Subclinical Signs of Retinal Microvascular Impairment in Obese Patients

Evidência Subclínica de Alterações Microvasculares na Retina em Doentes Obesos

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ABSTRACT

INTRODUCTION: Basic science research has shown that obesity is associated with enhanced oxidative stress, reduced nitric oxide availability and secondary microvascular endothelial dysfunction. However, the clinical demonstration of this microvasculature damage has been challenging because of the *in vivo* inaccessibility of the small capillaries. This study sought to evaluate the impact of obesity in the structure and microvasculature of the retina using a clinically available non-invasive technology.

METHODS: Obese patients (body mass index, BMI ≥35 kg/m^2) with no history of clinically evident microvascular disease and age-matched controls were consecutively recruited. Retina macular structure was evaluated as retinal thickness in the different retinal layers in the foveal, parafoveal and perifoveal regions using optical coherence tomography (OCT). Macular vascular properties were evaluated using OCT angiography. Foveal avascular zone properties and vascular density (in both superior (SVP) and deep vascular plexus (DVP)) were computed. Clinically relevant adjustments were performed.

RESULTS: A total of 79 participants were included (49 obese, 30 age-matched controls). Obese patients had subclinical signs of retinal microvascular impairment when compared to controls (increased retinal foveal avascular zone area and irregularity (area: 0.38±0.12 *vs* 0.32±0.10 mm^2, p=0.037; perimeter: 2.33±0.37 *vs* 2.08±0.35 mm, p=0.002; circularity: 0.85±0.09 *vs* 0.91±0.04, p<0.001) and decreased parafoveal vascular density in the DVP (62.4±10.1 *vs* 67.7±9.8, p=0.020), despite preserved structural retinal layers thickness. These differences were not influenced by the clinical diagnosis of diabetes.

CONCLUSION: Obese patients in our sample had subclinical signs of microvascular im-

pairment when compared to controls. This subclinical retinal microvascular impairment may reflect an initial stage of a systemic microangiopathic process that deserves our attention as obesity prevalence keeps growing worldwide.

KEYWORDS: Fluorescein Angiography; Microvessels; Obesity; Retinal Vessels; Tomography, Optical Coherence.

RESUMO

INTRODUÇÃO: Estudos de investigação não clínica suportam a evidência da associação direta entre a obesidade e o aumento de *stress* oxidativo, diminuição da disponibilidade de óxido nítrico e consequente disfunção endotelial. Contudo, o impacto destas alterações *in vivo* é difícil de avaliar devido à inacessibilidade destes microvasos. Com este estudo pretendeu-se avaliar o impacto da obesidade na microcirculação e estrutura da retina usando tecnologia não invasiva clinicamente disponível.

MÉTODOS: Foram recrutados doentes obesos (índice de massa corporal, IMC BMI ≥ 35 kg/m^2) sem história de doença microvascular clinicamente evidente. A estrutura da retina macular foi avaliada usando tomografia de coerência ótica (OCT), medindo-se a espessura das diferentes camadas. As propriedades microvasculares da mácula foram avaliadas usando OCT angiografia, avaliando-se as propriedades da zona avascular foveal e a densidade vascular no plexo superficial e profundo. Foram realizados ajustes para variáveis clinicamente relevantes.

RESULTADOS: Foram incluídos 79 participantes (49 obesos, 30 controlos matched para a idade). Foram detetados sinais de alterações da microvasculatura macular dos doentes obesos quando comparando com os controlos (aumento das dimensões e irregularidade da zona avascular foveal - área: 0,38±0,12 *vs* 0,32±0,10 mm^2, p=0,037; perímetro: 2,33±0,37 *vs* 2,08±0,35 mm, p=0,002; circularidade: 0,85±0,09 *vs* 0,91±0,04, p<0,001- e diminuição da densidade vascular parafoveal no plexo profundo, 67,7±9,8 *vs* 62,4±10,1, p=0,020). Estas diferenças mantiveram-se após ajuste para o diagnóstico clínico de diabetes. Não foram encontradas diferenças aquando da comparação do componente estrutural.

CONCLUSÃO: A obesidade no nosso estudo mostrou-se associada a sinais de atingimento da microvasculatura retiniana, quando comparando com o grupo de controlo. Estes sinais podem refletir um processo mais amplo de atingimento sistémico da microvasculatura que merece atenção, dada a crescente epidemia mundial da obesidade, pelo potencial lesivo de órgãos-alvo a longo-prazo.

PALAVRAS-CHAVE: Angiofluoresceinografia; Obesidade; Microvasos; Tomografia de Coerência Óptica; Vasos Retinianos.

