Apelin in Plasma and Vitreous and in Fibrovascular Retinal Membranes of Patients with Proliferative Diabetic Retinopathy

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PURPOSE. Apelin is an endogenous ligand for the angiotensin-1-like receptor APJ. Because apelin has been reported to regulate angiogenesis, the authors searched for associations between apelin and proliferative diabetic retinopathy.

METHODS. The study included 55 patients undergoing vitrectomy for proliferative diabetic retinopathy (study group) and 34 patients undergoing vitrectomy for idiopathic preretinal membranes or macular hole (control group). Using enzyme-linked immunosorbent assay, the authors measured the concentrations of apelin and vascular endothelial growth factor (VEGF) in the vitreous and plasma. The expression of apelin and angiotensin-1-like receptor APJ in the excised membranes was examined by fluorescence immunostaining and semiquantitative reverse transcription polymerase chain reaction.

RESULTS. Vitreous concentrations of apelin were significantly higher in the study group than in the control group ($P = 0.005$), whereas plasma concentrations of apelin did not vary significantly ($P = 0.66$). The vitreous concentrations ($P < 0.001$) and the plasma concentrations ($P = 0.03$) of VEGF were significantly higher in the study group than in the control group. Neither the vitreous concentrations of apelin and VEGF ($P = 0.47$) nor the plasma concentrations of apelin and VEGF ($P = 0.19$) were significantly associated with each other. In the fibrovascular membranes of the study group, colocalization of the endothelial markers CD31 with the markers for apelin and colocalization of the endothelial markers CD31 and APJ was observed. Expression of apelin mRNA ($P = 0.03$), APJ mRNA ($P = 0.02$), and VEGF mRNA ($P < 0.01$) was significantly higher in fibrovascular proliferative diabetic retinopathy membranes than in idiopathic epiretinal membranes.

CONCLUSIONS. The apelin/APJ system may be involved in retinal neovascularization during the development of proliferative diabetic retinopathy. (*Invest Ophthalmol Vis Sci.* 2010;51: 4237–4242) DOI:10.1167/iovs.09-4466

In the industrialized world, diabetic retinopathy is one of the leading causes of blindness in people of working age.[1,2] Retinal neovascularization, the hallmark of proliferative diabetic retinopathy, occurs at an advanced stage of the disease, and blindness can result from abnormal fibrovascular proliferation with subsequent intravitreal hemorrhage and tractional retinal detachment.[3] Since the landmark study by Aiello et al.[4] on the association between elevated intraocular concentrations of vascular endothelial growth factor (VEGF) and proliferative diabetic retinopathy, VEGF has been considered the most important primary mediator of retinal angiogenesis. It remains unclear, however, whether other substances also play a primary role in the process of ischemia-induced retinal neovascularization.

Apelin is an endogenous ligand for the angiotensin-1-like receptor APJ. It was first identified from bovine stomach extracts in 1998.[5] Before its isolation, the APJ receptor was referred to as an orphaned G-protein–coupled receptor because its endogenous ligand was not identified yet. Apelin mRNA and immunoreactive apelin were detected in various tissues and organs including brain, heart, lung, stomach, uterus, and ovary.[6–10] In rats, apelin was found to be localized within the endothelia of small arteries in organs such as liver, spleen, lung, pancreas, and adipose tissues.[11] Apelin was shown to affect many biological functions in mammals, such as adjusting the neuroendocrine, cardiovascular, and immune systems by autocrine, paracrine, endocrine, and exocrine signaling.[12] Apelin has various isoforms; the most widely studied are apelin-13 and apelin-36, with apelin-13 invariably exhibiting greater degrees of biological potency than apelin-36.[13]

In addition, apelin has been reported to regulate angiogenesis both in vitro and in vivo. In vitro, apelin was found to stimulate the proliferation and migration of retinal endothelial cells and vascular tube formation.[14] The evaluation of APJ and apelin expression patterns during embryogenesis in general and in the developing retina in particular suggested that autocrine signaling by apelin in endothelial cells provides a mechanism for regulating new blood vessel growth in embryonic angiogenesis.[15–17] The expression of apelin is upregulated during tumor neovascularization, and the overexpression of apelin increases in vivo tumor growth.[18,19]

Given that apelin has been recognized as a factor promoting angiogenesis and given that the role of apelin has not been examined in the development of proliferative diabetic retinop-

athy, we conducted the present study to examine the concentration of apelin in the blood, in the vitreous fluid, and in retinovitreal fibrovascular membranes of patients with proliferative diabetic retinopathy.

METHODS

This prospective comparative study included patients who consecutively underwent pars plana vitrectomy as treatment of proliferative diabetic retinopathy caused by diabetes mellitus type II (study group) or who underwent pars plana vitrectomy as treatment of idiopathic macular holes or preretinal membranes (control group). The study was conducted in accordance with the Declaration of Helsinki, and we received institutional approval from the review committee of Peking University People's Hospital. Informed consent for all examinations and procedures was obtained from each subject.

