



# The impact of macular edema on microvascular and metabolic alterations in retinitis pigmentosa

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## Abstract

**Purpose** The primary aim of our study was to evaluate retinal microvascular anomalies recorded with optical coherence tomography angiography (OCTA) and the retinal metabolic function measured with retinal oximetry (RO) in patients with retinitis pigmentosa (RP). The secondary aim of the study was to link the presence of macular edema to microvascular and metabolic parameters in RP. **Methods** OCTA and RO were performed on 94 eyes: 64 eyes diagnosed with RP with (ME-RP) and without (no-ME-RP) macular edema were compared with 30 control eyes. Study end points were as follows: mean superficial (FAZ-S) and deep foveal avascular zone (FAZ-D) determined by OCTA. In addition, we evaluated the mean arterial (A-SO<sub>2</sub>; %), venular (V-SO<sub>2</sub>; %) oxygen saturation, their difference (A-V SO<sub>2</sub>; %), as well as the corresponding mean diameter of the retinal arterioles (D-A; μm) and venules (D-V; μm). **Results** RP patients differed from controls by enlarged FAZ-S and FAZ-D ( $p \leq 0.001$ ), attenuated retinal vessels ( $p \leq 0.001$ ), and increased retinal vessel oxygen saturation ( $p \leq 0.010$ ). Subgroup analyses within RP patients revealed more pronounced alterations of microvascular parameters and metabolic function in the presence of macular edema. In the no-ME-RP subgroup, significant interactions were present between FAZ-S, A-SO<sub>2</sub>, and V-SO<sub>2</sub>, whereas in the ME-RP subgroup, we found significant correlations between FAZ-D and D-A.

**Conclusion** A combined microvascular structure–metabolic function approach enhances our understanding of inherited retinal diseases. The presence of macular edema in RP seems to be a result of more altered microvascular–metabolic function. Macular edema should thus be taken into consideration when evaluating microvascular and/or metabolic changes in RP.

## Key Messages

What was known before?

- Patients with retinitis pigmentosa show significant changes in the oxygen metabolic function; these biomarkers can be used in daily clinical practice.

What this study adds:

- RP patients with and without macular edema suffer from metabolic and microvascular alterations, however with different severity.
- A combined microvascular structure-metabolic function approach improves our understanding of inherited retinal diseases.

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**Keywords** Optical coherence tomography angiography · Inherited retinal diseases · Retinitis pigmentosa · Foveal avascular zone · Retinal oximetry · Retinal vessel diameter · Metabolic function · Macular edema

## Introduction

Retinitis pigmentosa (RP) represents a heterogeneous group of inherited retinal diseases marked by progressive photoreceptor cell degeneration [1–3]. Apart from pigmentary changes with a perivascular “bone spicule” configuration and the pale, prominent appearing optic disc, a universal clinical feature in eyes with RP is the attenuation of retinal vessels [3–8]. It is thought to reflect decreased metabolic demand of the degenerating retina [6, 7, 9–11].

Invasive measurements with microelectrodes in animals under normal conditions confirmed regional variations in the intraretinal oxygen distribution. Three oxygen consumption zones were identified and corresponded to the superficial and deep retinal capillary and choroidal beds [12]. According to published experiments on the oxygen metabolism of the retina, the metabolic supply of rod photoreceptors is mainly supported by choroidal vessels and to lesser extent by deep retinal capillaries [12–16]. Under dark adapted conditions, the metabolic activity of the highly energy-demanding rod photoreceptors is supported further by oxygen diffusion from the superficial retinal capillary vessels [16].

In vivo research in animal models for outer retinal degeneration revealed a reduction of the outer retinal oxygen consumption. Even if degenerated photoreceptors use less oxygen, the oxygen metabolism of the inner retina remained unaffected. These changes of the oxygen environment are thought to play a role in the progression of the degenerative process and neurovascular remodelling [17–19]. Furthermore, histopathological studies reported about sclerosis and atrophy of the retinal vasculature in advanced cases [20, 21]. In addition, in regions with destructed retinal pigment epithelium (RPE), a lack of choroidal capillaries was detected [21–24].

Optical coherence tomography angiography (OCTA) is a novel, contact-free and non-invasive imaging technique that allows for a depth-resolved visualization of both the retinal and the choroidal microvasculatures. It is based on motion contrast via mapping erythrocyte movement over time by comparing sequential OCT B-scans in a given cross section [25]. Several studies conducted with OCTA have reported about a reduction of retinal and choroidal blood flow, choroidal vascular defects, and an increase in the size of the foveal avascular zone (FAZ) in eyes of patients suffering RP [26–29]. However, some other studies reported contradictory results [30, 31].