INTRODUCTION

Obesity is a growing international public health problem. According to World Health Organization data, worldwide, more than 1.9 billion adults are overweight and, of these, 650 million are obese.[1] Obesity is a major risk factor for noncommunicable diseases. On the cardiovascular system, obesity accelerates atherosclerosis progression, inducing pathophysiological changes that are detectable already from young adulthood.[2] Moreover, obesity has also been linked to diabetes, cancer and other chronic diseases, representing around 4 million deaths and 120 million disability-adjusted life years worldwide.[3]

Microvascular dysfunction has been recently highlighted to be one of the potential hallmarks of obesity.[4] Basic science research has demonstrated that the excess of adiposity elicits a chronic inflammatory response with consequent dysregulation of paracrine and endocrine factors released by adipocytes causing the disruption of vascular homeostasis.[5,6] This process leads to a decreased blood fluidity and endothelial function characterized by decreased availability of nitric oxide, and enhanced bioactivity of vasoconstrictor and proatherogenic factors.[2] Both the systemic and local adipose tissue derived agents seem to contribute to the development of microvascular dysfunction by impairing these signaling pathways.[4,7] Currently, there is an unmet need to clinically study the microvascular damage in an early stage in people with obesity, so that further end-organ complications can be prevented. Although some clinical studies have demonstrat-

ed the microvascular impairment in obesity, using methods such as skin capillaroscopy and Doppler flowmetry or coronary histological analysis,[8–10] none of those strategies provides a rapid, reproducible, and non-invasive approach that would adapt to the real-world clinical practice.

The retina is a privileged tissue for observing microvascular processes *in vivo*. Optical coherence tomography angiography (OCTA) was recently introduced as a rapid, non-invasive technology that uses erythrocytes movement to detect blood flow in the different retinal capillaries.[11] Several studies have demonstrated the usefulness of OCTA in evaluating the natural history of retinal vascular diseases.[12–15] As OCTA allows us to track microvascular changes over time from a quantitative perspective, it is a crucial tool for understanding the natural history of several diseases, even before their clinical appearance. In addition, the combination of OCTA (and its microvascular insights) and structural OCT (that evaluate the retinal neuronal component) information may bring new knowledge on the mechanisms that lie behind the relationship between retinal vascular and neuronal components, that can be frequently translated to a systemic perspective.

Our group has previously demonstrated that obese patients have precocious subclinical neurodegeneration of the optic nerve, regardless of their co-morbidities (including diabetes)[16] that is not reversible after bariatric surgery.[17] These findings highlight the importance of understanding the impact of obesity in the retina and eye diseases, as the evaluation of the retina microcirculation may improve our knowledge on the effect of obesity not only in the eye but also in a systemic perspective.

The aim of this study was to evaluate the retinal microvasculature features in people with obesity using OCTA.

METHODS

This was a prospective study that was conducted in two Portuguese Reference Centers for Obesity Treatment (Centro Hospitalar e Universitário de São João, CHUSJ, Porto, Portugal and Centro Hospitalar de Entre o Douro e Vouga, CHEDV, Santa Maria da Feira Portugal). All procedures were approved by the Hospitals Ethics Committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants before inclusion.

SAMPLE

Participants were prospectively recruited from January 2018 and December 2018. Obese patients that were candidates to bariatric surgery after multidisciplinary evaluation were consecutively invited to participate in the study. Body mass index (BMI) was determined using the formula weight (kg)/height (m^2). We only included patients with BMI >35 kg/m^2 (severe/morbid and super obese patients) with no previous systemic disease besides hypertension, non-insulin-dependent diabetes, dyslipidemia, or obstructive sleep apnea. Controls consisted of consecutive non-obese (BMI ≤25 kg/m^2) volunteers, age-matched, and with no ocular or systemic disease besides hypertension, non-insulin-dependent diabetes, or dyslipidemia.

All included subjects underwent a complete ophthalmological exam. Individuals were excluded in both groups if they had any stage of diabetic retinopathy on indirect ophthalmoscopy, a personal or familial history of glaucoma or ocular hypertension, uveitis, optic neuropathy, age-related macular degeneration, vitreoretinal, optic nerve or choroidal vascular diseases, refractive error greater than 6/–6 diopters, recent history of decreased visual acuity, previous refractive or intraocular surgery, narrow anterior chamber angle, and secondary causes of glaucoma, such as of pigment dispersion syndrome or pseudoexfoliation syndrome.

CLINICAL DATA

For obese patients, additional clinical data was extracted from the preoperative evaluation that is routinely performed by a bariatric-specific multidisciplinary team. The clinical diagnosis of diabetes was established by Endocrinologists following the guidelines from the American Diabetes Association.[19] Fasting serum profile included serum hemoglobin (Hb, g/dL), total protein content (g/dL), albumin (g/dL), aspartate transaminase (AST, U/L), alanine transaminase (ALT, U/L), gamma glutamyl transferase (GGT, U/L), alkaline phosphatase (AP, U/L), total cholesterol (mg/dL), high-density lipoprotein cholesterol (HDL, mg/dL), low-density lipoprotein cholesterol (LDL, mg/dL), triglycerides (TG, mg/dL), glucose (Glu, mg/dL), glycosylated hemoglobin (HbA1c, %), urea (mg/dL), creatinine (Cr, mg/dL), inorganic phosphorus (P, mg/dL), sodium (Na, mmol/L), potassium (K, mmol/L), B12 vitamin (pg/mL), thyroid-stimulating hormone (TSH, μU/mL), free thyroxine (T4, pmol/L) and insulin (μU/mL). Glomerular filtration rate (GFR, mL/min/1.73 m^2) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[20]

IMAGE ACQUISITION

The imaging protocol has been previously described by our group.[17,18] The protocol was explained to the participants before any acquisition. All images were obtained by the same trained ophthalmic professional and in the same environment conditions. Acquisitions were repeated, if necessary, to obtain high-quality images. In contrast to the structural, the microvascular evaluation was only performed in the subsample of patients from CHEDV due to OCTA technology availability at the time of the study.

