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Ocular posterior segment microstructural and microvascular morphological changes in protein supplement-consuming bodybuilders

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A R T I C L E I N F O

Keywords:
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Retina

A B S T R A C T

Background: To determine the effects of protein supplement (whey protein powder (PP)) on retinal, choroidal and optic nerve head (ONH) microstructure and microvascular morphology in healthy bodybuilders.

Methods: This cross-sectional study included 23 male adults (consumers, 23 right eyes) who had been routinely consuming whey PP for bodybuilding purposes for $\geq$ 3 months, and 21 age- and gender-matched healthy volunteers (non-consumers, 21 right eyes) who also attended the gym but did not consume any nutritional supplements. Participants underwent standard ocular exams, enhanced depth imaging optical coherence tomography (EDI OCT), and optical coherence tomography angiography (OCTA) after $\geq$ 8 h of rest and fasting.

Results: Whey PP was consumed for a median of 9.5 (6–12) months. Whey PP consumers had a median age of 22 (21–22) years, while non-consumers had 21 (20–22) years (p = 0.067). Whey PP consumers had greater microstructural thickness than non-consumers, with subfoveal choroidal thickness (301.40 ± 38.91 versus 278.12 ± 33.58 μm; p = 0.035) being significantly different but not central macular thickness (270.55 ± 24.60 versus 265.85 ± 12.44 μm; p = 0.402). Despite a non-significant difference in superficial and deep capillary plexus vascular densities (VDs), whey PP consumers had relatively lower VDs than non-consumers in all macular regions (p > 0.05). Despite this, whey PP consumers displayed greater ONH VDs, as well as higher global RNFL thickness (116.75 ± 10.41 versus 114.50 ± 11.70 μm) than non-consumers (p > 0.05).

Conclusion: Protein supplements, particularly whey PPs, appear to be associated with different changes in the retina and choroid, as well as ONH microstructural and microvascular morphology, implying that paying attention to these clinical aspects when performing ocular tests in bodybuilders who consume nutritional supplements could be critical.

1. Introduction

Protein requirements increase in direct proportion to the intensity and duration of an athletic performance. In order to ensure an appropriate supply of essential, or indispensable, amino acids (AAs), protein should be included in diets before and after the performance, as well as during the day [1]. In fact, various foods and supplements have been produced to fulfill these unique dietary needs. These manufactured goods are used by mouth in the form of a powder, pill, capsule, tablet, or liquid to complement the diet. Whey protein powder (PP) supplements (a component of milk protein) are the popular protein source for athletes due to their ease of digestion and absorption. Actually, dietary proteins are the most important source of an active and healthy lifestyle [2]. Athletes frequently use branched-chain AA (BCAA) supplementation, which reduces muscle soreness after strenuous exercise and increases training performance [3]. Besides, regular gym goers (bodybuilders) also frequently ingest protein supplements [4].

The retinal microvascular system consists of a central retinal artery, which branches into four major intraretinal arteries extending toward the peripheral retina and feed into the capillary bed. The retinal venous system is structured similarly, with a central retinal venule draining into the cavernous sinus. Typically, retinal capillaries form a two-layer network that is interconnected. While, superficial capillary plexus (SCP) is a microvascular network in the nerve fiber and ganglion cell layers that contains arterioles, venules, and capillaries, deep capillary plexus (DCP) is a second microvascular network that lies deeper in the inner nuclear and outer plexiform layers, and is primarily composed of capillary-sized vessels [5]. Owing to the retinal thinness in the avascular fovea, choroidal circulation allows for adequate retinal oxygenation [6]. The retinal and choroidal layers, the most vascularized ocular tissue,

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play a significant role in the pathogenesis of many ocular diseases [7]. It is consequently critical to monitor retinal and choroidal microvascular morphological changes. Despite this, measuring these changes has been extremely challenging due to the poor resolution and consistency of traditional imaging techniques.