Retinal vessel oximetry (RO) allows for contact-free in vivo measurements of the retinal oxygen saturation and thus the exploration of metabolic alterations [13, 32–35]. Recent RO studies on patients suffering from RP showed a significant increment of oxygen saturation in the retinal arterioles and venules, presumably the consequence of reduced oxygen consumption [6, 7, 36–41]. Furthermore, the oxygen saturation alterations correlated well with structural and functional changes [7, 8, 37, 38]. Thus, as the highest amount of oxygen is used by retinal photoreceptors, a reduction in retinal oxygen demand with secondary increase in the retinal vessel saturation values following cellular apoptosis has been hypothesized to explain the observed vascular oxygen alterations in RP patients [6, 7, 36–41]. Notably, the application of transcorneal electrical stimulation in patients with RP induced a measurable increase in oxygen consumption, the latter supposed to be the result of an improvement in oxygen demand of the preserved photoreceptors [42].

Given the known microvascular and metabolic alterations in RP, it is conceivable that also the superficial and deep retinal vascular structures may exhibit metabolic dysfunction. Thus, the main goal of this prospective observational study was to investigate the superficial and deep foveal avascular zones and RO parameters of eyes affected by RP and to compare these measurements to healthy control eyes. Furthermore, the presence of macular edema in RP has been found to reflect a stronger impairment of metabolic function and indicated a different relationship between altered oxygen saturation parameters against functional and structural parameters [39, 40]. Therefore, our secondary objective of the study was to link the presence of macular edema to retinal microvascular anomalies and metabolic functional parameters.

## Methods

Prospective, observational study of 94 eyes was performed: 64 eyes diagnosed with RP and 30 healthy control eyes were examined from January 2018 to September 2019 in the University Eye Hospital Basel, Switzerland. This study was approved by the local IRB (Ethics Commission of Central and Northern Switzerland, EKNZ Basel Switzerland; trial number EKNZ BASEC 2017-00937).

## Subjects

All patients underwent all required standard evaluations by experienced fellowship-trained specialists for inherited retinal diseases (M.G.T and H.P.N.S.) and presented with a typical clinical picture of retinitis pigmentosa. All participants underwent a detailed ophthalmic examination, which included among others refraction, best corrected visual acuity (BCVA) obtained under standardized Early Treatment Diabetic Retinopathy Study (ETDRS) conditions, intraocular pressure examination by applanation, biomicroscopy, and funduscopy. The clinical phenotype of RP patients was characterized following a detailed ophthalmic examination as described above and additional OCT of the macula, fundus autofluorescence, visual field testing with semi-automated kinetic perimetry (V4e, III4e, I4e, III3e isopters tested with Octopus 900®, Haag-Streit AG Bern, Switzerland), and full-field electroretinography (ffERG, Diagnosys LLC Espion system; ISCEV standard [43]). The RP group was divided in two subgroups: with (ME-RP) and without appearance of macular edema (no-ME-RP), defined by the presence of intraretinal spaces situated within and around the foveal center. The inclusion criteria for patients and controls included the following: stable fixation and refractive error (spherical equivalent) of < 6 diopters. Patients and controls who had previous ocular surgery or ocular and systemic pathology (as for instance, diabetes mellitus or neurological disease) that may influence the OCTA and RO measurements were not included in this study. Additional exclusion criteria were images with inadequate quality or an expressed unwillingness to participate in the study. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional/national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

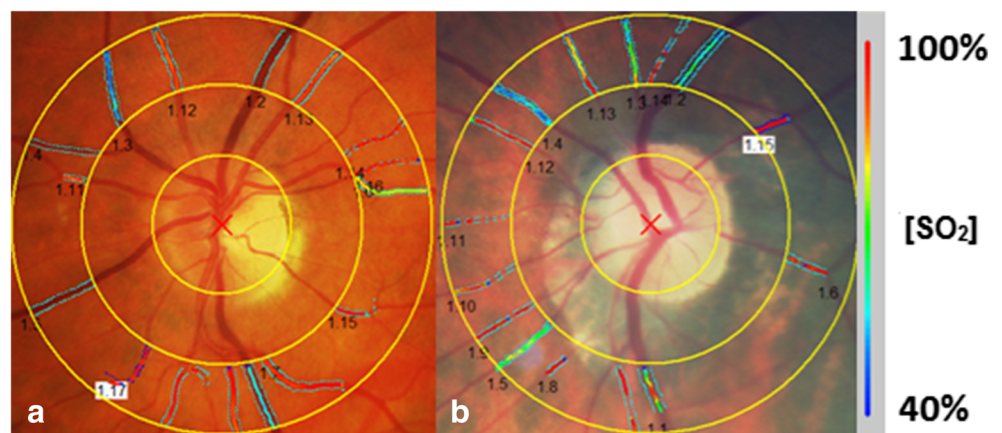
Prior to OCTA measurements, pupils were dilated (Tropiphen 10 ml eye drops prepared in our institutional

pharmacy as a combination of tropicamide 0.5% and phenylephrine 1%).

