Ocular Blood Flow and Retinal Metabolism in Abyssinian Cats with Hereditary Retinal Degeneration

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Purpose. To investigate if retinal blood flow decreases with progression of the disease in Abyssinian cats with progressive retinal atrophy (PRA), to examine if the choroidal blood flow was affected by the disease, and to determine the uptake of glucose and formation of lactate in the outer retina.

Methods. Local blood flow in different parts of the eye was determined with radioactive microspheres, in 9 normal cats and in 10 cats at different stages of PRA. Three blood flow determinations were made in each animal, during control conditions, after IV administration of indomethacin and after subsequent administration of N^{ω}-nitro-L-arginine (L-NA). Blood samples from a choroidal vein and a femoral artery were collected to determine the retinal formation of lactate and uptake of glucose.

Results. In Abyssinian cats with PRA ($n = 10$), the retinal blood flow was significantly ($P \leq 0.01$) lower than in normal cats ($n = 9$) during control conditions, 6.4 ± 1.7 compared with 14.1 ± 1.9 g min^{-1} (100 g)$^{-1}$. The vascular resistance in the iris and ciliary body was significantly higher in the cats at a late stage of PRA, both compared with normal cats and to cats at an early stage of the disease, whereas the choroidal vascular resistance was not significantly affected. Indomethacin had no effect on ocular blood flows in normal cats, but in cats with PRA, iridal blood flow was more than doubled after indomethacin. The retinal formation of lactate was significantly ($P \leq 0.001$) lower in cats with PRA than in normal cats, 0.111 ± 0.035 ($n = 8$) compared with 0.318 ± 0.024 ($n = 8$) μmol min^{-1}. The uptake of glucose was not significantly different in cats with PRA.

Conclusions. Retinal blood flow is severely decreased in Abyssinian cats at a late stage of retinal degeneration, whereas the choroidal microcirculation is not significantly affected by the disease. At a late stage of retinal degeneration, vascular resistance in the iris is significantly increased, which at least in part could be caused by cyxlooxygenase products. (*Invest Ophthalmol Vis Sci.* 2001;42:1038–1044)

The recessively inherited progressive rod/cone degeneration, also called progressive retinal atrophy (PRA), observed in Abyssinian cats has clinical features that greatly resemble those found in patients with retinitis pigmentosa (RP). The disease is slow in onset; usually no changes are observed by ophthalmoscopic examination until the age of 1.5 to 2 years.[1] However, affected kittens can be disclosed by electroretinography at a much earlier stage.[2] The degeneration starts in the peripheral and midperipheral retina and affects initially only the rods, but with progression of the disease, the degeneration spreads centrally and affects cones as well. At an advanced stage of the disease the rods and cones are lost, and the outer plexiform layer is reduced in thickness, whereas the inner retina has an almost normal appearance.[1,3]

In Abyssinian cats with PRA, as well as in RP patients, the retinal blood vessels become attenuated with progression of the disease,[1] indicating decreased retinal blood flow. This effect could be secondary to the degeneration of the photoreceptors, due to the reduced metabolic demand of the retina. However, the retinal blood vessels supply the inner retina,[4] which seems to be almost unaffected by the retinal atrophy in the Abyssinian cat,[1,3] whereas the photoreceptors and the outer layers of the retina are supplied with oxygen and nutrients by diffusion from the choroidal microcirculation.[4] The retinal and choroidal microcirculation have very different characteristics. Retinal blood flow is low and autoregulated, with a high extraction of oxygen and nutrients, whereas choroidal blood flow is high and without autoregulation, with a low extraction of nutrients.[4] The significance of the high choroidal blood flow is not clear; is it necessary to regulate the temperature in the retina or is it due to the metabolic demands of the outer retina? In the former case, one would expect choroidal blood flow to be unaffected in Abyssinian cats with PRA.

Unlike most other tissues, the retina produces large amounts of lactate even under hyperoxic conditions. A recent study has shown that in the outer retina most of the glucose is metabolized to lactate by the way of aerobic glycolysis, whereas the inner retina uses mainly glucose oxidation for energy production.[5] In vitro, the outer segments of photoreceptors[6–8] as well as the Müller cells,[9] and the retinal pigmented epithelial cells[10,11] have been shown to produce lactate, but the major source of retinal lactate production is not known.

The aim of the present study was to investigate if retinal blood flow decreased with the progression of the disease in Abyssinian cats with PRA and to examine if choroidal blood flow was affected by the disease. Furthermore, we examined if the uptake of glucose and formation of lactate in the outer retina was affected in the Abyssinian cats with PRA.