Macular Structure: Spectral Domain Optical Coherence Tomography

Heidelberg Spectralis spectral-domain optical coherence tomography (SD-OCT Heidelberg Engineering, Heidelberg, Germany) was employed to all patients to evaluate macular ultrastructure by acquiring a horizontal raster cube scan centered

at the fovea with 61 lines (120 μm apart) covering the 30ºx25º posterior pole area. All scans were averaged nine times using the TruTrack technology from Heidelberg. Images were manually reviewed, and low-quality scans were excluded.

Macular Microvasculature: Spectral Domain Optical Coherence Tomography Angiography

The individuals were scanned in both eyes using a 10ºx10º scan high resolution protocol centered on the fovea with the Heidelberg Spectralis OCTA system (Spectralis; Heidelberg Engineering, Heidelberg, Germany). The device uses an 870 μm central wavelength and images at 85,000 per second with an isotropic lateral resolution of 5.7 μm/pixel. The image cubes were acquired using 5 repeated scans with the TruTrack technology from Heidelberg. Images were manually reviewed, and low-quality scans were excluded.

IMAGE PROCESSING AND QUANTIFICATION

Macular Structure: Retinal and Choroidal Thickness

Seven layers were defined using an automated proprietary software algorithm (Spectralis; Heidelberg Engineering, Heidelberg, Germany): the retinal nerve fiber layer, the ganglion cell layer, the inner plexiform layer (IPL), the inner nuclear layer (INL), the outer plexiform layer (OPL), the outer nuclear layer (ONL) including Henle's layer and the choroid. Each image was reviewed in a masked fashion. If necessary, segmentation lines were adjusted to enhance accuracy. For the retinal layers, three concentric regions were defined based in the Early Treatment Diabetic Retinopathy Study (ETDRS) macula grid centered on the umbo: the fovea of 1mm diameter, the parafoveal ring 1-3 mm from the umbo and the perifoveal ring 3-6 mm from the umbo. The automated quantification of retinal thickness included in the device was used. Choroidal thickness (CT) was measured using the calipers provided in the Spectralis Heidelberg software in the subfoveal area and at 500 μm intervals from the fovea to 1,500 μm in the nasal and temporal regions.

Macular Microvasculature and foveal avascular zone

Automated segmentation of the superficial vascular plexus (SVP) and deep vascular plexus (DVP) was performed using the software provided within the Spectralis® (Heidelberg Engineering). The retinal projection artifacts were removed using the projection artifact removal tool from Heidelberg (Software Version 6.14.1) before the images were further processed for quantification. The algorithm removes flow projection from the normally avascular outer retinal slab and preserves *in situ* flow signal of the deeper vessels. The en face angiograms were exported for analysis as Tagged Image File Format (tiff) format. Image analysis was performed using Image J V. 1.51 (National Institutes of Health, Bethesda).[21] Raw data was cut using the same frame (960x960 pixels) in order to exclude artifacts that sometimes occur in the margin of the scan. Brightness and contrast adjustments were not performed, the images were manipulated in the native form. Before any conversion, the initial pixel values were coded as 8-bit values, ranging from 0-255.

The foveal avascular zone was evaluated in the SVP *en face* angiogram. Foveal avascular zone area (mm^2) and perimeter (mm) were manually outlined two times using ImageJ polygon selection tool (Image J V. 1.51, National Institutes of Health, Bethesda).[21] The average of the two measurements was used. Foveal avascular zone circularity was then calculated using the following equation: *Foveal avascular zone circularity = (4π × foveal avascular zone area)/foveal avascular zone perimeter*, in mm. Circularity is the expression of the regularity of a shape: the closer the value is to 1, the more the shape is similar to a perfect circle.[22]

To calculate vascular density in SVP and DVP, each *en face* angiogram was processed using Image J (Image J V. 1.51, National Institutes of Health, Bethesda)21 Phansalkar's local threshold method with a 15-pixel radius. Vascular density was defined as the ratio between the area with vasculature (white pixels) and the total area (total number of pixels), multiplied by 100. Overall vascular density, foveal vascular density (1mm diameter centered at the umbo), and parafoveal vascular density (ring 1-3mm from the umbo) were considered for analysis.