All patients underwent ophthalmic and medical examination. The ophthalmic examination included the history of previous ocular treatments, slit-lamp biomicroscopy, gonioscopy, ophthalmoscopy, fundus fluorescein angiography, and fundus color photography (fundus camera TRC-50EX; Topcon; Tokyo Optical Co., Ltd., Tokyo, Japan). The severity of diabetic retinopathy was assessed on the standardized fundus color photographs and on the fluorescein angiogram, or by ocular ultrasound if a vitreous hemorrhage or dense cataract prevented an ophthalmoscopic examination of the ocular fundus. The medical examination included a blood examination for the fasting serum concentrations of blood glucose and glycosylated hemoglobin (HbA1c). Systolic and diastolic blood pressures were measured with a mercury sphygmomanometer with the patient in the sitting position, after the patient had rested for at least 15 minutes. Arterial hypertension was defined as systolic blood pressure of 140 mm Hg or higher, diastolic blood pressure of 90 mm Hg or higher, or treatment with antihypertensive medication.

Pars plana vitrectomy was performed in a standardized technique involving three pars plana sclerotomy incisions. At the start of the removal of the vitreous body, approximately 1 mL undiluted vitreous gel was aspirated though the vitreous cutter under simultaneous inflation of the vitreous cavity with air through the infusion canula. In 14 eyes with proliferative diabetic retinopathy, fibrovascular membranes were surgically obtained during vitrectomy. In a similar manner, in six eyes with macular holes or preretinal membranes, fibrous membranes were obtained and served as control.

Blood samples were collected and the vitreous samples were immediately centrifuged for 10 minutes at 4°C at 3000 rpm. The liquid component without sediment was immediately stored at $-80°C$. Concentrations of apelin and VEGF were measured by using enzyme-linked immunosorbent assays (human Apelin-13 ELISA Kit; Uscnlife Science & Technology Company, Double Lake, Missouri City, TX; and human VEGF ELISA Kit, RapidBio Laboratory, Calabasas, CA). The sensitivity of apelin ELISA assays was 1 ng/mL, the interassay coefficient was 8%, and the intra-assay coefficient was 8%. The sensitivity of VEGF ELISA assays was 16 pg/mL, the interassay coefficient was 10%, and the intra-assay coefficient was 10%.

Dual-color immunofluorescence staining was performed on the frozen sections of the fibrovascular membranes and of the control membranes by staining with rabbit anti–apelin polyclonal IgG (1:200 dilution; ab59469; Abcam, Cambridge, MA) or rabbit anti–APJ receptor polyclonal IgG (1:200 dilution; ab66218; Abcam) and with mouse anti–CD31 polyclonal IgG (1:150 dilution; Zhongshan Goldenbridge Biotechnology Co., Ltd., Beijing, China). Apelin and APJ-receptor were examined with cyanogen (Cy) 3-conjugated goat anti–rabbit-fluorescein isothiocyanate IgG (1:200 dilution; BA1032; Sigma-Aldrich, St. Louis, MO), and CD31 was examined with FITC-conjugated goat anti–mouse-tetramethyl rhodamine isothiocyanate IgG (1:400 dilution; Zhongshan Goldenbridge Biotechnology). The samples were counterstained with DAPI (1:1000 dilution; D9542; Sigma-Aldrich). Sections were examined with a fluorescence microscope (DS-Ril-U2; Nikon, Tokyo, Japan) and photographed (DS-U2; Nikon).

RNA extraction, reverse transcription, and semiquantitative reverse transcription polymerase chain reaction (RT-PCR) were performed for fibrovascular membranes of six patients with proliferative diabetic retinopathy and for membranes of six patients with idiopathic epiretinal membranes. The following primers were used: human apelin (119 bp): sense, 5′-CACCTCGCACCTGCTGTA-3′; antisense, 5′-GAACGG-GAATCATCCAAAC-3′; APJ (587 bp): sense, 5′-TGGCTGACCTGACCT-TCGTG-3′; antisense, 5′-TTCACCAGGTGGTAGGGCAT-3′; VEGF (209 bp): sense, 5′-TTCTGCTGTCTTGGGTGCAT-3′; antisense, 5′-ATTCT-GCCCTCCTCCTTCTG-3′; and GAPDH (120 bp): sense, 5′-GAGTC-CACTGGCGTCTTCAC-3′; antisense, 5′-GTTCACACCCATGACGAACA-3′. GAPDH, one of the housekeeping genes, served as the positive control.