## Retinal vessel oximetry acquisition

RO measurements were performed with the spectrophotometric oximetry unit for retinal vessel oximetry (IMEDOS Systems UG, Jena, Germany; Fundus Camera FF450, Carl Zeiss Meditec AG, Jena, Germany) (Fig. 1). Fundus images were recorded using a camera system, DCC Digital Camera KY-F75 (JVC Inc., Yokohama, Japan), coupled to the Zeiss fundus camera from a camera angle of 50°. The software operating the system (VISUALIS; IMEDOS Systems UG) differentiates simultaneously between oxygenated and deoxygenated hemoglobin based on the differential light imaging characteristics at different wavelengths, measuring the oxygen saturation level in the examined retinal vessel. In summary, RO was performed at two different wavelengths: at the green channel ( $548 \pm 10$  nm) to record the oxygen insensitive image and at the red channel ( $610 \pm 10$  nm) to record the oxygen sensitive image [33, 44]. An optic disc-centered image protocol was used where two concentric rings were created in the peripapillary area: one with a radius of 1.0 optic disc diameters and the other with a radius of 1.5 optic disc diameters. The region between these two circles defined the area of interest where all measurements were obtained. Four test–retest fundus images for each eye were performed [8, 36, 37, 45]. One image with optimal illumination, red channel illumination < 160 steps of the scale, and green channel illumination > 60 steps of the scale was further selected for analysis, as described in details in one of our previous publications [45]. For oximetry analyses, all main arterioles and venules were selected manually within the area of interest. The software (Vesselmap) calculates then the optical density ratio (ODR) of the two images and, thus, the mean oxygen saturation of the evaluated retinal vessel. The global mean oxygen saturation in

**Fig. 1** An example for retinal oximetry (RO) measurements in left eyes of (a) a healthy subject and (b) a patient suffering from retinitis pigmentosa (RP). RO parameters were calculated in all main first and second branch retinal arterioles and venules within 1.0–1.5 optic disc diameter distances from the optic disc margin



retinal arterioles (A-SO<sub>2</sub>) and venules (V-SO<sub>2</sub>) was measured, and their difference, the A-V SO<sub>2</sub>, was calculated. In addition, we determined the diameters of the retinal arterioles (D-A) and venules (D-V).

### Optical coherence tomography angiography acquisition

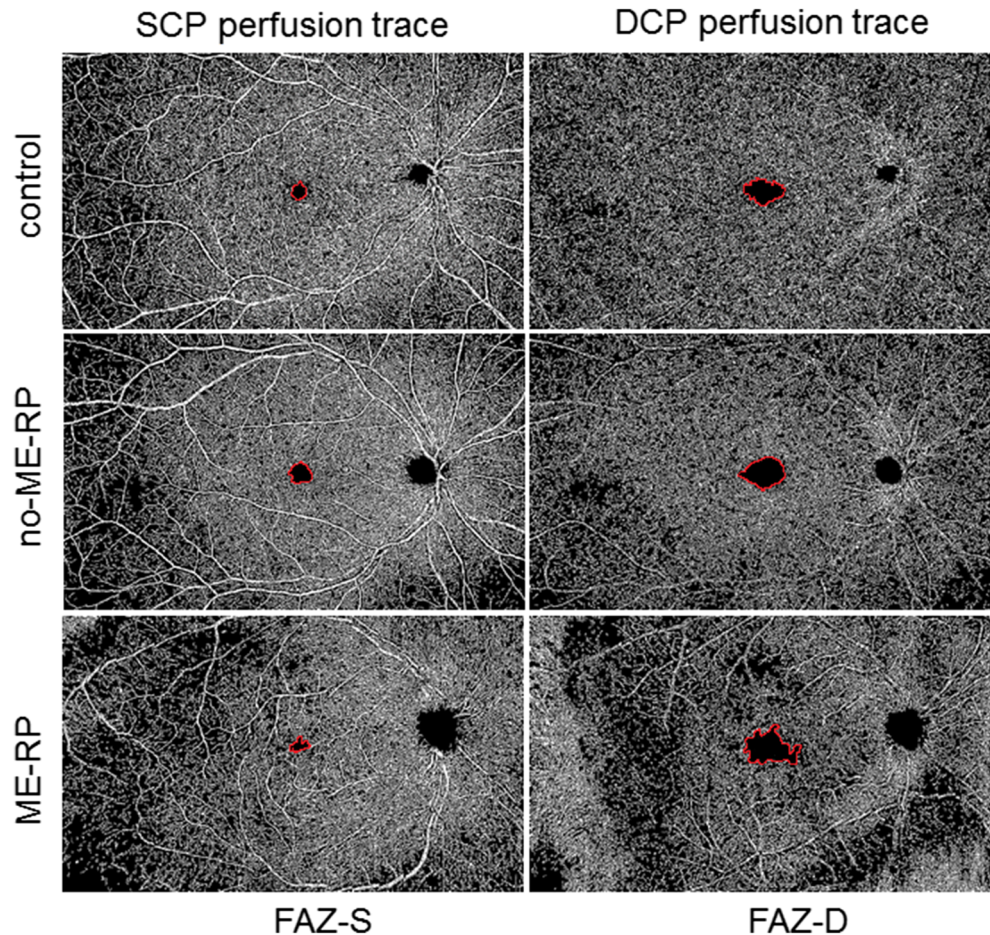
We performed 9 × 15 mm OCTA scans centered around the fovea for an evaluation of both, the superficial (FAZ-S), and the deep retinal foveal avascular zone (FAZ-D) with a PLEX Elite 9000 OCTA device (Carl Zeiss Meditec AG, Jena). The superficial retinal foveal avascular zone was defined as the vessel-free zone within the superficial vascular plexus localized between the inner limiting membrane (ILM) and the inner plexiform layer (IPL) of the retina. The retinal foveal avascular zone was defined as the vessel-free zone within the deep vascular plexus localized between IPL and the outer plexiform layer (OPL) of the retina. The measurement of the avascular area in square millimeters was performed via the software's ruler tool of ImageJ (1.52a, Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA). All scans were recorded masked by one experienced operator (M.D.V.W.). The FAZ