STATISTICS

The sample size was estimated for the two primary outcomes. 1) To detect a minimal difference in macular thickness between obese and non-obese patients of 15 μm, assuming a standard deviation of 20 μm and a ratio of 2 cases/1 control. Using these criteria, at least 21 controls and 42 cases were used as the reference sample size (this outcome was evaluated in both Hospital Centers). 2) Considering a 10% clinically significant difference in vascular density between groups, and a standard deviation of 5%. Accordingly, for a power of 90%, an alpha value of 0.05, a minimum of 17 obese patients and 17 controls were necessary.

Descriptive statistics for continuous variables are presented as mean and standard deviation (SD) or as median (interquartile range or range), according to the skewness of the distribution. Categorical variables are presented as number (n) and percentage (%) of total and were compared using Fisher test. For continuous variables, intergroup comparisons were performed using Mann-Whitney or Independent t-test depending on the skewness of the distributions, and the association between variables was accessed using the Pearson's Correlation Coefficient (r). For the purpose of evaluating possible associations with microvascular features, variables were grouped as 1) clinical (age, sex, BMI, hypertension, diabetes, dyslipidemia and OSA), and 2) serum profile (Hb, proteins, albumin, AST, ALT, GGT, AP, total cholesterol, HDL, LDL, TG, HbA1c, urea, creatinine, P, Na, K, B12 vitamin, TSH, T4, insulin and GFR. These two groups were then evaluated for associa-

tions with preset OCTA microvascular features (FAZ area, FAZ perimeter, FAZ circularity, SVP vascular density at the fovea and parafovea, DVP vascular density at the fovea and parafovea). A linear regression model was used for adjusting identified factors to a possible relationship with microvasculature using a backward approach. Only variables with significant association with microvascular features in the exploratory analysis were included. All statistical analysis was performed using IBM SPSS Statistics v. 25 (SPSS Inc., Chicago, IL, USA). Significance was set at 0.05.

RESULTS

RETINA STRUCTURE AND MICROVASCULATURE: COMPARISON OF OBESE GROUP VS CONTROLS

A total of 79 participants were included: 49 in the obese group and 30 controls. From these, a sub-sample included in the macular microvascular evaluation. The total population and subsample are characterized in Table 1.

Table 1. Clinical and demographic characteristics.

	Total (n=79 participants)			Subsample (n=40 participants)		
	Controls (n=30)	Obese (n=49)	*p*-value	Controls (n=20)	Obese (n=20)	*p*-value
Age, years - Median (IQR)	50 (15.0)	50.0 (13.0)	0.971[a]	46.5 (16.8)	49.5 (14.3)	0.10[a]
Male, n (%)	11 (37)	9 (18)	0.014[¥]	6 (30)	6 (30)	1.0[¥]
BMI, kg/m^2 - Median (range)	23. (18.6-24.9)	42.9 (35.1-59.5)	<0.001[a]	22 (19.5-24.5)	42 (36.5-51.4)	<0.001[a]
Hypertension, n (%)	7 (23)	25 (51)	<0.001[¥]	1 (5)	11 (55)	<0.001[¥]
Diabetes, n (%)	3 (10)	22 (45)	<0.001[¥]	2 (10)	7 (35)	0.014[¥]
Dyslipidemia, n (%)	4 (13)	25 (51)	<0.001[¥]	0 (0)	12 (60)	<0.001[¥]
OSA, n (%)	2 (7)	14 (29)	0.002[¥]	0 (0)	7 (35)	<0.001[¥]

[a] Mann-Whitney test. [¥] Fisher test. IQR: interquartile range. BMI: body mass index. OSA: obstructive sleep apnea.

Supplementary Table 1. Retinal layers and choroid thickness (µm) in obese group (n=98 eyes) and controls (n=60 eyes).

Retinal Layer	Location	Obese	Controls	*p*-value
All layers, µm	Foveal	275±17	278±22	0.386
	Parafoveal	337±15	339±17	0.569
	Perifoveal	294±14	297±13	0.315
RNFL, µm	Foveal	13±1.7	12.7±2.2	0.249
	Parafoveal	22.0±1.9	21.5±2.0	0.122
	Perifoveal	36.8±5.6	35.3±5.1	0.104
GCL, µm	Foveal	15.7±4.7	16.2±6.4	0.577
	Parafoveal	50.2±6.5	50.5±5.0	0.729
	Perifoveal	35.8±3.8	36.1±3.3	0.641
IPL, µm	Foveal	21.5±3.2	21.2±4.5	0.715
	Parafoveal	41.6±3.8	41.7±3.2	0.536
	Perifoveal	29.8±2.8	29.9±2.6	0.929
INL, µm	Foveal	20.2±4.8	20.1±5.1	0.914
	Parafoveal	41.0±3.7	40.9±3.6	0.87
	Perifoveal	33.6±2.3	33.4±2.5	0.715
OPL, µm	Foveal	26.5±6.4	27.7±6.6	0.251
	Parafoveal	33.7±4.6	33.8±6.1	0.941
	Perifoveal	27.8±2.2	27.3±2.8	0.206
ONL, µm	Foveal	91.7±9.3	95.1±17.4	0.168
	Parafoveal	68.5±8.1	68.9±10.8	0.841
	Perifoveal	54.2±6.4	56.1±7.4	0.092
Choroid, µm	Subfoveal	298±97.1	289±73.3	0.554
	500 µm, nasal	298±94.0	278±71.2	0.173
	1000 µm, nasal	284±102	265±82.9	0.265
	1500 µm, nasal	262±102	248±79.3	0.385
	500 µm, temporal	297±92.7	282±68.6	0.274
	1000 µm, temporal	280±86.6	275±64.8	0.722
	1500 µm, temporal	273±75.6	272±70.8	0.924