Statistical analysis was performed using a commercially available statistical software package (SPSS for Windows, version 17.0; SPSS, Chicago, IL). For primary analyses, we performed an independent sample t-test to compare the concentrations of apelin and VEGF in the study group with proliferative diabetic retinopathy and in the control group with nondiabetic patients. In a second step, measurements of the apelin concentration and the VEGF concentration were transformed into a decadic logarithm scale, and Mann-Whitney rank-sum test was performed. Where appropriate, Pearson and Spearman correlation tests were used. Two-tailed $P < 0.05$ was considered to indicate statistical significance.

RESULTS

The study included 89 patients, with 55 patients in the study group and 34 patients in the control group (Table 1). The groups did not vary significantly in sex, body mass index, waist-to-hip ratio, and prevalence of arterial hypertension. Patients of the study group were significantly younger than the patients in the control group (Table 1). In the study group, panretinal photocoagulation had been performed in 18 (33%) patients at least 4 months before inclusion into the study.

Vitreous concentrations of apelin were significantly higher in the study group than in the control group (12.5 ± 9.7 ng/mL vs. 7.4 ± 4.9 ng/mL; $P = 0.005$; Fig. 1). In a similar manner, vitreous concentrations of VEGF were significantly higher in the study group than in the control group (411 ± 609 pg/mL vs. 74 ± 65 pg/mL; $P < 0.001$; Fig. 2). Because data on the concentration of apelin and VEGF did not show a normal distribution according to a Gaussian distribution curve ($P = 0.003$ and $P < 0.001$, respectively), we transformed the data into a decadic logarithm scale. After this logarithmic transformation of the concentration measurements, a similar result was obtained (Mann-Whitney rank-sum test): the vitreous concentrations of apelin and of VEGF were significantly higher in the study group with proliferative diabetic retinopathy than in the control group with the nondiabetic patients (mean rank: apelin, 52 vs. 33, $P = 0.001$; VEGF, 55 vs. 29, $P < 0.001$). When the outliers presented by asterisks in Figures 1 and 2 were dropped, the differences between the two study groups remained statistically significant ($P = 0.006$ and $P < 0.001$, respectively). To further exclude the possibility that the increased apelin concentrations in the vitreous were caused by vitreous hemorrhage in patients with proliferative diabetic retinopathy and vitreous hemorrhage, we performed a bivariate correlation test and an independent Student's t-test. The bivariate correlation test revealed that the vitreous concentrations of apelin were not significantly associated with the presence of a vitreous hemorrhage ($P = 0.47$). The Student's t-test showed that the vitreous concentrations of apelin did not vary significantly between patients with proliferative diabetic retinopathy and vitreous hemorrhage compared with patients with proliferative diabetic retinopathy without vitreous hemorrhage ($P = 0.25$).

TABLE 1. Composition of the Study Population

	Proliferative Diabetic Retinopathy (*n* = 55)	Nondiabetic Ocular Diseases (*n* = 34)	*P**
Age, y	52.2 ± 9.8	62.1 ± 10.4	<0.001
Female sex, *n* (%)	27 (49)	21 (62)	0.24
Body mass index, kg/m²	24.6 ± 2.8	23.8 ± 2.9	0.20
Waist-to-hip ratio	0.90 ± 0.06	0.89 ± 0.06	0.24
Hypertension, *n* (%)	22 (40)	8 (24)	0.11
Duration of diabetes, y	13.4 ± 7.0	—	—
Fasting blood glucose, mmol/L	8.3 ± 3.2	—	—
Glycosylated hemoglobin, %	7.3 ± 1.2	—	—
Subgroups, *n*, %			
Panretinal photocoagulation history	18 (33)	—	—
Anterior chamber neovascularization	1 (2)	—	—
Vitreous hemorrhage	46 (84)	—	—
Fibroneovascular membranes	36 (66)	—	—
Tractional retinal detachment	28 (51)	—	—
Idiopathic macular hole	—	23 (68)	—
Idiopathic epiretinal membrane	—	11 (32)	—

Values are mean ± SD except where indicated.

* Statistical significance of the difference between both groups.

Plasma concentrations of VEGF were significantly higher in the study group than in the control group (1142 ± 558 pg/mL vs. 861 ± 582 pg/mL; *P* = 0.03; Fig. 3). In contrast, plasma concentrations of apelin did not differ significantly between the study group and the control group (16.9 ± 12.5 ng/mL vs. 18.1 ± 13.4 ng/mL; *P* = 0.66). Again, after the logarithm transformation of the concentration measurements, the Mann-Whitney *U* test showed similar results: plasma concentrations of VEGF were significantly higher in the study group than in the control group (mean rank, 50 vs. 37; *P* = 0.03), whereas plasma concentrations of apelin did not vary significantly between the groups (mean rank, 44 vs. 47; *P* = 0.59).

Comparison of concentrations of apelin in the vitreous and in the plasma revealed that the parameters were not significantly correlated with each other, neither as raw data (Pearson's correlation coefficient: *r* = −0.10; *P* = 0.36) nor after logarithmic transformation (*r* = −0.11; *P* = 0.29; Fig. 4).