Materials and Methods

Local blood flow in different parts of the eye was determined with radioactive microspheres in 10 cats at different stages of PRA, 9 Abyssinian and 1 Abyssinian/mixed breed. Nine normal cats, eight mixed breed (European) and one Abyssinian, of comparable ages served as control group (Table 1). All cats with PRA and most of the normal cats were bred at the Department of Medicine and Surgery, Swedish University of Agricultural Science (Uppsala, Sweden) and were kept there until the day of the experiment. Four normal cats, which were obtained from a licensed breeder, were kept at the animal department at the Biomedical Center (University of Uppsala, Uppsala,

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TABLE 1. Basic Data for Cats with PRA and Normal Cats

Cats with PRA						Normal Cats				
Exp. No.	Breed	Sex	Weight (kg)	Age (years)	Stage*	Exp. No.	Breed	Sex	Weight (kg)	Age (years)
96-005	A	F	2.2	2.7	S2	96-041	MB	F	2.7	0.5
96-006	A	M	2.6	7.8	S4	96-043	MB	F	2.6	0.5
96-007	A	M	3.1	4.0	S3	97-003	MB	F	3.3	0.8
96-039	A	M	4.2	5.8	S4	97-005	A	F	2.2	1.8
96-040	A	M	3.2	5.8	S4	97-007	MB	M	6.3	1.1
97-004	A	M	2.4	0.9	S0	97-011	MB	F	4.0	3.1
97-006	A	F	2.7	3.5	S3	97-012	MB	F	4.2	4.2
97-008	A	F	2.3	2.0	S1	97-013	MB	F	3.9	4.8
97-009	A/MB	F	3.2	8.1	S4	97-014	MB	F	3.0	3.4
97-010	A	M	2.8	0.9	S0					

A, Abyssinian; MB, mixed breed (European); F, female; M, male.
* Disease stage, as classified by Ref. 1.

Sweden) for 2 weeks before the experiments. The experiments were approved by the local ethics committee in Uppsala and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Clinical Examination and Classification into Disease Stages

Within a month before the blood flow determination, the cats with PRA were examined by ophthalmoscopy and classified into disease stages (S1–S4) as previously described in detail[1] (for a brief description see Table 2). In addition, two cats, homozygous for the recessively inherited disease and therefore known to be affected but yet not showing any clinical symptoms of PRA (S0), were included in the study (Table 1).

Anesthesia and General Surgical Procedures

Induction of anesthesia was achieved by IM injection of a mixture (1:1) of ketamine (Ketalar, 50 mg ml^{-1}; Parker-Davis, Morris Plains, NJ) and xylazine (Rompun, 20 mg ml^{-1}; Bayer AG, Leverkusen, Germany), approximately 0.3 ml (kg body wt)$^{-1}$. The induction of anesthesia was made in the research animal quarters, usually by the animal technician. The cat was then placed in a cage, wrapped in a blanket, and transported to the laboratory (Department of Physiology, University of Uppsala), where it immediately was placed on a servo-controlled heating pad. A femoral vein was cannulated with polyethylene tubing and α-chloralose, 75 mg (kg body wt)$^{-1}$, was slowly given IV. A tracheotomy was made, to insert a tracheal cannula, and the other femoral vein and both femoral arteries were cannulated with polyethylene tubings. For the injection of microspheres, the right brachial artery was cannulated by a catheter, which was advanced into the left heart ventricle by monitoring the pulse pressure curve. The cat was then placed prone, and one femoral artery was connected to a pressure transducer for continuous recording of the arterial blood pressure on a chart recorder (SE 460; ABB Goerz Instruments, Vienna, Austria). The other femoral artery was used for blood sampling during the experiment. One femoral vein was used for continuous infusion of a sodium bicarbonate solution (5%; ~10 μl kg^{-1} min^{-1}). The other femoral vein was used for infusion of drugs during the experiment. The tracheal cannula was connected to a Palmer pump for artificial ventilation. Arterial blood samples were collected during the preparation as well as during the experiment to determine the arterial pH, P_{CO_2}, and P_{O_2}. The samples were analyzed in an ABL 300 (Radiometer, Copenhagen, Denmark), and the values were adjusted to normal by changing the ventilation and/or IV administration of sodium bicarbonate.

In most experiments, a choroidal vein was cannulated for collection of venous blood for determination of blood glucose and lactate. Therefore, during the initial surgical procedures, an incision was made in the upper eyelid of the left eye, at the one o'clock position, and parts of the conjunctiva and extraocular muscles were removed to expose the intrascleral venous plexus and a choroidal vein. The wound was then closed by a clamp until the vein was to be cannulated.

TABLE 2. Classification of Abyssinian with PRA into Disease Stages Based on Ophthalmoscopy

Stage	Ophthalmoscopy
S0	No symptoms.
S1: stage of suspected disease	Subtle gray parapapillary discoloration on one or both side of the optic disc.
S2: early stage	Tapetal discoloration on both sides of the optic disc and always also present in the peripheral fundus. Peripheral blood vessels slightly attenuated.
S3: moderately advanced stage	Generalized diffuse tapetal discoloration, with dark and light gray areas. Parts of tapetal fundus hyperreflective. Prominent thinning of blood vessels, particularly in the periphery.
S4: advanced stage	Generalized hyperreflectivity in tapetal fundus. Severe degenerative changes with heavily pigmented lesions in the form of streaks or clumps in non-tapetal fundus. Blood vessels severely attenuated or not visible.