Retinal nerve fiber layer (RNFL), the ganglion cell layer (GCL), the inner plexiform layer (IPL), the inner nuclear layer (INL), the outer plexiform layer (OPL), the outer nuclear layer (ONL) including Henle's layer.

When evaluating the retina structure, we found no significant differences between the thickness of the retina layers from controls and obese individuals in the foveal, parafoveal or perifoveal locations (results detailed in supplementary Table 1).

Regarding retina microvasculature, we found signs of retina microvascular impairment in obese patients when compared to controls (Table 2). The mean retina foveal avascular zone area and perimeter were significantly superior in the obese group whereas foveal avascular zone circularity and parafoveal vascular density at the level of the DVP were significantly reduced in this group. In the obese group, there was a correlation between increased BMI and decreased parafoveal vascular density at the level of the DVP (r=-0.395, p=0.012), whereas in controls this correlation was not verified (r=-0.048, p=0.793).

FACTORS ASSOCIATED WITH MICROVASCULAR IMPAIRMENT IN OBESE PATIENTS

An exploratory analysis on the potential factors (clinical and serological) associated with microvascular impairment in obese patients was performed. Table 3 summarizes the

Table 2. Comparison of the microvascular networks among obese individuals (n=40 eyes) and controls (n=40 eyes).

	Control	Obese	*p*-value
FAZ area, mm²	0.323±0.104	0.376±0.118	0.037*
FAZ perimeter, mm	2.079±0.346	2.334±0.368	0.002*
FAZ circularity	0.913±0.039	0.853±0.087	<0.001*
SVP foveal VD, %	29.5±6.6	28.8±6.0	0.578
SVP parafoveal VD, %	62.2±12.8	60.4±11.5	0.521
DVP foveal VD, %	43.2±7.6	40.9±8.1	0.177
DVP parafoveal VD, %	67.7±9.8	62.4±10.1	0.020*

FAZ: foveal avascular zone. SVP: superficial vascular plexus. DVP: deep vascular plexus. VD: vascular density. * statistically significant, independent t-test.

Table 3. Association between clinical characteristics of the participants and microvascular features.

	FAZ Area, mm²		FAZ perimeter, mm		FAZ circularity		SVP foveal VD		DVP foveal VD		SVP parafoveal VD		DVP parafoveal VD	
Correlations	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
BMI, kg/m²	-.09	.58	-.06	.72	-.03	.87	-.09	.59	-.03	.85	-.15	.25	**-.33**	**.04**
Age, years	.10	.53	.07	.67	.07	.66	**-.38**	**.02**	-.13	.42	-.27	.10	-.29	.07
Group Comparisons	**Value**	***p***	**Value**	***p***	**Value**	***p***	**Value**	***p***	**Value**	***p***	**Value**	***p***	**Value**	***p***
Sex														
Male	.39(.16)	.85	2.3(0.6)	.85	.91(.17)	.76	.26(.11)	.36	.36(.19)	.81	.64±.13	.97	.69±.23	.83
Female	.35(.16)		2.3(0.5)		.88(.14)		.24(.06)		.37(.10)		.64±.12		.70±.08	
Diabetes														
Yes	.40±.12	.25	2.5(.7)	.24	.87±.10	.50	.23±.05	.91	.37±.07	.62	.67±.11	.08	.68±.05	.82
No	.36±.11		2.3(.4)		.85±.09		±23.06		.36±.09		.61±.12		.69±.15	
Hypertension														
Yes	**.42±.13**	**.04**	**2.5±.4**	**.02**	.86(.36)	.44	**.22±.05**	**.04**	.34(.12)	.15	.62(.16)	.18	**.68(.09)**	**.03**
No	**.31±.07**		**2.1±.2**		.86(.08)		**.25±.05**		.40(.06)		.68(.19)		**.74(.15)**	
OSA														
Yes	.43(.22)	.16	2.4±.4	.32	.90(.10)	.53	.21(.12)	.94	.33(.18)	.94	.64±.13	.92	.71(.11)	.36
No	.35(.11)		2.3±.36		.87(.14)		.25(.06)		.39(.09)		.64±.12		.69(.09)	
Dyslipidemia														
Yes	.40±.11	.10	**2.4(.4)**	**.03**	.89(.13)	.46	.23±.05	.72	.36±.07	.73	.63±.09	.81	.68±.09	.61
No	.34±.12		**2.1(.4)**		.88(.13)		.24±.07		.37±.09		.64±.16		.70±.15	

Correlations are presented as the Pearson's correlation coefficient (r) and the correspondent p value. Group comparisons are presented as mean±standard deviation or as median (interquartile range) and the correspondent p value for independent t-test or Mann-Whitney test, respectively. BMI: body mass index. OSA: obstructive sleep apnea. FAZ: foveal avascular zone. VD: vascular density. SVP: superficial vascular plexus. DVP: deep vascular plexus. Values in bold and underlined correspond to significant associations (p<0.05).