In the study group, the vitreous concentrations of apelin were not significantly associated with the vitreous concentra-

tions of VEGF (*r* = −0.10; *P* = 0.47; Fig. 5), nor were the plasma concentrations of apelin significantly associated with the plasma concentrations of VEGF (*r* = −0.18 in plasma samples (*P* = 0.19).

Expression of APJ and apelin was detected in the specimens of all fibrovascular membranes of the study group with proliferative diabetic retinopathy with moderate staining intensities for APJ (Fig. 6A) and strong staining for apelin (Fig. 7A). Colocalization of endothelial markers CD31 (Fig. 6B) and APJ (Fig. 6) or CD31 (Fig. 7B) and apelin (Fig. 7) were observed in all specimens of the study group. None of the membranes removed from the eyes of the control group showed specific staining of CD31, APJ, or apelin (Figs. 6, 7E–H). Results of semiquantitative RT-PCR showed that the expression of apelin mRNA (*P* = 0.03), APJ mRNA (*P* = 0.02), and VEGF mRNA (*P* < 0.01) was significantly higher in the fibrovascular membranes of the six patients with proliferative diabetic retinopathy than in the membranes of the six patients with idiopathic epiretinal membranes (Fig. 8).
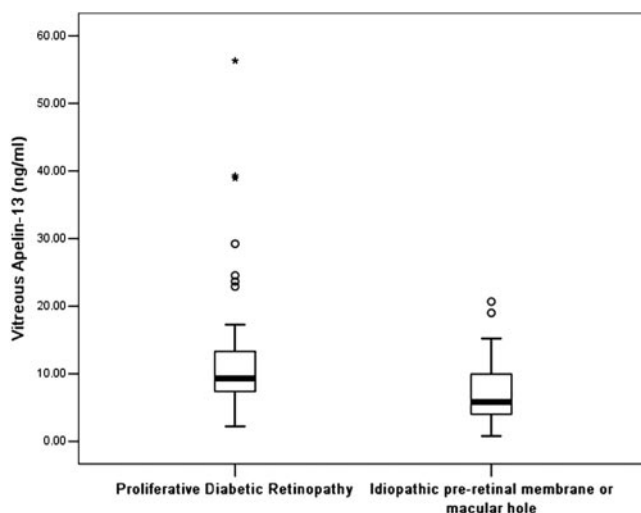

FIGURE 1. Box plots showing the vitreous concentration of apelin in patients with proliferative diabetic retinopathy and in patients with idiopathic preretinal membrane or macular holes, with a statistically significant difference between both groups (*P* = 0.005).
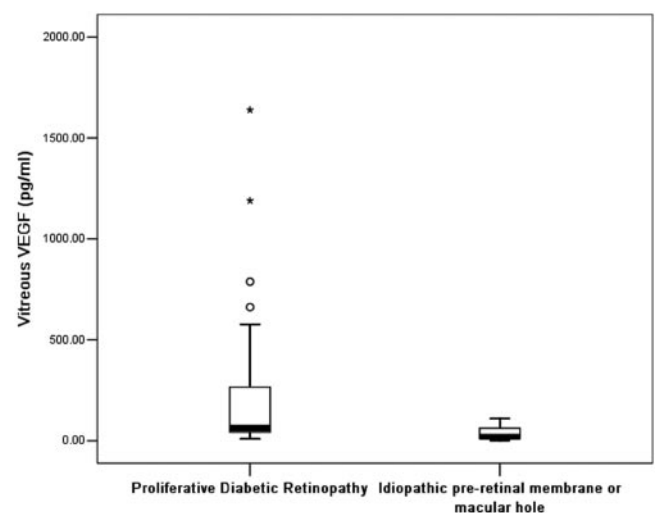

FIGURE 2. Box plots showing the vitreous concentration of VEGF in patients with proliferative diabetic retinopathy and in patients with idiopathic preretinal membrane or macular holes, with a statistically significant difference between both groups (*P* < 0.001).
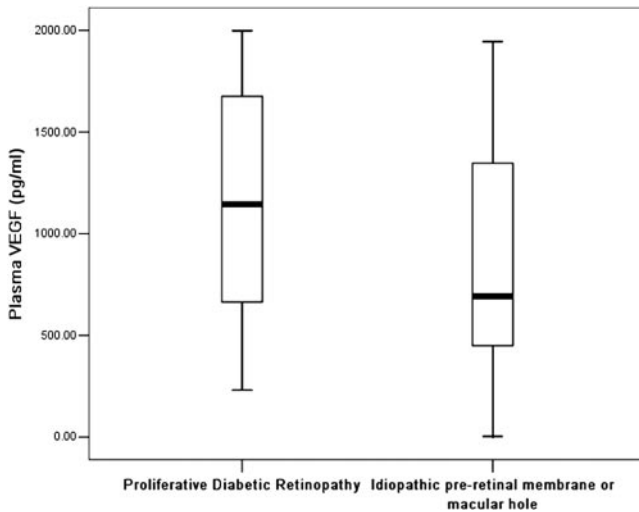
FIGURE 3. Box plots showing the plasma concentration of VEGF in patients with proliferative diabetic retinopathy and in patients with idiopathic preretinal membrane or macular holes, with a statistically significant difference between both groups ($P = 0.03$).

