R, Simó R, Audi L, Mateo C, Mesa J, García-Ramírez M, Carrascosa A: Vitreous levels of vascular endothelial growth factor are not influenced by its serum concentrations in diabetic retinopathy. *Diabetologia* 40:1107-1109, 1997
 29. Grant MB, Mames RN, Fitzgerald C, Ellis EA, Abou-Friekha M, Guy J: Insulin-like growth factor I acts as an angiogenic agent in rabbit cornea and retina: comparative studies with basic fibroblast growth factor. *Diabetologia* 36:282-291, 1993
 30. Danis RP, Bingaman DP: Insulin-like growth factor-1 retinal microangiopathy in the pig eye. *Ophthalmology* 104:1661-1669, 1997
 31. Takagi H, Yoshimura N, Tanihara H, Honda Y: Insulin-like growth factor-related genes, receptors and binding proteins in cultured human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 35:916-923, 1994
 32. Moriarty P, Boulton M, Dickson A, McLeod D: Production of IGF-I and IGF binding proteins by retinal cells in vitro. *Br J Ophthalmol* 78:638-642, 1994
 33. Ocran I, Fay CT, Parmelee JT: Expression of insulin and insulin-like growth factor receptors and binding proteins by retinal pigment epithelium. *Exp Eye Res* 52:581-589, 1991
 34. Tanihara H, Inatani M, Honda Y: Growth factors and their receptors in the retina and pigment epithelium. *Prog Ret Eye Res* 16:271-301, 1997
 35. Grant M, Jerdan J, Merimee TJ: Insulin-like growth factor-I modulates endothelial cell chemotaxis. *J Clin Endocrinol Metab* 65:370-371, 1987
 36. Grant M, Guay C, Marsh R: Insulin-like growth factor I stimulates proliferation, migration and plasminogen activator release by human retinal pigment epithelial cells. *Curr Eye Res* 9:323-335, 1990
 37. Grant MB, Guay C: Plasminogen activator production by human retinal endothelial cells of non-diabetic and diabetic origin. *Invest Ophthalmol Vis Sci* 32:53-64, 1991
 38. Grant MB, Caballero S, Millard WJ: Inhibition of IGF-I 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