Thanks to advancements in enhanced depth imaging optical coherence tomography (EDI-OCT), a far greater understanding of the retinal and choroidal layers is now possible [8]. Furthermore, OCT angiography (OCTA) is a unique non-invasive method that uses motion contrast imaging to generate angiographic images in seconds based on high-resolution volumetric blood flow data. This technique produces volumetric data that has the clinical potential of precisely localizing and diagnosing disease while also disclosing functional and microstructural characteristics.

We aimed to provide an up-to-date assessment of the effects of protein supplement (whey PP) consumption on the retinal, choroidal, and optic nerve head (ONH) microstructure and microvascular morphology in young healthy bodybuilders using multimodal imaging modalities, including EDI-OCT and OCTA.

2. Materials and methods

2.1. Study design and ethics approval

This cross-sectional study was performed at Afyonkarahisar Health Sciences University between January and May 2023 in compliance with the Helsinki Declaration, and it was approved by the Afyonkarahisar Health Sciences University Ethic Committee (*Approval code: 2011-KAEK-2: 2022/16-603*).

2.2. Participant selection

Our study included 23 young male healthy adults who had been regularly ingesting whey PP for bodybuilding purposes for ≥3 months (consumers) and 21 age- and gender-matched healthy volunteers who also attended the gym but did not consume any nutritional supplements (non-consumers). These participants had a healthy weight, a body mass index of 18–5–24.9, were emmetropes with 20/20 uncorrected visual acuity, and no systemic or ocular diseases. None of them drank or ate caffeine-containing beverages or foods regularly, nor did they smoke, drink alcohol or energy drinks, wear cosmetic or therapeutic contact lenses, or recently use ocular lubricants. Participants with the following characteristics were excluded from the study: (a) a history of regular smoking and alcohol consumption, (b) an intraocular pressure (IOP) > 21 mmHg, (c) a presence of any systemic vasculopathy, i.e., diabetes and systemic hypertension, (d) any glaucomatous findings, including glaucomatous optic disk changes and visual field defects, (e) a history of intraocular surgery or refractive laser therapy, or contact lens use and any medications within the preceding 3 months (f) a history of ocular trauma, amblyopia, cataract, or ocular inflammatory diseases, any of which could interfere with imaging of the ocular posterior segment and/or the microstructural and microvascular morphological findings. All participants had their tests, including EDI OCT and OCTA, carried out between 0800 and 1100 A.M. in standard settings after at least 8 h of rest and fasting.

2.3. Ophthalmological assessment

Following collection of demographics such as age, a single senior ophthalmologist performed a thorough ophthalmological assessment, including measurements of auto-refraction (average of three values, Topcon Auto-refractometer model KM 8900, Japan), uncorrected visual acuity, average of the three IOPs (mmHg) (Goldmann; Haag-Streit AG, Köniz, Switzerland), and axial length (AXL) (AL-Scan, Nidek CO., Gamagori, Japan), as well as anterior and posterior slit-lamp biomicroscopy (HaagStreit, Bern, Switzerland) both before and after cycloplegia with Tropicamide 1% and Phenylephrine 10%. Manifest refraction spherical equivalent was applied to define refractive errors, which was determined mathematically by combining the sphere power and half of the cylinder power. Emmetropia with astigmatism was therefore described as an absolute cylindrical error of ≥ ± 0.50 Diopter (D) cylinder but emmetropia when the spherical equivalent was considered (manifest refraction spherical equivalent; > −0.5 to <+0.5 D).

2.4. Enhanced depth imaging optical coherence tomography

EDI-OCT scanning (Spectralis SD-OCT; Heidelberg Engineering, Heidelberg, Germany) was performed as previously described [8]. All procedures were done under mydriasis by a single masked trained technician while a video image of the central retina was monitored. Foveal center was determined to be a point of greatest depression within a 500-μm radius. Central macular thickness (CMT) was defined as the distance between the vitreoretinal interface and the anterior surface of the retinal pigment epithelium in the central 1 μm to indicate an average CMT. Furthermore, two senior ophthalmologists blindly classified and demarcated all EDI-OCT images according to the proposed technique. Subfoveal choroidal thickness (SFCT) was manually quantified as a vertical distance from the outer surface of a hyper-reflective line denoting the retinal pigment epithelium and Bruch's membrane complex to a hypo-reflective line denoting the sclero-choroidal interface centered on the fovea using a measuring tool with an integrated linear caliber. The average SFCT was calculated by averaging two blind ophthalmologists' SFCT measurements at the fovea, as well as 1500 and 3000 μm nasal and temporal distances from the foveal center (Fig. 1).