DISCUSSION

The results showed that the concentration of apelin in the vitreous of patients with proliferative diabetic retinopathy was significantly higher than the level of apelin in the vitreous of patients without diabetes. In the patients with proliferative diabetic retinopathy, the vitreous concentrations of apelin were not significantly associated with the vitreous concentrations of VEGF (Figs. 4, 5) or with the apelin concentrations in the plasma. Correspondingly, the plasma concentrations of apelin did not vary between the study group with patients with proliferative diabetic retinopathy and the control group with the nondiabetic patients. The finding of increased intraocular apelin concentrations, in contrast to normal apelin concentrations in the plasma, was paralleled by immunohistochemical findings of positive immunofluorescence staining of apelin and APJ in the endothelial cells of fibrovascular membranes of the patients with proliferative diabetic retinopathy, and it was
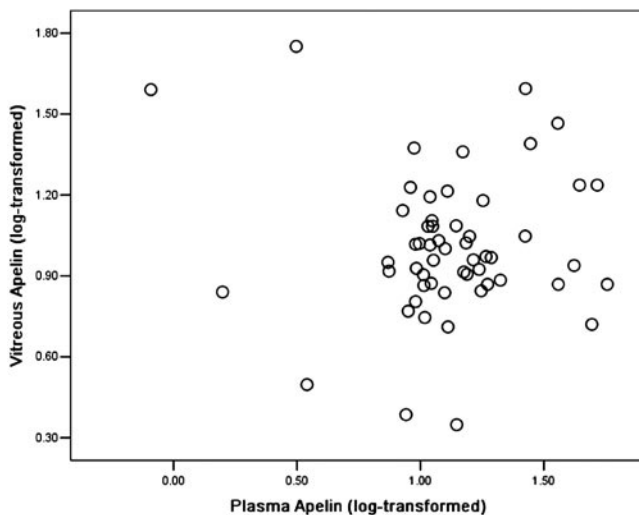

FIGURE 4. Scatter plot showing the association between the vitreous concentration of apelin and the plasma concentration of apelin in patients with proliferative diabetic retinopathy, without a statistically significant correlation between both parameters ($P = 0.29$).
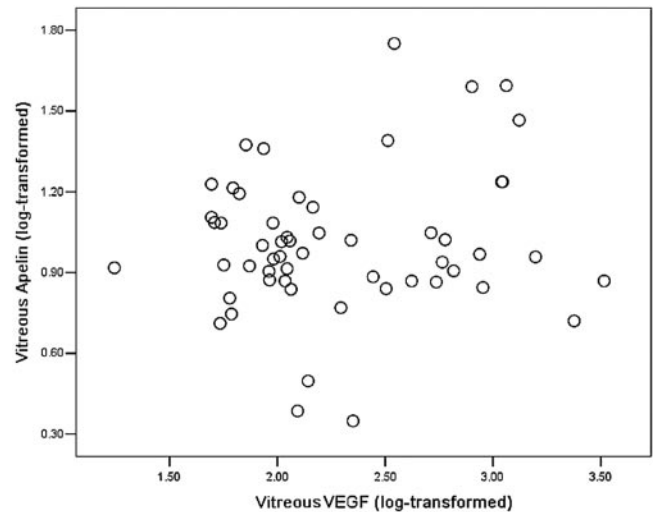

FIGURE 5. Scatter plot showing the association between the vitreous concentrations of apelin and of VEGF levels in patients with proliferative diabetic retinopathy, without a statistically significant correlation between both parameters ($P = 0.47$).

paralleled by a significantly higher expression of apelin mRNA and APJ mRNA in the fibrovascular membranes of patients with proliferative diabetic retinopathy than in the membranes of patients with idiopathic epiretinal membranes.