Experimental Protocols

Before the start of the experiment, heparin (KabiVitrum, Stockholm, Sweden), 500 IU/kg body wt, was given IV. Three blood flow determinations were made in each animal, during control conditions, after IV administration of indomethacin, 5 mg kg^{-1}, and after IV administration of N^{ω}-nitro-L-arginine (L-NA), 20 mg kg^{-1}, an inhibitor of nitric oxide synthase. Indomethacin was given to simplify the subsequent cannulation and collection of venous blood samples from the choroidal vein, because without indomethacin, the tip of the cannula is often clogged. Because nitric oxide seems to contribute to the normal vascular tone in both the retinal[12] and the uveal microcirculation[12–15] of the cat, we wanted to examine if the response to L-NA was different in cats with PRA.

Before each blood flow measurement, the heart rate was determined by running the recorder at a high chart speed, which made it possible to see each pulse pressure wave. After each blood flow measurement, an arterial blood sample was collected for determination of the arterial pH, P_{O_2}, and P_{CO_2}.

Table 3. Weight of Intraocular Tissues in Normal Cats and in Abyssinian Cats with PRA

	Normal (n = 12)	PRA (n = 11)
Retina	122 ± 9	104 ± 12
Choroid	93 ± 5	87 ± 4
Iris	88 ± 5	68 ± 5*
Ciliary body	264 ± 22	259 ± 29

Values are expressed as wet weight in milligrams; n = number of eyes.

* ($P \leq 0.05$) statistically significant difference from normal cats (Student's t-test; unpaired, two-tailed).

After the first blood flow determination, indomethacin was given as soon as possible and 20 minutes later, the choroidal vein was cannulated by a tapered polyethylene tubing. Venous blood was then collected by free flow into a plastic tube, and simultaneously arterial blood was sampled from a femoral artery. The arterial and venous blood samples were immediately frozen, by placing the tubes in a mixture of ethanol and dry ice. The second blood flow measurement was then made, and after this a slow infusion of L-NA was started. L-NA was given during 10 minutes, and 30 minutes later, arterial and venous blood samples for determination of glucose and lactate were collected. The third blood flow determination was then made and immediately afterward, the animal was killed by intracardiac injection of a potassium chloride solution.

In most experiments, the left eye was immediately enucleated and sectioned along ora serrata, and the anterior and posterior segments were the placed in fixative, to be used for electron microscopy or immunohistochemistry. The morphology and immunohistochemical data will be presented elsewhere. The right eye was dissected into iris, ciliary body, choroid, and retina, to determine local blood flows in the eye.

Determination of Regional Blood Flows with Radioactive Microspheres

Regional blood flows were determined using radioactive microspheres (15.5 ± 0.1 μm), according to the reference flow method, described in detail elsewhere.[16–18] Microspheres labeled with three different radionuclides, ^{141}Ce, ^{113}Sn, and ^{103}Ru (NEN, Boston, MA), were used, allowing three blood flow determinations in each animal. The microspheres, 1×10^6 to 2×10^6 per injection, were injected into the left heart ventricle over 15 to 20 seconds, and simultaneously, reference samples were collected from a femoral artery, by free flow into preweighed plastic tubes over a 1-minute period (10 seconds per tube).

After the experiment, the chest wall was opened to verify the proper location of the intracardiac catheter, and tissue samples were collected from the heart muscle and the lung, in addition to the samples from the eye. The tissues samples were placed into preweighed plastic tubes, and together with the reference blood samples were then weighed and counted in a three-channel γ-spectrometer. Regional blood flows and cardiac output (CO) were then calculated as previously described.[19] The blood flow values for the intraocular tissues are best expressed as total flow in the tissue, as it may be difficult during dissection to completely remove the vitreous from the retinal preparation, which may cause large variability in the blood flow values, if expressed as flow per gram wet tissue weight. However, while analyzing the data, we observed that the weight of the iris was significantly lower in the cats with PRA and the weight of the retina appeared to be lower as well, although not statistically significant (Table 3). Thus, blood flow values were expressed in grams per minute per 100 g tissue. Furthermore, vascular resistance was calculated as MABP divided by the flow, because the arterial blood pressure tended to be slightly higher in the cats with PRA (see Table 4).

Determination of Glucose Uptake and Lactate Formation in the Outer Retina

After the experiment, the venous and arterial blood samples were thawed, and their concentrations of glucose and lactate were determined by a dual analyzer (Model 2700; YSI, Yellow Springs, OH). Each sample was analyzed at least twice, and the mean value of these determinations was used in the calculations. Glucose uptake and lactate formation was calculated as their arterio-venous differences multiplied by the choroidal blood flow in the right eye (data obtained with the microspheres). In one experiment, the cannulation of the choroidal vein failed completely, and in two experiments, choroidal blood flow was too low after L-NA to obtain blood samples for the analysis of glucose and lactate.

Drugs

Indomethacin and L-NA were purchased from Sigma Chemical Co. (St. Louis, MO). Indomethacin was dissolved in ~5 ml PBS, to which 3 drops of 2 M NaOH was added immediately before use, and L-NA was dissolved in saline (6 mg/ml) with 2 M NaOH added (~1 drop/2 ml) to result in a pH of ~9.