2.5. Optical coherence tomography angiography

All OCTA (Optovue, Inc., Fremont, California, USA) procedures were undertaken by a single trained technician in Angio Retina mode with a scanning area of 6 × 6 mm^2. This non-invasive system is based on the split-spectrum amplitude-decorrelation angiography algorithm, with blood flow acting as intrinsic contrast. The blood flow is defined as a time-dependent speckle pattern change caused by erythrocyte light scattering and tissue interference. OCTA can generate volumetric scans of 304 × 304 A-scans at 70,000 A-scans per second while using an 840 nm light source and an axial resolution of 5 mm. The 'Auto Adjust' mode was used to automatically adjust the subject's AXL, refraction correction, and image polarization during image acquisition. The scan quality index (SQI), which is a score from 1 to 10 assigned by the device to angiograms at the end of each acquisition by combining signal strength, ocular movements, and image focusing, was used as well. Images with low-quality scans (SQI < 7) exhibiting fixation loss or incorrect foveal centration, segmentation errors, or motion artifacts were discarded, and the shots were repeated until the recording quality was satisfactory. An eye-tracking and a motion correction technology were used to minimize and correct ocular motion artifacts.

Both vessel densities (VDs) and flow area were automatically quantified using AngioVue Analytics (software version 2018.1.1.63), a quantitative analysis software. Typically, SCP appears as defined silhouette morphology with a linear and continuous white shape against a black background in an angiogram, and it is located in the retinal nerve fiber layer (RNFL) and ganglion cell layer. DCP, on the other hand, is depicted as a regular distribution around foveal avascular zone (FAZ), with many complex, tiny radial and horizontal interconnections located in the boundary plane between the inner nuclear layer and outer plexiform layer [9]. The retinal capillary VDs were determined using a 6 × 6 mm^2 macular angiogram of whole, foveal, parafoveal, perifoveal SCP and DCP. This was measured by the percentage of pixels in the particular region that were covered by vessels and microvasculature. The SCP angiogram was divided with an inner boundary 3 μm beneath the internal limiting membrane and an outer boundary 16 μm beneath the