Our results are consistent with previous studies that suggested a relationship between apelin and angiogenesis.[14,20–22] Apelin mRNA was highly expressed in the vascular system, particularly in the vascular endothelial cells.[20] In mouse retinas, in situ hybridization showed an expression and upregulation of the apelin receptor during the embryogenic formation of the retinal vessels and was associated with the centrifugal extension of the superficial vasculature.[16] In another developmental study, the APJ gene was an early and specific marker of the venous phenotype in the retinal vasculature.[21] The link between receptor expression and vessel formation correlated with the mitogenic properties of apelin on endothelial cells.[22] Furthermore, a recent report showed the angiogenic activity of apelin in basement membrane matrix (Matrigel; BD Biosciences, Franklin Lakes, NJ) experiments and indicated that apelin was a novel angiogenic factor in retinal endothelial cells.[14]

The finding in our study that the plasma concentration of apelin did not vary between the diabetic study group with proliferative retinopathy and the nondiabetic control group agrees and disagrees with previous investigations. In the study by Erdem et al.,[23] plasma concentrations of apelin were significantly lower ($P < 0.001$) in patients with newly diagnosed type 2 diabetes mellitus than in healthy control patients. In another investigation, however, the basal plasma apelin concentrations were significantly higher in patients with impaired glucose tolerance and in diabetic patients than in nondiabetic control patients.[24] Reasons for the discrepancies between the studies may be differences in the patient inclusion criteria and in study composition, such as advanced diabetes mellitus with proliferative retinopathy as in our study, in contrast to patients with newly diagnosed type 2 diabetes mellitus in the study by Erdem et al.[23]

The findings that vitreous concentrations of apelin were higher in eyes with proliferative diabetic retinopathy than in nondiabetic eyes, that the increase in apelin concentration was found in the intraocular compartment but not in the plasma compartment, and that immunohistochemistry revealed immunofluorescence staining of apelin and APJ in the endothelial
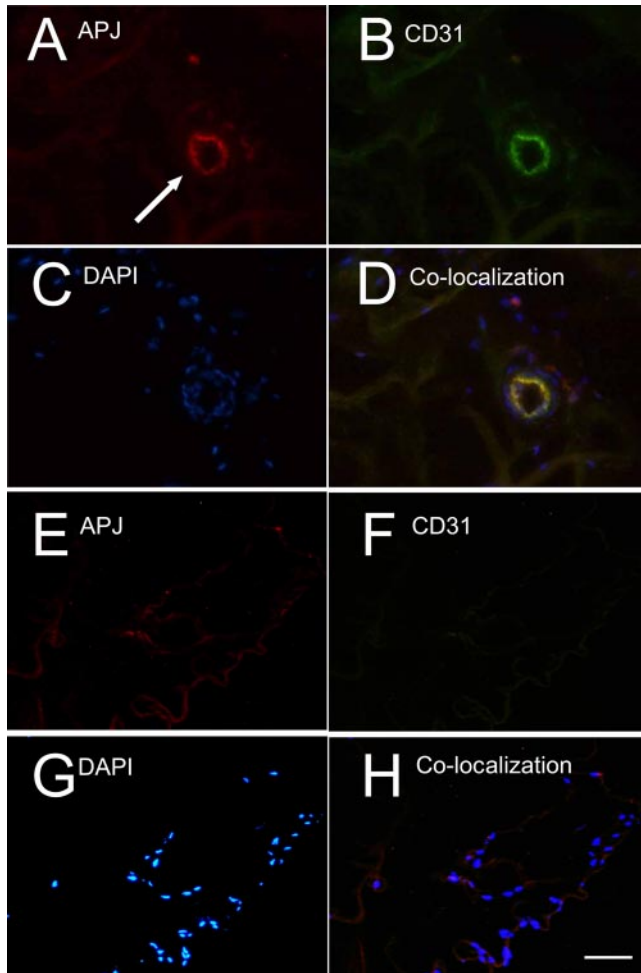
FIGURE 6. (**A–D**) Immunostaining for APJ (**A**), endothelial cell marker CD31 (**B**), and DAPI (**C**) in fibrovascular membranes from eyes with proliferative diabetic retinopathy. Staining intensities of APJ were moderate (**A**, *arrow*) and were colocalized with endothelial cells, as identified by CD31 (**D**). (**E–H**) Staining of APJ (**E**), CD31 (**F**), and DAPI (**G**) in preretinal membranes of control patients without diabetic retinopathy. Only weak and insignificant staining of APJ (**E**) and CD31 (**F**) was observed. Scale bar, 50 μm.

cells of the fibrovascular membranes of patients with proliferative diabetic retinopathy may lead to the inference that the increased apelin concentrations in the vitreous of the eyes with proliferative diabetic retinopathy was caused by the local production of apelin, presumably as an autocrine function of the retinal vascular endothelial cells. They suggest that apelin potentially plays a role in retinal neovascularization and in the development of proliferative diabetic retinopathy. Because the vitreous concentrations of apelin were not correlated with the vitreous concentrations of VEGF in the study group with proliferative diabetic retinopathy, it may be inferred that apelin and VEGF do not act in a directly synchronized manner in the angiogenesis of proliferative diabetic retinopathy.