Table 4. Association between serum characteristics of the participants and microvascular features.

	FAZ Area, mm²		FAZ perimeter, mm		FAZ circularity		SVP foveal VD		DVP foveal VD		SVP parafoveal VD		DVP parafoveal VD	
Correlations	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Hb (g/dL)	.01	.95	-.05	.79	.14	.47	.20	.30	.01	.96	.13	.51	.15	.43
Protein (g/dL)	.26	.16	.24	.21	.10	.60	-.03	.90	-.07	.73	-.13	.48	.07	.70
Albumin (g/dL)	.36	.09	.36	.09	.04	.85	.04	.86	-.06	.77	.10	.63	.20	.35
AST (U/L)	.03	.89	.02	.92	.06	.77	-.12	.53	-.21	.26	-.12	.52	-.03	.88
ALT (U/L)	.05	.78	.10	.59	-.09	.65	-.09	.62	-.30	.11	-.15	.42	-.06	.76
GGT (U/L)	**.38**	**.04**	**.51**	**.004**	-.32	-.09	-.27	.15	-.24	.21	-.23	.23	-.20	.29
AP (U/L)	.32	.10	**.44**	**.02**	-.31	.11	-.22	.27	**-.40**	**.03**	-.24	.22	-.08	.68
Chol. (mg/dL)	-.02	.90	-.05	.81	.25	.20	.07	.73	-.05	.81	.36	.06	-.28	.15
HDL (mg/dL)	-.08	.67	-.07	.71	.06	.76	-.09	.66	-.006	.97	-.05	.79	-.29	.12
LDL (mg/dL)	-.04	.85	-.06	.77	.22	.26	.08	.70	.11	.56	**.43**	**.02**	-.27	.17
TG (mg/dL)	-.05	.81	-.06	.74	-.18	.35	.19	.30	.002	.99	-.27	.17	.12	.54
Glu (mg/dL)	**.43**	**.02**	**.58**	**.001**	.32	.09	**-.37**	**.05**	**-.40**	**.03**	-.01	.95	**-.50**	**.01**
HbA1c (%)	**.65**	**<.001**	**.75**	**<.001**	-.23	.24	**-.51**	**.01**	**-.45**	**.02**	-.29	.13	**-.45**	**.02**
Insul (µU/mL)	-.003	.99	.19	.33	**-.45**	**.02**	-.04	.85	-.16	.42	-.03	.65	-.13	.52
Urea (mg/dL)	-.24	.23	-.12	.55	-.26	.19	.16	.42	.23	.24	.09	.66	-.08	.69
Cr (mg/dL)	-.003	.99	.07	.70	-.13	.48	.03	.89	.05	.78	.09	.64	-.09	.63
P (mg/dL)	-.12	.55	-.20	.32	.30	.13	.05	.80	.06	.76	-.02	.91	-.02	.99
Na (mmol/L)	-.19	.32	-.24	.21	.31	.10	.16	.41	-.001	.99	-.28	.13	-.26	.17
K (mmol/L)	-.02	.94	-.15	.44	.26	.17	-.16	.40	-.10	.59	-.15	.43	-.09	.63
B12 (pg/mL)	.16	.41	.16	.41	.03	.90	.21	.29	.01	.96	-.09	.63	.24	.22
TSH (µU/mL)	.12	.53	.21	.28	-.17	.40	-.18	.36	-.16	.41	.15	.45	-.24	.23
T4 (pmol/L)	.04	.85	-.06	.75	.22	.23	.28	.15	.12	.56	**.55**	**.003**	**.39**	**.04**
GFR (mL/min/1.73m²)	-.05	.81	-.05	.78	-.11	.58	.32	.08	.15	.44	.10	.59	**.42**	**.02**

Correlations are presented as the Pearson's' correlation coefficient (r) and the correspondent p value. Hb: hemoglobin; Protein: total protein content, AST: aspartate transaminase; ALT: alanine transaminase; GGT: gamma glutamyl transferase; AP: alkaline phosphatase; Chol: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; TG: triglycerides; Glu: glucose; HbA1c: glycosylated hemoglobin; Cr: creatinine; P: inorganic phosphorus; Na: sodium; K: potassium; B12: B12 vitamin; TSH: thyroid-stimulating hormone; T4: free thyroxine; Insul: insulin; GFR: glomerular filtration rate.

Table 5. Multiple linear regression models for significant factors associated with microvascular features.