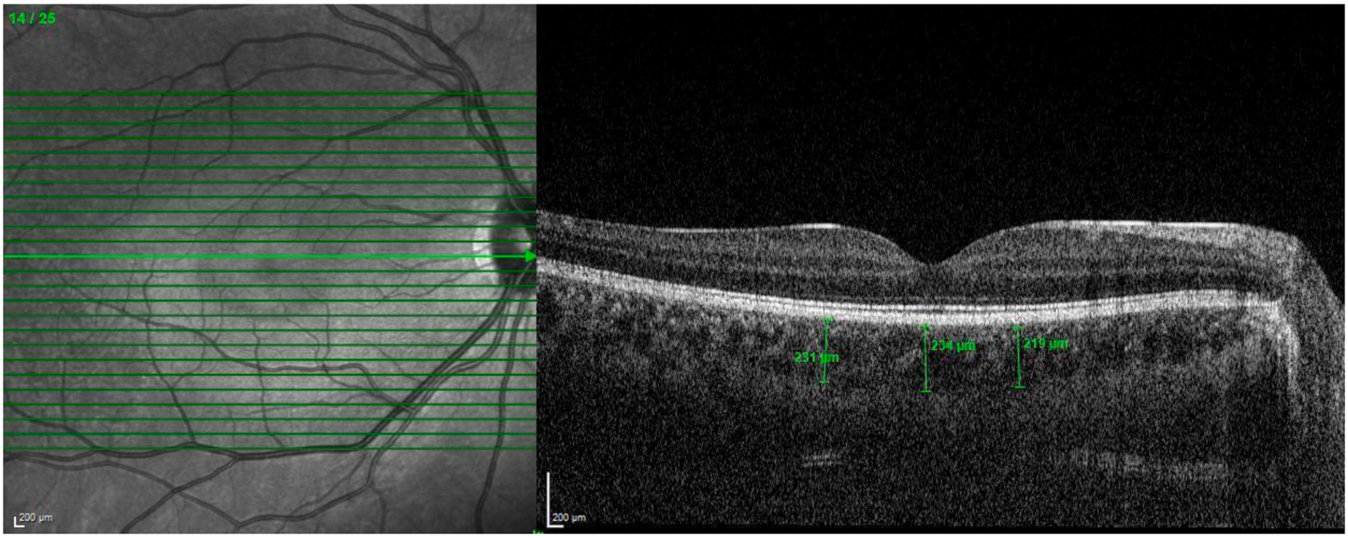

Fig. 1. Choroidal thickness measured by enhanced depth imaging optical coherence tomography subfoveally and 1500 and 3000 μm nasal and temporal to the fovea.

inner plexiform layer (IPL). The DCP angiogram was separated from 16 μm to 70 μm beneath the IPL (Fig. 2).

The FAZ is typically a significant zone devoid of flow signal on an en face macular angiogram. This zone was analyzed automatically with FAZ mode software to determine parameters such as FAZ area (mm^2), FAZ perimeter (PERIM) (mm), and foveal VDs in a 300-μm wide region surrounding the FAZ (FD-300) (%). Maximum circular area that could be captured by the fovea-centered image section with a radius of 2.97 mm was manually created in the outer retinal and choriocapillaris layers. The outer retina was located 70 μm beneath the IPL and 30 μm beneath the retinal pigment epithelium. The AngioVue software calculated the outer retinal flow automatically as the percentage of vessel area covered in the selected area centered on the FAZ. The choriocapillaris layer was located between the retinal pigment epithelium and the deeper layer, with retinal pigment epithelium offsets of 31 μm and 59 μm, respectively [10]. Using Optovue software with a flow function, the choriocapillaris flow was calculated automatically by dividing choriocapillaris vessel areas by selected area (Fig. 3).

Previously described Angio Analytics 2.0 (software version 2018.1.1.63) was used to quantify the ONH in a 4.5 × 4.5-mm^2 area [11]. The wavelength was 840 nm, the scanning frequency was 70.000 Hz, and the distances to discriminate between lateral and axial directions were 15 μm and 5 μm, respectively. The scanning depth was 2–3 mm with 304 B-scans × 304 A-scans per B-scan. Throughout the process, the motion correction technique and DualTrac were employed. The VDs were quantified automatically as the proportion of the measured area occupied by flowing blood, which is identified as pixels with decorrelation values above the threshold level (as determined by the split-spectrum amplitude-decorrelation angiography algorithm). In this context, the HD Angio Disc 4.5 mm mode was used to scan a 4.5 × 4.5 mm area around the ONH. This mode automatically produced the whole image VD (wiVD), as well as inside disk and peripapillary VDs. A 0.75 mm-wide elliptical annulus projecting from the ONH boundary (inner elliptical contour) was used to define peripapillary area and was also used to calculate peripapillary VD. Furthermore, using optical disk 200 × 200 mode, RNFL thickness results, including ONH global RNFL thickness, were achieved. Two senior ophthalmologists evaluated scans and exempted images with SQI < 7, poor clarity, residual motion artifacts visible as an anomalous vessel pattern or disk boundary on the en face angiogram, local weak signal, or serious segmentation failures. Manual adjustments were made to the modifiable segmentation failure as necessary (Fig. 4).