Potential limitations of our study should be mentioned. First, the patients in the study group were younger than the patients in the control group (Table 1). Given that the likelihood of an abnormal condition usually increases with age, it may be inferred that the difference in age between study group and control group potentially serves only to strengthen the conclusions drawn. Second, the study group consisted of patients with proliferative diabetic retinopathy who were in need of surgery; therefore, the results and conclusions drawn in our

study are valid only for the proliferative stage of diabetic retinopathy. It has remained undetermined whether similar observations may be made in the early stages of the disease. Third, the absence of a direct correlation between the VEGF concentration in the vitreous and the apelin concentration in the vitreous does not provide evidence that these two factors are totally independent in their mode of an increased expression or their actions. Further experiments are needed to reveal whether there is a network of cooperation between VEGF and apelin in the development of proliferative diabetic retinopathy.

In conclusion, the vitreous concentrations of apelin were significantly higher in eyes with proliferative diabetic retinopathy than in nondiabetic eyes, whereas the plasma concentrations of apelin did not vary significantly between both groups. In contrast, both the vitreous concentrations and the plasma concentrations of VEGF were significantly higher in the study group than in the control group. Neither the vitreous concentrations of apelin and VEGF nor the plasma concentrations of apelin and VEGF were significantly associated with each other. In the fibrovascular membranes of the eyes with proliferative diabetic retinopathy, colocalization of the endothelial markers CD31 with the markers for apelin and colocalization of the
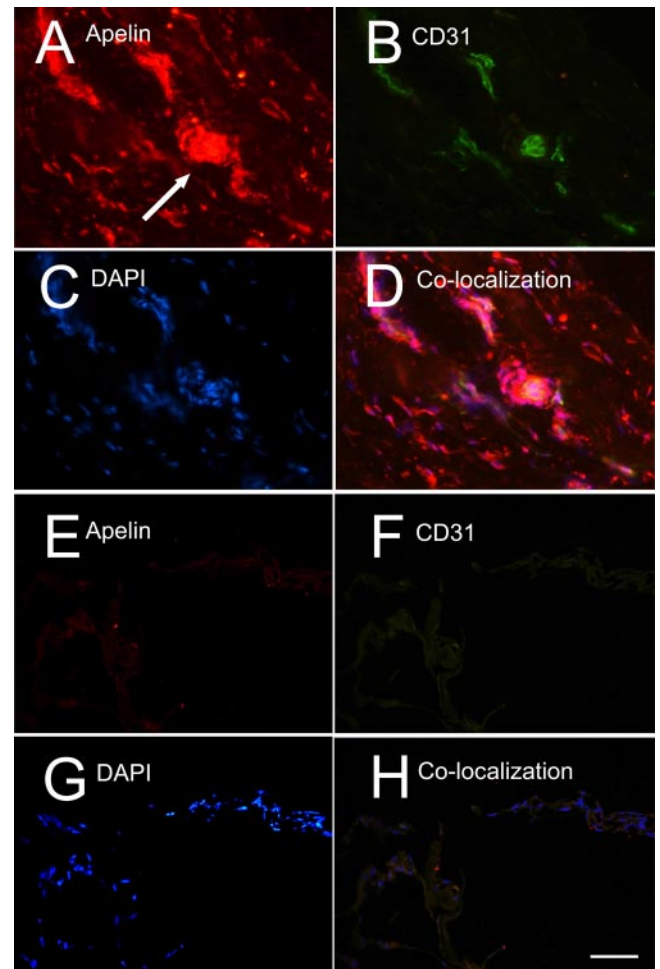

FIGURE 7. Immunostaining for apelin (**A**), endothelial cell marker CD31 (**B**), and DAPI (**C**) in fibrovascular membranes from proliferative diabetic retinopathy. Staining intensities of apelin were strong (**A**, *arrow*) and were colocalized with endothelial cells, as identified by CD31 (**D**). (**E–H**) Staining of apelin (**E**), CD31 (**F**), and DAPI (**G**) in the preretinal membranes of control patients without diabetic retinopathy. Only weak and insignificant staining of apelin (**E**) and CD31 (**F**) was observed. Scale bar, 50 μm.
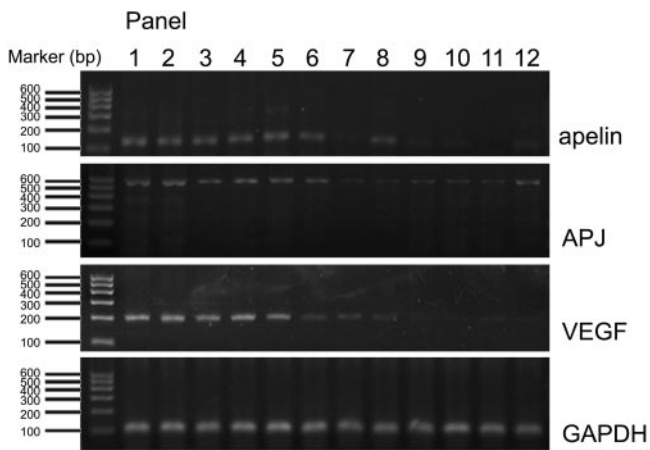
FIGURE 8. RT-PCR amplification of apelin-13, APJ receptor, VEGF, and GAPDH in the fibrovascular membranes of six patients with proliferative diabetic retinopathy (*panels 1–6*) and in the membranes of six patients with idiopathic epiretinal membrane (*panels 7–12*).