measurements were performed independently by two experienced retina specialists (M.D.V.W. and M.G.T.). Cases with non-agreement were discussed thoroughly and brought into alignment. Within these measurements we aimed to find both similarities and differences between diseased and healthy eyes. Furthermore, central retinal thickness (CRT) was measured automated in micrometers from the ILM to the RPE by using the Zeiss ETDRS retina thickness algorithm (version 0.1) within the central sector of a standardized ETDRS chart centered over the fovea with a diameter of 1 mm. In addition, the maximal retinal thickness was measured manually in micrometers from the ILM to the RPE in the ME-RP subgroup with the software's ruler tool (Fig. 2).

In order to avoid the effect of continuous light exposure on retinal vessel diameter and oxygen saturation, the RO was performed before the OCTA measurement.

Study end points were as follows: the mean superficial (FAZ-S) and deep retinal foveal avascular zone (FAZ-D) determined by optical coherence tomography angiography (OCTA). In addition, we evaluated the mean arterial (A-SO<sub>2</sub>; %) and venular (V-SO<sub>2</sub>; %) oxygen saturation, their difference (A-V SO<sub>2</sub>; %), as well as the mean diameter of the retinal arterioles (D-A; μm) and venules (D-V; μm).

**Fig. 2** Image examples of the superficial and deep perfusion trace obtained with 9 × 15 mm OCTA scans centered around the fovea showing the superficial (FAZ-S, left side) and deep retinal foveal avascular zones (FAZ-D, right side) in right eyes of a healthy control subject and two patients suffering from retinitis pigmentosa both in absence (no-ME-RP) or presence (ME-RP) of cystoid macular edema. The red marker represents the measurement of the avascular area in mm<sup>2</sup>



## Statistical analysis

Retinitis pigmentosa may be presented asymmetrically or even unilaterally [46, 47]. Therefore, for statistical evaluation, ANOVA-based linear mixed effects models were performed with SPSS® (IBM SPSS Statistics®, version 22.0.0.0) which allows taking the dependency of the left and right eye in the same subject into account. The model is thus suitable for repeated measurements also when considering both eyes in the analysis.  $P < 0.05$  was defined as statistically significant. For statistical evaluation, normal distribution of all parameters was ensured with histograms and Shapiro–Wilk test. In order to compare subgroups against one another, ANOVA with all pairwise comparisons was performed; all presented  $p$  values were Bonferroni adjusted. To predict the effect of the underlying disease on OCTA and RO measurements, the eye and the group were treated as a fixed factor and the subject as a random factor. In order to exclude an age- or refraction-related bias, we took into account the spherical equivalent and the age as covariates. In order to predict the interaction effect of structural OCTA parameters on metabolic functional RO measurements, linear mixed effects models were applied. In this context, a significant interaction indicates a different relationship of structural OCTA parameters with the RO variables between subgroups. Results are presented as arithmetic mean and standard deviation ( $\pm$  SD) over all examined subgroups, each with their corresponding  $p$  values.