Statistical Analysis

Student's t-test for unpaired data (two-tailed) was used when the cats with PRA were compared with normal cats. One-way ANOVA was used to compare the vascular resistance in normal cats (n = 9) with the vascular resistance in cats at an early (S0–S2; n = 4) and a late stage (S3–S4; n = 6) of retinal degeneration. Repeated-measures ANOVA was used when the blood flow after indomethacin and L-NA was compared with blood flow during control conditions, within the different groups. Tukey's test was used as post-ANOVA test in both cases. $P < 0.05$ was

Table 4. MABP, HR, CO, TPR, and Arterial pH, P_{CO_2}, and P_{O_2} during the Three Blood Flow Determinations, in Normal Cats and in Abyssinian Cats with PRA

	MABP (mm Hg)	HR (beats min^{-1})	CO (g min^{-1})	TPR (PRU)*	pH	P_{CO_2} (kPa)	P_{O_2} (kPa)
Normal (n = 9)							
Control	104 ± 4	119 ± 6	239 ± 22	45.7 ± 3.7	7.42 ± 0.005	5.3 ± 0.05	13.6 ± 0.31
Indomethacin	105 ± 5	137 ± 10	246 ± 23	45.1 ± 4.1	7.42 ± 0.002	5.3 ± 0.04	12.9 ± 0.33†
L-NA	123 ± 5†‡	158 ± 7†	137 ± 18†‡	102 ± 14.3†‡	7.42 ± 0.007	5.1 ± 0.13	13.1 ± 0.34
PRA (n = 10)							
Control	115 ± 6	124 ± 5	265 ± 16	45.0 ± 4.0	7.41 ± 0.005	5.4 ± 0.06	13.1 ± 0.16
Indomethacin	121 ± 9	131 ± 7	314 ± 39	42.3 ± 4.5	7.41 ± 0.005	5.4 ± 0.07	12.3 ± 0.25†
L-NA	133 ± 9†	151 ± 5†‡	200 ± 45‡	89.1 ± 14.4†‡	7.43 ± 0.007	5.2 ± 0.12	12.9 ± 0.39

* PRU, peripheral resistance units (100 mm Hg min g^{-1}).

† Statistically significant difference from control; ‡ statistically significant difference from indomethacin ($P \leq 0.05$; repeated-measures ANOVA, with Tukey's test as post-ANOVA test).

TABLE 5. Ocular Blood Flows under Control Conditions, in Normal Cats and in Abyssinian Cats with PRA

	Normal (n = 9)	All PRA (n = 10)	S0–S2 (n = 4)*	S3–S4 (n = 6)†
Retina	14.1 ± 1.9	6.4 ± 1.7‡	9.6 ± 3.4	4.3 ± 1.1§
Choroid	746 ± 104	832 ± 116	1142 ± 169	626 ± 86‖
Iris	23.9 ± 3.8	21.4 ± 7.3	41.2 ± 12.7	8.2 ± 2.8‖
Ciliary body	74.8 ± 5.5	75.3 ± 9.2	103 ± 9.6§	57.0 ± 7.2‖

Values are expressed in [g min^{-1} (100 g tissue)$^{-1}$].

* Cats at an early stage of PRA.

† Cats at an advanced stage of PRA.

‡ ($P \leq 0.01$) statistically significant difference from normal cats (Student's t-test; unpaired, two-tailed).

§ Statistically significant difference from normal cats; ‖ statistically significant difference from cats at an early stage (S0–S2) of retinal degeneration ($P \leq 0.05$; one-way ANOVA, with Tukey's test as post-ANOVA test).

considered significant. All values are given as the mean ± SEM. Linear regression analysis was used to determine whether blood flow, formation of lactate, or uptake of glucose was significantly correlated with age (progression of disease).

RESULTS

MABP, HR, CO, TPR, and Arterial pH, P_{CO_2}, and P_{O_2}

There was no significant difference in mean arterial blood pressure (MABP), heart rate (HR), cardiac output (CO), total peripheral resistance (TPR), or arterial pH, P_{CO_2} and P_{O_2} between the normal cats and the cats with PRA during control conditions (Table 4). Indomethacin had no effect on these parameters, except the arterial P_{O_2}, which was slightly reduced in both groups (Table 4). In Abyssinian cats with PRA as well as in normal cats, L-NA caused the expected increase in MABP,[13,15] concomitant with a decrease in cardiac output and an increase in total peripheral resistance and an increase in heart rate (Table 4).

Ocular Blood Flow during Control Conditions

During control conditions, retinal blood flow was significantly lower in Abyssinian cats with PRA than in normal cats, whereas local blood flow in the choroid, iris, and ciliary body was not significantly different (Table 5). There was no significant correlation between retinal blood flow and age (progression of disease), either in cats with PRA (Fig. 1A) or in normal cats (data not shown). However, local blood flows in the different parts of the uvea were negatively correlated with age in Abyssinian cats with PRA (Figs. 1B through 1D) but not in normal cats (data not shown).