endothelial markers CD31 and APJ were observed. The data suggest that the apelin/APJ system may be involved in retinal neovascularization during the development of proliferative diabetic retinopathy, independently of the role of VEGF.

References

1. Negi A, Vernon SA. An overview of the eye in diabetes. *J R Soc Med.* 2003;96:266–272.
2. Tielsch JM, Sommer A, Witt K, et al. Blindness and visual impairment in an American urban population: the Baltimore Eye Survey. *Arch Ophthalmol.* 1990;108:286–290.
3. Simó R, Carrasco E, García-Ramírez M, Hernández C. Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. *Curr Diabetes Rev.* 2006;2:71–98.
4. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med.* 1994;331:1480–1487.
5. Tatemoto K, Hosoya M, Habata Y, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun.* 1998;251:471–476.
6. De Falco M, De Luca L, Onori N, et al. Apelin expression in normal human tissues. *In Vivo.* 2002;16:333–336.
7. Habata Y, Fujii R, Hosoya M, et al. Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum. *Biochim Biophys Acta.* 1999;1452:25–35.
8. Kawamata Y, Habata Y, Fukusumi S, et al. Molecular properties of apelin: tissue distribution and receptor binding. *Biochim Biophys Acta.* 2001;1538:162–171.
9. Shirasuna K, Shimizu T, Sayama K, et al. Expression and localization of apelin and its receptor APJ in the bovine corpus luteum during the estrous cycle and prostaglandin F2α-induced luteolysis. *Reproduction.* 2008;135:519–525.
10. De Falco M, Fedele V, Russo T, et al. Distribution of apelin, the endogenous ligand of the APJ receptor, in the lizard *Podarcis sicula. J Mol Histol.* 2004;35:521–527.
11. Tatemoto K, Takayama K, Zou MX, et al. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul Pept.* 2001;99:87–92.
12. Sorli SC, van den Berghe L, Masri B, et al. Therapeutic potential of interfering with apelin signalling. *Drug Discov Today.* 2006;11:1100–6110.
13. Simpkin JC, Yellon DM, Davidson SM, et al. Apelin-13 and apelin-36 exhibit direct cardioprotective activity against ischemia-reperfusion injury. *Basic Res Cardiol.* 2007;102:518–528.
14. Kasai A, Shintani N, Oda M, et al. Apelin is a novel angiogenic factor in retinal endothelial cells. *Biochem Biophys Res Commun.* 2004;325:395–400.
15. Cox CM, D'Agostino SL, Miller MK, et al. Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. *Dev Biol.* 2006;296:177–189.
16. Saint-Geniez M, Masri B, Malecaze F, et al. Expression of the murine msr/apj receptor and its ligand apelin is upregulated during formation of the retinal vessels. *Mech Dev.* 2002;110:183–186.
17. Kasai A, Shintani N, Kato H, et al. Retardation of retinal vascular development in apelin-deficient mice. *Arterioscler Thromb Vasc Biol.* 2008;28:1717–1722.
18. Kälin RE, Kretz MP, Meyer AM, et al. Paracrine and autocrine mechanisms of apelin signaling govern embryonic and tumor angiogenesis. *Dev Biol.* 2007;305:599–614.
19. Sorli SC, Le Gonidec S, Knibiehler B, Audigier Y. Apelin is a potent activator of tumour neoangiogenesis. *Oncogene.* 2007;26:7692–7699.
20. Masri B, van den Berghe L, Sorli C, et al. Apelin signalisation and vascular physiopathology. *J Soc Biol.* 2009;203:171–179.
21. Saint-Geniez M, Argence CB, Knibiehler B, Audigier Y. The msr/apj gene encoding the apelin receptor is an early and specific marker of the venous phenotype in the retinal vasculature. *Gene Expr Patterns.* 2003;3:467–472.
22. Masri B, Morin N, Cornu M, et al. Apelin (65–77) activates p70 S6 kinase and is mitogenic for umbilical endothelial cells. *FASEB J.* 2004;18:1909–1191.
23. Erdem G, Dogru T, Tasci I, et al. Low plasma apelin levels in newly diagnosed type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes.* 2008;116:289–292.
24. Li L, Yang G, Li Q, et al. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. *Exp Clin Endocrinol Diabetes.* 2006;114:544–548.