## Results

Altogether, 94 eyes were enrolled in the study: 64 eyes of 32 patients diagnosed with RP (15 ♀ 17 ♂;  $49.7 \pm 15.4$  years, range 20–81 years) and 30 age-matched control eyes from 15 healthy participants (8 ♀ 7 ♂;  $43.8 \pm 16.6$  years, range 21–71 years). We divided the RP group into two subgroups: with (ME-RP) and without appearance of macular edema (no-ME-RP), defined by the presence of intraretinal spaces situated within the foveal center. In the no-ME-RP subgroup, 29 patients participated with 53 eyes (27 right and 26 left) without macular edema, and in the ME-RP subgroup, 8 patients participated with 11 eyes (5 right and 6 left) with macular edema. Five patients were part of both subgroups, each having one eye with ME (2 right and 3 left eyes). Depending on the presence of macular edema, mean CRT was reduced in the no-ME-RP subgroup ( $220.00 \pm 37.80$   $\mu\text{m}$ ), while it was increased in the ME-RP subgroup ( $312.55 \pm 39.54$   $\mu\text{m}$ ) when compared with controls ( $247.08 \pm 17.16$   $\mu\text{m}$ ,  $p < 0.001$ ; Table 1). In the ME-RP subgroup, the average maximal retinal thickness was  $394.55 \pm 70.54$   $\mu\text{m}$ . All demographic characteristics of our controls and RP patients are summarized in Table 1.

## Comparison of FAZ-S and FAZ-D from OCTA measurements in RP against controls and within RP subgroups with and without macular edema

In general, compared with controls, eyes suffering from RP showed significantly enlarged FAZ-S ( $p = 0.001$ ) and FAZ-D ( $p < 0.001$ ; Table 2). However, evaluated in subgroups, only the ME-RP differed from controls by both parameters: FAZ-S and FAZ-D ( $p < 0.001$ ). When evaluated between RP subgroups, ME-RP showed significantly wider FAZ-S and FAZ-D than no-ME-RP ( $p < 0.001$ ; Table 2).

## Comparison of RO parameters in RP against controls and within RP subgroups with and without macular edema

Compared with controls, eyes suffering from RP showed significant differences, when the A-SO<sub>2</sub> and V-SO<sub>2</sub> parameters were taken into account: Both in the presence (ME-RP) and in the absence (no-ME-RP) of cystoid macular edema, the A-SO<sub>2</sub> and V-SO<sub>2</sub> in RP patients were significantly increased ( $p < 0.03$ ). Even showing slightly higher V-SO<sub>2</sub> values in the ME-RP, neither A-SO<sub>2</sub> nor V-SO<sub>2</sub> reached statistically significant difference between the ME-RP and no-ME-RP subgroups ( $p > 0.17$ ; Table 2).

## Comparison of retinal vessel diameters in RP against controls and within RP subgroups with and without macular edema

In comparison with controls, eyes suffering from RP showed significantly attenuated retinal vessels, when the D-A and D-V were taken into account: Both in the presence (ME-RP) and in the absence (no-ME-RP) of cystoid macular edema, the D-A and D-V were significantly thinner ( $p < 0.001$ ). Again, even showing slightly more attenuated D-A in the ME-RP, there was no statistically significant difference for neither D-A nor D-V between the RP subgroups ( $p > 0.35$ ; Table 2).

## Interactions between retinal vessel structure (OCTA parameters) and metabolic function (RO parameters) in retinitis pigmentosa patients with and without macular edema

RP eyes without macular edema (no-ME-RP) showed significant structure–function correlations between the FAZ-D and the arterial and venular oxygen saturations (A-SO<sub>2</sub> and V-SO<sub>2</sub>). This means that an enlarged FAZ-D correlated positively not only with increased A-SO<sub>2</sub> ( $p = 0.004$ ; with the slope of  $48.951$  [CI  $99.12$ – $102.52$ ]) but also with increased V-SO<sub>2</sub> ( $p = 0.012$ ; with the slope of  $48.954$  [CI  $59.234$ – $65.961$ ]). In RP, eyes with macular edema (ME-RP) interactions were significant between the FAZ-D and the diameter of the retinal

**Table 1** The demographic characteristics including age, gender, spherical equivalent (SE), best corrected visual acuity (BCVA) in logMAR units, the oximetry (A-SO<sub>2</sub>, V-SO<sub>2</sub> and A-V SO<sub>2</sub>), and retinal vessel diameter (D-A and D-V) results acquired with RO and the superficial (FAZ-S) and deep foveal avascular zone (FAZ-D) results as measured with OCTA in eyes of controls and patients suffering from retinitis