The negative correlation between uveal blood flow and age (Fig. 1), the significantly lower tissue weight of the iris (Table 3), and the large variability in iridal blood flow (Table 5) in the cats with PRA prompted us to divide the cats with PRA into two groups: animals at an early stage (S0–S2) of retinal degeneration and animals at a late stage (S3–S4). This showed that uveal blood flows were significantly higher in the cats at an early stage of retinal degeneration than in cats at a late stage, but only blood flow in the ciliary body was significantly higher than in normal cats (Table 5). Because MABP was slightly higher in the animals with PRA, especially in the younger ones (early stage), we also calculated the vascular resistances. This showed that the vascular resistance in the iris was markedly higher in cats at an advanced stage of PRA, when compared both with cats at an early stage of the disease and with normal cats (Fig. 2C). The vascular resistance in the ciliary body was also significantly increased in cats at a late stage of PRA (Fig. 2D), whereas choroidal vascular resistance was not significantly different between the three groups (Fig. 2B). The vascular resistance in the retina was significantly higher in cats at a late stage of PRA than in normal cats (Fig. 2A).

Effects of Indomethacin and L-NA on Ocular Blood Flows

In normal cats, ocular blood flows were not affected by indomethacin, whereas L-NA caused a significant reduction of blood flow in the choroid, iris, and ciliary body (Fig. 3A).

In cats with PRA, local blood flow in the iris was more than doubled after indomethacin, both at an early (Fig. 3B) and a late (Fig. 3C) stage of retinal degeneration, whereas local blood flows in the choroid, ciliary body, and retina were unaffected. Subsequent administration of L-NA caused a significant reduction of uveal blood flows in both groups (Figs. 3B, 3C).

Formation of Lactate and Uptake of Glucose in the Outer Retina

In cats with PRA, the retinal formation of lactate was 0.111 ± 0.035 μmol min^{-1} (n = 8) compared with 0.318 ± 0.024 μmol min^{-1} (n = 8) in normal cats ($P \leq 0.001$; unpaired t-test) after indomethacin only. This difference persisted also after the subsequent administration of L-NA (Fig. 4A). The formation of lactate was negatively correlated with age in cats with PRA (Fig. 5A), but not in normal cats (data not shown).

The retinal uptake of glucose appeared to be slightly higher in cats with PRA than in normal cats, but the difference was not statistically significant (Fig. 4B). There was no correlation between glucose uptake and age either in cats with PRA (Fig. 5B)

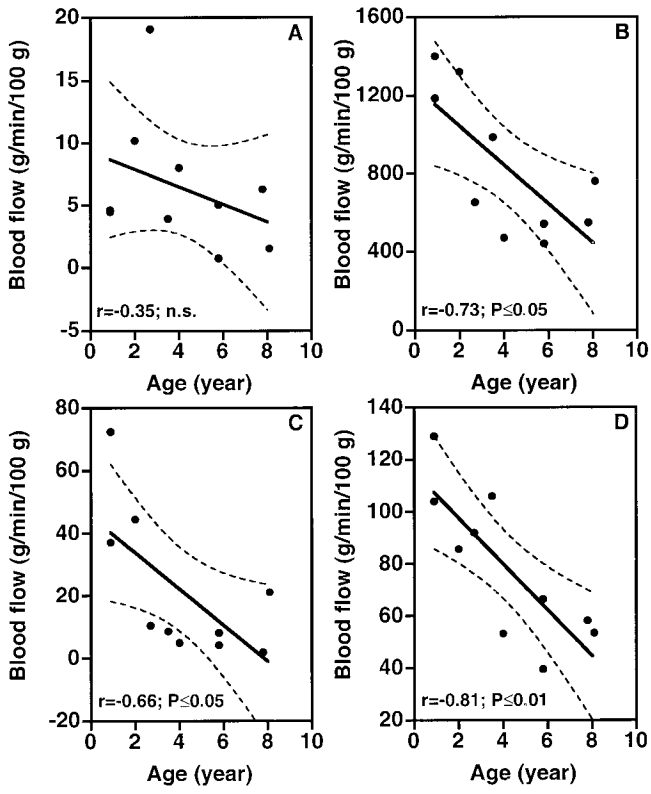

FIGURE 1. Blood flow in the retina (**A**), choroid (**B**), iris (**C**), and ciliary body (**D**) as a function of age in cats with progressive retinal atrophy (PRA). *Dotted lines,* 95% confidence intervals.