2.6. Data analysis

The Statistical Package for the Social Sciences program (SPSS Inc., version 26.0, Chicago, IL, USA) was used to conduct the statistical analysis. Normally distributed data were displayed as mean standard deviation, while abnormally distributed data were given as median and quartiles. Categorical variables were represented as percentages and frequencies, and the Kolmogorov Smirnov test was used to assess their conformity to a normal distribution. To compare continuous variables between groups, the Student *t*-test or Mann-Whitney U test were used, albeit on the basis of normal distribution conformance. A statistical significance level was set at $p < 0.05$.

3. Results

We compared data from the right eyes of bodybuilders who consumed just whey PP for a median of 9.5 (6–12) months to those of non-consumers. Whey PP consumers were 22 (21–22) years old, while non-consumers were 21 (20–22) years old ($p = 0.067$). Both AXL (23.87 ± 1.10 versus 23.68 ± 1.12 mm, $p = 0.574$) and IOP (15.60 ± 1.85 versus 15.31 ± 2.29 mmHg, $p = 0.644$) did not differ statistically significantly between whey PP consumers and non-consumers.

3.1. Enhanced depth imaging optical coherence tomography: microstructural analysis

Whey PP consumers had statistically significantly higher SFCT of 301.40 ± 38.91 μm compared to non-consumer, who had 278.12 ± 33.58 μm ($p = 0.035$) (Fig. 5). Despite statistically non-significant differences, whey PP consumers had larger CMT (270.55 ± 24.60 versus 265.85 ± 12.44 μm, $p = 0.402$) compared to non-consumers (Table 1).

3.2. Optical coherence tomography angiography: microvascular analysis

The SQI in whey PP consumer and non-consumer bodybuilders was (9 (8–9)) and (9 (7–9)), respectively ($p = 0.056$). There were no statistically significant differences in whole, foveal, parafoveal, and perifoveal superficial and deep capillary plexus VDs between the two groups; nonetheless, VDs in all regions in whey PP consumers were comparatively lower ($p > 0.05$). Whey PP consumers also had lower outer retinal (7.860 ± 1.549 versus 7.931 ± 1.358) and choriocapillaris (20.294 ± 0.900 versus 21.569 ± 5.948) flow areas than non-consumers ($p > 0.05$). The FAZ area, PERIM, and FD-300 did not differ statistically