Demographic characteristics	Controls	no-ME-RP subgroup	ME-RP subgroup	<i>p</i> values (univariate ANOVA or $\chi^2$ test†)
Number of eyes (patients)	30 (15)	53 (29)	11 (8)	0.797†
Age, mean ± SD (years)	43.78 ± 16.55	49.19 ± 15.59	51.91 ± 14.99	0.117
Sex: ♀/♂	8/7	14/15	4/4	0.951†
BCVA logMAR, mean ± SD	0.01 ± 0.07	0.44 ± 0.66	0.46 ± 0.25	<i>0.004*</i>
Spherical equivalent (SE), mean ± SD (diopters)	- 0.72 ± 1.88	- 1.33 ± 2.18	- 0.70 ± 0.69	0.612
CRT, mean ± SD (µm)	247.08 ± 17.16	220.00 ± 37.80	312.55 ± 39.54	< <i>0.001*</i>
FAZ-S, mean ± SD (mm <sup>2</sup> )	0.37 ± 0.24	0.43 ± 0.28	1.59 ± 2.58	<i>0.001*</i>
FAZ-D, mean ± SD (mm <sup>2</sup> )	1.14 ± 0.51	1.11 ± 0.48	2.49 ± 2.59	< <i>0.001*</i>
A-SO <sub>2</sub> , mean ± SD (% SO <sub>2</sub> )	95.44 ± 7.13	100.03 ± 8.65	101.63 ± 5.37	<i>0.010*</i>
V-SO <sub>2</sub> , mean ± SD (% SO <sub>2</sub> )	57.31 ± 11.84	62.16 ± 12.83	67.94 ± 6.49	<i>0.014*</i>
A-V SO <sub>2</sub> , mean ± SD (% SO <sub>2</sub> )	38.13 ± 9.11	37.86 ± 12.36	33.69 ± 7.20	0.281
D-A, mean ± SD (µm)	99.87 ± 12.00	74.05 ± 13.78	70.14 ± 7.72	< <i>0.001*</i>
D-V, mean ± SD (µm)	120.58 ± 15.49	94.72 ± 20.14	94.86 ± 12.95	< <i>0.001*</i>

pigmentosa with (ME-RP) and without (no-ME-RP) the presence of cystoid macular edema as mean ± standard deviation (SD). Statistical results of univariate ANOVA analysis or  $\chi^2$  tests are given as *p* value; statistical significance is defined as *p* < 0.05 (presented in italics and marked with an asterisk)

arterioles (D-A). This means that enlarged FAZ-D correlated positively with attenuated retinal arterioles (*p* = 0.039; with the slope of 69.668 [CI 65.818–73.519]).

**Interactions between OCTA parameters and visual acuity in retinitis pigmentosa patients with and without macular edema**

When analyzed with linear mixed effects models, we found significant interactions between the logMAR BCVA and the FAZ-S in no-ME-RP eyes (*p* = 0.003; Table 3). However, for ME-RP eyes, we found no significant interactions. Also, the

FAZ-D showed no significant impact on the logMAR BCVA in patients suffering from RP.

Thus, both the superficial and deep foveal avascular zone measurements were correlated with oxygen metabolic function in RP.

**Discussion**

The present study confirms not only an altered metabolic function in RP but also a different metabolic pattern in those RP patients suffering from macular edema. In addition, we

**Table 2** The oxygen saturation parameters (A-SO<sub>2</sub>, A-V SO<sub>2</sub>, and V-SO<sub>2</sub>), retinal vessel diameters (D-A and D-V), and the superficial (FAZ-S) and deep foveal avascular zones (FAZ-D) as calculated mean differences with their corresponding *p* values between controls and patients

Parameters	Pairwise comparisons between subgroups		
	mean difference ( <i>p</i> values)		
	Controls vs. no-ME-RP subgroup	Controls vs. ME-RP subgroup	no-ME-RP vs. ME-RP subgroup
FAZ-S (mm <sup>2</sup> )	- 0.11 (0.555)	- 1.01 (< <i>0.001*</i> )	- 0.90 (< <i>0.001*</i> )
FAZ-D (mm <sup>2</sup> )	- 0.09 (0.731)	- 1.44 (< <i>0.001*</i> )	- 1.35 (< <i>0.001*</i> )
A-SO <sub>2</sub> (%)	- 4.23 ( <i>0.005*</i> )	- 5.23 ( <i>0.017*</i> )	- 1.00 (0.608)
V-SO <sub>2</sub> (%)	- 6.28 ( <i>0.026*</i> )	- 11.30 ( <i>0.007*</i> )	- 5.02 (0.175)
A-V SO <sub>2</sub> (%)	1.44 (0.592)	6.18 (0.116)	4.74 (0.183)
D-A (µm)	24.86 (< <i>0.001*</i> )	28.83 (< <i>0.001*</i> )	3.97 (0.358)
D-V (µm)	26.00 (< <i>0.001*</i> )	25.54 (< <i>0.001*</i> )	- 0.46 (0.940)