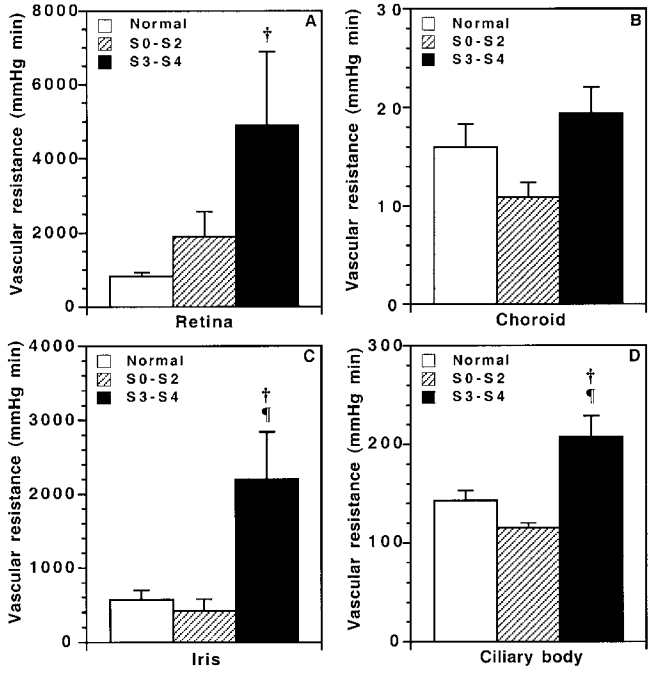

FIGURE 2. Vascular resistance in the retina (**A**), choroid (**B**), iris (**C**), and ciliary body (**D**), during control condition, in normal cats ($n = 9$) and in cats at an early (S0–S2, $n = 4$) and late (S3–S4; $n = 6$) stage of retinal degeneration. Values are means; error bars, SEM. †Significantly different from normal cats; ¶significantly different from cats at an early stage (S0–S2) of retinal degeneration ($P \leq 0.05$; one-way ANOVA, with Tukey's test as post-ANOVA test).

or in normal cats (data not shown). The glucose uptake was significantly reduced after the administration of L-NA, in cats with PRA as well as in normal cats (Fig. 4B).

DISCUSSION

The present study shows that retinal blood flow is severely decreased in Abyssinian cats with retinal degeneration, whereas the choroidal microcirculation is not significantly affected. Furthermore, iridal blood flow is markedly decreased in cats with PRA, at advanced stages of the disease.

The retinal arterio-venous passage time is increased in RP patients,[20] and measurement of blood flow velocities, with laser Doppler or color Doppler, have given lower values in RP patients than in normal subjects,[21–23] indicating that retinal blood flow is decreased in patients with RP. This suggestion is further supported by the present experiments, with actual measurement of retinal blood flow, in an animal model of RP. It has also been reported that pulsatile ocular blood flow is significantly decreased in RP patients, which was suggested to be due to decreased choroidal blood flow.[24] In the present experiments, the choroidal blood flow was negatively correlated with age in the cats with PRA, but the choroidal vascular resistance was not significantly different between cats at an early and a late stage of retinal degeneration, nor was it significantly different from that in normal cats. This indicates that the negative correlation between choroidal blood flow and age in the cats with PRA was mainly due the higher blood pressure and hence higher choroidal blood flow in the younger cats. Thus, we found no evidence of a disturbed choroidal microcirculation in the Abyssinian cats with PRA. Whether this is a true difference between RP patients and the cats with PRA or is due to differences in methodology remains to be established. Interestingly, vascular resistance in the anterior uvea was significantly increased, especially in the iris, in cats at a late stage of retinal degeneration, indicating that the vessels of the anterior uvea are affected by the disease as well as the retinal vessels.

One may suspect that the attenuation of the retinal blood vessels[1] and the diminished retinal blood flow are secondary effects due to the degenerative process in the outer retina, because the degeneration most likely will cause hyperoxia and hence vasoconstriction in the inner retina. This has recently been confirmed by determination of retinal oxygen tension profiles in Abyssinian cats with PRA.[25] At least two factors would contribute to the development of hyperoxia in the inner retina. First, as the photoreceptors degenerate, the oxygen