	FAZ Area, mm²		FAZ perimeter, mm		FAZ circularity		SVP foveal VD		DVP foveal VD		SVP parafoveal VD		DVP parafoveal VD	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Age (years)	-	-	-	-	-	-	-.28	.11	-	-	-	-	-	-
BMI (kg/m²)	-	-	-	-	-	-	-	-	-	-	-	-	.01	.95
Hypertension	.28	.23	-.10	.72	-	-	-.36	.17	-	-	-	-	**-.10**	**.02**
Dyslipidemia	-	-	.21	.08	-	-	-	-	-	-	-	-	-	-
HbA1c (%)	**.71**	**<0.001**	**.18**	**.002**	-	-	**-.02**	**.01**	-.21	.51	-	-	.05	.10
Glu, mg/dL	-.36	.20	.06	.81	-	-	.06	.87	**-.001**	**.002**	-	-	**-.002**	**.04**
Ins (µU/mL)	-	-	-	-	**-.01**	**.02**	-	-	-	-	-	-	-	-
GFR (mL/min/1.73m²)	-	-	-	-	-	-	-	-	-	-	-	-	.002	.06
GGT (U/L)	.18	.92	-.07	.66	-	-	-	-	-	-	-	-	-	-
AP (U/L)	-	-	.15	.29	-	-	-	-	-.14	.53	-	-	-	-
T4 (pmol/L)	-	-	-	-	-	-	-	-	-	-	**.04**	**.003**	.14	.38
LDL (mg/dL)	-	-	-	-	-	-	-	-	-	-	.24	.19	-	-

GGT: gamma glutamyl transferase; AP: alkaline phosphatase; LDL: low-density lipoprotein cholesterol; Glu: glucose; HbA1c: glycosylated hemoglobin; T4: free thyroxine; Insul: insulin; GFR: glomerular filtration rate..

associations between clinical characteristics of the participants (age, sex, BMI, diabetes, hypertension, dyslipidemia, OSA) and the microvascular features.

The clinical diagnosis of diabetes was not associated with any of the microvascular features. Patients with hypertension presented an increased FAZ area and perimeter, decreased vascular density at the fovea in the SVP and decreased vascular density at the parafovea in the DVP. Increased BMI correlated with decreased vascular density in parafoveal DVP. Patients with dyslipidemia had an increased FAZ perimeter.

Table 4 details the associations between serum characteristics of the participants and the microvascular features. Increased fasting serum glucose and increased HbA1c were both significantly correlated with increased FAZ area (r=0.43, p=0.02 and r=0.65, p<0.01), increased FAZ perimeter (r=0.58, p=0.001 and r=0.75, p<0.001), decreased vascular density in the foveal SVP (r=-0.37, p=0.05; r=-0.51, r=0.01) and DVP (r=-0.40, p=0.03 and r=-0.45, p=0.02) and decreased vascular density in the parafoveal DVP (r=-0.50, p=0.01 and r=-0.45, p=0.02).

We performed a multivariate linear adjustment for clinical and serum factors that demonstrated a significant association with the microvascular features in the previous analysis (Table 5). After multivariate adjustment, HbA1c was the main factor associated with both FAZ area, perimeter and foveal vascular density in SVP. FAZ circularity was only found to be associated with serum insulin whereas vascular density in foveal DVP was influenced by serum glucose. These results were independent of the previous clinically classification of the patients as diabetic/non-diabetic. Serum glucose, along with the presence of hypertension, were the major conditioners for vascular density in parafoveal DVP.

DISCUSSION

In this study we explore the impact of obesity in the retina structure and microvasculature. Whereas no significant differences were found when comparing the different retinal layers thickness, we found obese patients to have subclinical signs of impaired retinal perfusion expressed as an increased foveal avascular zone area, perimeter, and irregularity as well as decreased parafoveal vascular density at the level of the deep vascular plexus, when compared to controls. This may represent an initial stage of a developing systemic vasculopathy process that deserves our attention as obesity prevalence keeps growing worldwide.

Interestingly, retinal microvasculature did not demonstrate distinct features when dividing patients according to the previous clinically classification into present/absent diabetes. However, when considering obese individuals with and without clinically evident diabetes in a conjunct analysis, there was a correlation between increased fasting serum glucose and decreased vascular density in DVP and between increased glycosylated hemoglobin and increased foveal avascular zone area and perimeter and decreased foveal vascular density in SVP. In our study, the group of patients with non-insulin dependent diabetes included individuals with different levels of metabolic control, and the group of patients without diabetes included individuals without and with pre-diabetes. Our results highlight that the microvascular damage in obese patients may start before the clinical diagnosis of diabetes, and hyperglycemia in these individuals should be a concern.