suffering from retinitis pigmentosa with (ME-RP) and without (no-ME-RP) the presence of cystoid macular edema. Statistical significance is defined as *p* < 0.05 (presented in italics and marked with an asterisk)

**Table 3** Significant interactions between retinal vessel oximetry parameters (A-SO<sub>2</sub>, A-V SO<sub>2</sub>, V-SO<sub>2</sub>, D-A, and D-V), best corrected visual acuity (BCVA), and optical coherence tomography angiography parameters (FAZ-S and FAZ-D) as corresponding *p* values betweenpatients suffering from retinitis pigmentosa with (ME-RP) and without (no-ME-RP) the presence of cystoid macular edema. Statistical significance is defined as *p* < 0.05 (presented in italics and marked with an asterisk)

Interactions		A-SO <sub>2</sub>	V-SO <sub>2</sub>	A-V SO <sub>2</sub>	D-A	D-V	BCVA
Between OCTA parameters (FAZ-S and FAZ-D) and RO parameters (SO <sub>2</sub> in % or D in μm) or BCVA within RP subgroups, indicated as <i>p</i> values							
FAZ-S	no-ME-RP	0.324	0.484	0.822	0.531	0.058	<i>0.003*</i>
	ME-RP	0.651	0.878	0.823	0.142	0.635	0.589
FAZ-D	no-ME-RP	<i>0.004*</i>	<i>0.012*</i>	0.196	0.166	0.190	0.476
	ME-RP	0.449	0.989	0.570	<i>0.039*</i>	0.281	0.792

detected microvascular alterations in RP which were presented differently depending on the presence or absence of macular edema. Furthermore, we found that both the superficial and deep foveal avascular zone measurements correlate with the oxygen metabolic function in retinitis pigmentosa. Also, these correlations seem to depend on the presence of macular edema.

There are several possible explanations for our findings: Altered retinal metabolism in RP is proposed to be the main cause for compromised vasculogenesis and neurovascular remodelling of the degenerated retina. As the intraretinal oxygen is mainly used by the highly energy-demanding rod photoreceptors, a reduction in retinal oxygen consumption with secondary increase in the retinal vessel saturation values following cellular apoptosis has been proposed to explain the observed vascular oxygen alterations in RP patients [6, 7, 36–41]. Oxidative stress following apoptosis of rod photoreceptors is currently discussed to be the trigger for the secondary loss of cones and inner retinal cells [48–50]. Therefore, the presence of macular edema in RP is supposed to represent a more advanced stage of photoreceptor degeneration [39, 40]. In agreement, we found in our study more altered metabolic function in ME-RP patients.

Consistent with the neurovascular remodelling occurring with degeneration, attenuated vessels are regarded as a clinical hallmark in eyes with RP [3–6, 51]. The degree of retinal vessel attenuation seems to correlate with the remaining central retinal function as measured by multifocal ERG [6] and BCVA [39].

However, not only vessel diameters but also ocular hemodynamics are altered in RP. Ocular blood flow alterations, either retrobulbar, choroidal or retinal, have been confirmed in RP. For instance, hemodynamic studies in RP patients, using laser Doppler velocimetry [9], color Doppler imaging [52, 53], laser speckle flowgraphy [54], and retinal functional imaging [55], have revealed significant reduction of retinal blood flow. Based on MRI imaging, reduced retino-choroidal blood flow has been noted [56]. Furthermore,

choroidal hemodynamic disturbance has shown to correlate with worsening in the retinal function measured by full-field ERG, multifocal ERG, or perimetry in RP [53, 54, 57].

Regarding retinal vessel attenuation and ocular blood flow alterations in RP, it is not an unexpected finding that RP patients present with microvascular anomalies, which can be documented nowadays using OCTA, where the reduction of retinal and choroidal blood flow and an increase in the size of the foveal avascular zone (FAZ) have been reported [26–29].