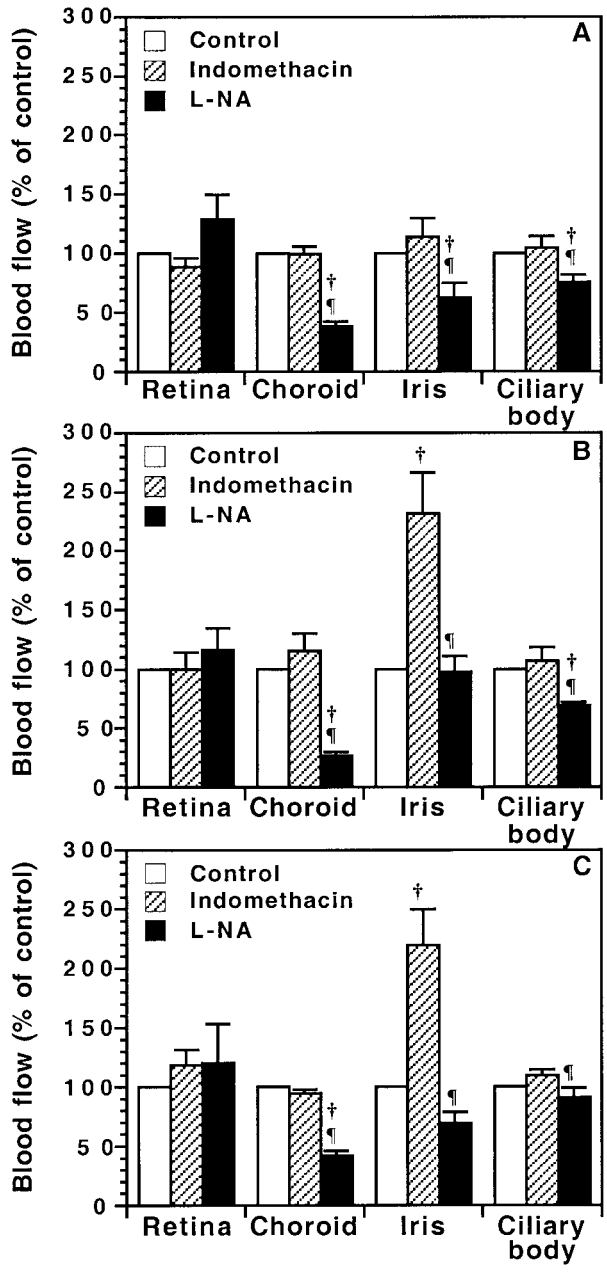

FIGURE 3. Effects of indomethacin and L-NA on ocular blood flows, in normal cats (**A**) and in cats at an early (**B**) and late (**C**) stage of retinal degeneration. Values are means; error bars, SEM ($n = 9$, 4, and 6, respectively). †Significantly different from control conditions; ¶significantly different from indomethacin ($P \leq 0.05$; repeated-measures ANOVA, with Tukey's test as post-ANOVA test).

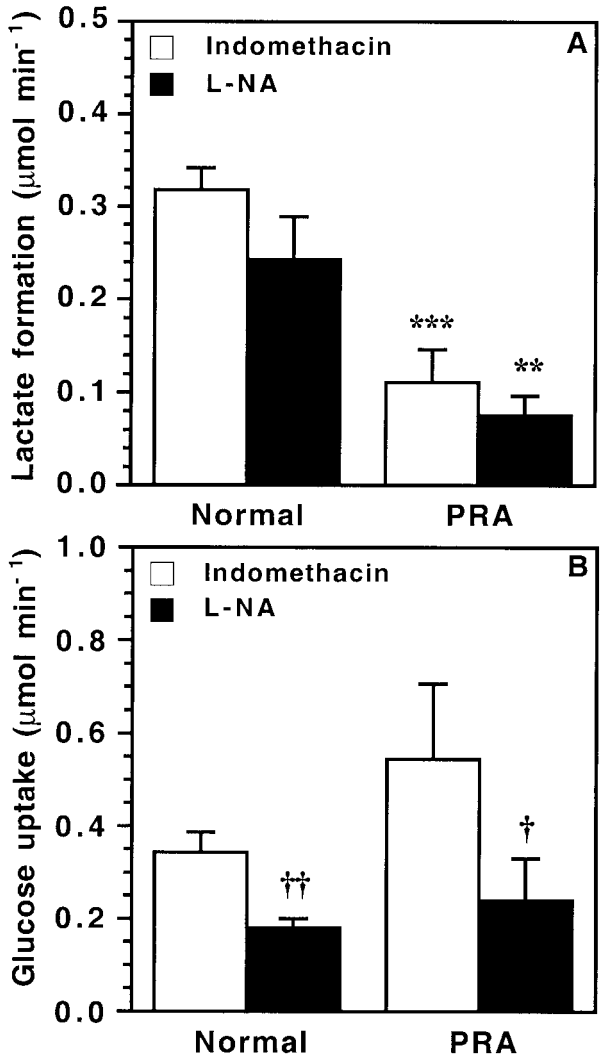

FIGURE 4. Formation of lactate (**A**) and uptake of glucose (**B**) in the outer retina, in normal cats ($n = 8$) and in Abyssinian cats with PRA ($n = 8$), after administration of indomethacin (□) and after subsequent administration of L-NA (■). Values are means; error bars, SEM.$^{***}P \leq 0.001$ and $^{**}P \leq 0.01$ denote significantly different from normal cats (Student's *t*-test; unpaired, two-tailed); $\dagger\dagger P \leq 0.01$ and $\dagger P \leq 0.05$ denote significantly different from indomethacin (Student's *t*-test; paired, two-tailed).

demand in the outer retina will diminish, which will increase the gradient for diffusion of oxygen from the outer to the inner retina. Second, with progression of the disease, the outer nuclear layer decreases in thickness and the outer plexiform layer becomes thinner as well.[1,3] Thus, the total thickness of the outer retina decreases, thereby decreasing the diffusion distance from the choroidal microcirculation to the inner retina. However, it is worth noting that the two cats that did not yet show any clinical signs of the disease (S0) had surprisingly low blood flow in the retina (4.5 and 4.6 g min^{-1} $(100\ g)^{-1}$, respectively), whereas the two other cats (stages S1 and S2) in the early group had retinal blood flow values comparable to normal cats, indicating that retinal blood flow may be differently affected at different stages of the disease.

The maintained high blood flow through the choroid in the cats with PRA indicate that the choroidal blood flow is not regulated by the metabolic demand of the outer retina, suggesting that the high choroidal blood flow may have other functions, such as controlling the retinal temperature. However, only a few percent of the oxygen is extracted as the blood passes through the choroicapillaries,[26] which ought to create a high oxygen tension in the healthy choroid. A decreased oxygen extraction, which could be expected in the cats with PRA, may therefore have a little or no effect on the oxygen tension in the choroid and hence on choroidal blood flow.