The role of obesity as a driver for microvascular dysfunction has been recently highlighted. Obesity, and the related changes in the secretion of adipokines, have shown to impair functional capillary density, endothelium-dependent vasodilation, vasomotion, and insulin-induced microvascular dilation and recruitment.[8,23] At least some of these effects correlate with BMI thus supporting a continuous relationship between fat mass and microvascular function.[24,25] Thus, in the presence of obesity, the relationship between microvascular dysfunction and hyperglycemia is bidirectional: by impairing insulin-mediated glucose disposal, microvascular dysfunction precedes and contributes to hyperglycemia in type 2 diabetes that will, by its turn, promote additional microvascular dysfunction.[23,26]

In our study, we found a correlation between blood-sugar related factors (increased fast serum glucose and glycosylated hemoglobin) and signs of retinal microvascular impairment (increased FAZ area, perimeter, and decreased vascular density) in patients with obesity. Our findings support the recent evidence that the relationship between hyperglycemia and microvascular dysfunction may be continuous and progressive. Accumulating evidence suggests that prediabetes (that is, the condition before the criteria for clinical diagnosis are met) is not simply a state of abnormal glucose concentration, but a state in which pathophysiological processes may already be damaging microvasculature.[27] Thresholding values that are used to clinically classify individuals with diabetes may not reflect the underlying pathological vascular process that individuals with obesity have, as they demonstrate microvascular dysfunction even before the clinically established diabetes.[23] Whether the biochemical changes induced by diabetes that are thought to be responsible for vascular damage have been densely studied,[28,29] the analysis of the initial pre-diabetic functional and structural organ changes that provide the scaffolding for the subsequent development of complications has lagged behind. As mentioned, obesity drives microvascular dysfunction that, per se, precedes and predisposes to diabetes complications. The analysis of the phenomenon that initiates this cascade since the prediabetic stage may provide the full comprehension and prediction of irreversible stages of microvascular disease, such as retinopathy or nephropathy. This is an important field for research as recent evidence stresses the microvascular disease in diabetes and obesity is a widespread phenomenon, and not organ specific.[30–33] For instance, although stroke, dementia and depression are not usually considered classic microvascular complications of diabetes, evidence is growing that microvascular dysfunction is one of the key underlying mechanisms.[34] Cerebral microvascular dysfunction can start before the onset of diabetes in individuals with obesity, following the path we have mentioned, and long-

term cerebral outcomes might be improved or prevented by targeting the pathways through which the microcirculation is damaged in an early stage.

The retina offers a unique opportunity to study biomarkers for both neural and microvascular changes in other tissues such as the brain and kidney, as it allows the direct visualization of microvasculature that is otherwise inaccessible using non-invasive tools. OCTA is a non-invasive clinically available technology that allows the reproducible and quantitative follow-up of retinal capillaries and is bringing new insights not only to the eye, but also to systemic diseases that target the microvasculature in their subclinical stages.

We acknowledge that our study has several limitations. Firstly, we are aware that obese patients' metabolic status may vary according to several non-controllable factors: the duration of the exposure to high-fat diet, the severity and duration of the co-morbidities (and the treatment that was applied), the genetic and familiar environment, the individual predisposition to a pro-inflammatory response, among others. Secondly, OCTA is a novel tool and artifacts are not despicable in this early phase of implementation of the technology. Long-term populational studies are needed to design further epidemiological and objective clinical trends that may justify the monitoring of this high-risk group of patients from an ophthalmological perspective. Besides all the potential limitations, our study has an important strength: by using a non-invasive technology available in the current clinical practice we demonstrate what may correspond to the first signs of a subclinical microvascular pathologic process in obese patients, regardless of the clinical diagnosis of diabetes. With obesity prevalence continuing to grow worldwide, it is essential to monitor its impact at a multisystemic level so that we can act before end-organ complications develop. As retinal vessels reflect the systemic microcirculation, OCTA may have a future relevant role in the way that it may enable the non-invasive monitorization of the systemic microangiopathy.

CONCLUSION

Obese patients in our study had subclinical retinal microvascular impairment when compared to controls that may reflect a more generalized systemic microvascular dysfunction. After multivariate adjustment, HbA1c, serum insulin, serum glucose, and the clinical diagnosis of hypertension, were the major factors associated with microvascular impairment in the obese group. Interestingly, the clinical diagnosis of diabetes was not a predictor of microvascular changes, suggesting that hyperglycemia and insulin resistance may damage the microcirculation earlier than the diagnosis is made.

The retinal examination using OCTA allows the clinical non-invasive monitoring of the microcirculation in these patients and may be used in future studies to monitor broader microvascular changes in individuals with obesity, and thus clarify and predict the routes that could be targeted for preventing long-term morbidities.

CONTRIBUTORSHIP STATEMENT / DECLARAÇÃO DE CONTRIBUIÇÃO:

RL and PC: reponsible for gathering data, presenting results and creating the manuscript.

MG, HSS, JP, MN, JC, FFR and MF: Supervised this this project and contributed with their expertise to its conclusion.

All authors read and approved the final manuscript.

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Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki as revised in 2013).

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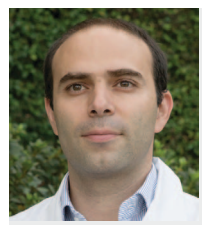

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