According to the results of Ma et al., reduced retinal blood flow and attenuated vessel diameters are related to increased oxygen tension in the retinal extracellular space [5]. Other studies suggested the higher extracellular oxygen level to lead to further vasoconstriction and diminished blood flow in retinal arteries [57] with further alterations in hemodynamics [53, 58–60]. Thus, enlarged FAZ in RP and reduced arteriole diameters as determined in our study could well be linked to a more progressive stage of outer retinal degeneration with the following: loss of photoreceptors, reduced barrier function of the RPE, and corresponding retinal and choroidal vascular remodelling.

Therefore, we studied further the relationship between metabolic and microvascular alterations in RP patients and we found that these are correlated. Furthermore, in RP without macular edema, there were significant interactions between the arterial and venular oxygen saturations (A-SO<sub>2</sub> and V-SO<sub>2</sub>) and the superficial FAZ. As RO especially measures, the oxygenation in retinal vessels located in the inner retina our results confirm an altered metabolic function of the inner retina resulting from a degenerative process of the outer retina in adults suffering from RP. Thus, this result seems to have a higher impact on the superficial vascular layer, as measured in our study with the superficial FAZ.

Contrary to the results of the studies cited before [26–29], other publications reported to have no significant changes in the size of the FAZ between diseased and healthy eyes [30, 31]. Based on the results of the present study in a genetically heterogenic RP group with different age and stage of

degeneration, one reason for discrepancy in FAZ findings in RP might probably be the presence of macular edema. The latter could be assumed not only because of the artifacts due to scattering and enlargement of the evaluated macular space as a result of edema but also due to a different altered metabolic stage of degeneration.

Furthermore, in RP eyes with macular edema, we found the vessel diameter of the retinal arterioles (D-A) to present with significant interactions with the deep FAZ. Here, more attenuated arterial vessels in ME-RP presented with a higher level of FAZ-D enlargement. These results are in accordance with recent studies that found the arterial vessel diameters to be significantly rarefied in RP [7, 40]. Those findings were even more pronounced in the presence rather than in the absence of macular edema and correlated well with the degree of the residual central retinal function as measured by multifocal ERG [7]. This vessel attenuation seems to have a significant impact on the deep rather than the superficial FAZ in ME-RP. Furthermore, even if rods are thought to be the main players in the pathogenesis of outer retinal degeneration, cones are also involved even if occurring later in the course of degeneration. Taking the normal human topographic distribution of photoreceptors with higher cone density in the fovea [61] into account, it is plausible that the presentation of macular edema in RP may reflect the compromised resistance of cones to oxidative stress.

In addition, we found the FAZ-S to have a significant effect on the visual acuity in no-ME-RP patients. This phenomenon could only be found for the superficial but not for the deep FAZ. Also, these findings are in agreement with current studies [62, 63] which found the FAZ-S but not the FAZ-D to correlate with visual function in RP.

Our analyses revealed significantly enlarged superficial and deep FAZ in RP only in eyes in presence, however, not in the absence of macular edema. Thus, our findings might explain why recent OCTA studies in RP found controversial results regarding the FAZ: While several studies reported about an increase in the size of the FAZ in eyes of patients suffering from RP [26–29], others published results contrary to these findings [30, 31].

Furthermore, RP patients without macular edema presented with less FAZ enlargement and slightly better BCVA. Also, the FAZ-S correlated well with BCVA. These results are in agreement with the study of Konieczka et al. where no-ME-RP patients showed a clear relationship between the residual BCVA and structure–function parameters evaluated by OCT and multifocal ERG [39]. Thus, one may suppose more preserved cone photoreceptors and less vascular remodelling in no-ME-RP patients. On the contrary, in the presence of macular edema (ME-RP subgroup), these results might be influenced by scattering artifacts [64] and therefore bias the evaluation of retinal microvasculature and its effect on BCVA.

The study has limitations that include the genetic heterogeneity of our RP patients, possible artifacts due to the refractive error, and the small sample size in the ME-RP subgroup.

In conclusion, our study showed that RP patients with macular edema show more pronounced alterations in microvascular structure and metabolic function. Increased retinal vessel oxygen saturation, narrower retinal vessels, and more disrupted FAZ seem to be linked to the clinical appearance of macular edema. As photoreceptors die during progression of the degenerative process, the normal microvascular structure and metabolic homeostasis in the RP retina seem to suffer from a significant alteration with enlargement of FAZ. This probably leads to higher levels of free oxygen radicals in the intercellular space, causing persistent macular thickening, clinically identified as macular edema. Whether a significant cone decimation in advanced stages of RP is also marked by a higher grade of neurovascular remodelling and, therefore, leads to a severe metabolic dysfunction remains to be investigated. Furthermore, studies are needed to evaluate to what extent the altered retinal and choroidal perfusion in RP patients may contribute to the metabolic dysfunction and to the presence of macular edema in the future.

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**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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