In the cats at an advanced stage of PRA, the iridal blood flow was significantly increased by indomethacin, indicating that the high vascular resistance and hence low iridal blood flow during control conditions were at least in part caused by formation of cyclooxygenase products. Most prostaglandins act as vasodilators in the eye,[27] whereas the thromboxane analogue U-46619 causes vasoconstriction.[28] Normally, there is a balance between the vasoconstrictor thromboxane A_2 (TXA_2) and the vasodilator prostacycline (PGI_2). An increased ratio of TXA_2/PGI_2 has previously been suggested to be involved in the development of diabetic retinopathy[29,30] as well as retinopathy of prematurity.[31,32] Although indomethacin had no effect on retinal blood flow in the present experiments, the increase in iridal blood flow indicates that an imbalance between TXA_2 and PGI_2 also may play a role in the progressive retinal atrophy in the Abyssinian cat. Interestingly, indomethacin increased iridal blood flow in Abyssinian cats at an early stage of the disease, although blood flow in the iris was normal or higher than normal in these animals under control conditions. This indicates that there may be an imbalance in the ratio of TXA_2/PGI_2 and hence disturbances of the iridal microcirculation even before there are any clinical signs of the disease.

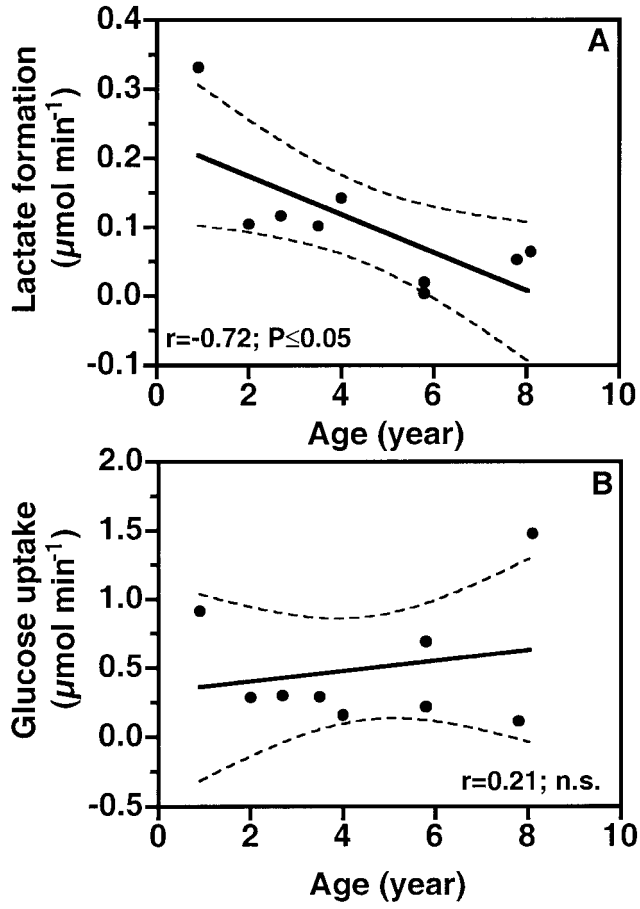

FIGURE 5. Formation of lactate (**A**) and uptake of glucose (**B**) in the outer retina as a function of age in cats with progressive retinal atrophy (PRA). *Dotted lines,* 95% confidence intervals.

In the Abyssinian cats with PRA, the formation of lactate in the outer retina was approximately one third that observed in the normal cats. The decreased formation of lactate was apparent already at early stages of the disease; only one cat, which carried the disease but did not show any clinical symptoms of it (S0), had a lactate production comparable to normal cats (see Fig. 5). In addition to the outer segments of the photoreceptors, the retinal pigmented epithelial cells and the Müller cells contribute to the formation of lactate in the outer retina. Furthermore, the Müller cells have been shown to produce lactate that is taken up and used by the photoreceptors.[9] The results of the present experiments, indicate that this metabolic pathway is either downregulated as the degeneration of the photoreceptors progresses or it is normally not a major metabolic pathway, because then one would have expected the arteriovenous difference for lactate to be increased in the Abyssinian cats with PRA. Considering the reduced metabolic demands in the outer retina in the cats with PRA, it may seem surprising that the decreased formation of lactate was not accompanied by a decreased uptake of glucose. Actually, the uptake of glucose appeared to increase (not statistically significant) in the cats with PRA, which could be due to increased diffusion of glucose from the outer to the inner retina, because the supply of glucose to the inner retina will decrease with the diminished retinal blood flow.

In conclusion, the present investigation shows that retinal blood flow is severely decreased in Abyssinian cats at a late stage of retinal degeneration, whereas the choroidal microcirculation is not significantly affected by the disease. The vascular resistance in the iris was significantly higher in cats at a late stage of PRA, indicating that the disease affects the iridal blood vessels as well. Indomethacin increased iridal blood flow in cats with PRA, but not in normal cats, which suggests that the high vascular resistance in the iris at least in part is caused by cyxlooxygenase products. Further studies are needed to determine whether the vascular changes *merely* are secondary to the retinal degeneration or if they contribute to the progression of the disease as an aggravating factor.

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