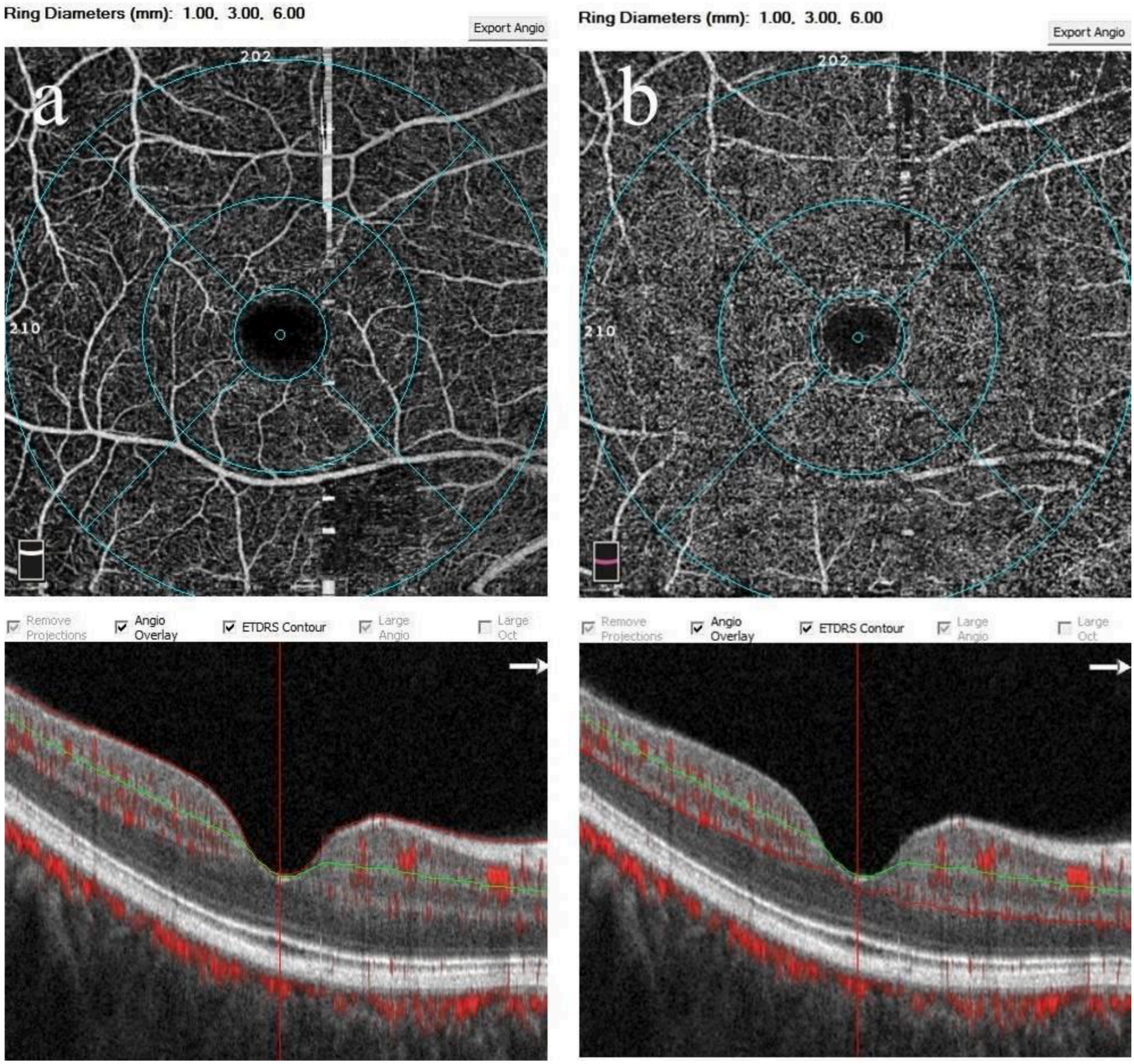

Fig. 2. The 6 × 6 mm^2 optical coherence tomography angiograms of the right eye of a young healthy bodybuilder who has been consuming whey protein powder for at least 3 months demonstrating the quantification of vessel densities in whole, foveal, parafoveal, and perifoveal superficial (a) and deep (b) capillary plexus as depicted by Early Treatment of Diabetic Retinopathy Study (ETDRS) contours. The image below shows a cross-section through the macula center with normal foveal microstructural anatomy. Scan quality index: 9/10.

significantly ($p > 0.05$) (Table 1).

Unlike retinal microcirculation, the ONH analysis revealed that whey PP consumers had much higher wiVD ($p = 0.196$), as well as inside disk ($p = 0.673$) and peripapillary ($p = 0.408$) VDs than non-consumers, though the differences were statistically non-significant. Moreover, whey PP consumers had a lower cup-disk ratio (0.105 ± 0.090 versus 0.130 ± 0.096, $p = 0.371$), which was accompanied by a higher global RNFL thickness (116.750 ± 10.412 versus 114.500 ± 11.704 μm, $p = 0.502$) relative to non-consumers (Table 2).

4. Discussion

Many studies have been conducted to establish a relationship between nutrition and ocular problems [12]. However, no study has been conducted to determine any ocular posterior segment microstructural and microvascular morphological changes associated with PP, particularly whey PP, using EDI-OCT and OCTA in young healthy PP consumers versus age- and gender-matched non-consumer bodybuilders.

In general, dietary supplements, whose safety and labeling criteria have been set by the European Commission, are nutrients that are taken from food and synthesized to improve the quality of their consumption [13]. Biochemically, AAs are the building blocks for muscular growth that proteins give. Protein is essential because, unlike fats and carbs, the human body does not store it. Thus, dietary supplements offer all AAs that the human body cannot produce. Certainly, protein can be extracted from a number of dietary sources and *"concentrated"* by removing non-protein components, resulting in a PP that is 70 to 85% pure protein (with the remaining 15–30% made up largely of carbs and fat).

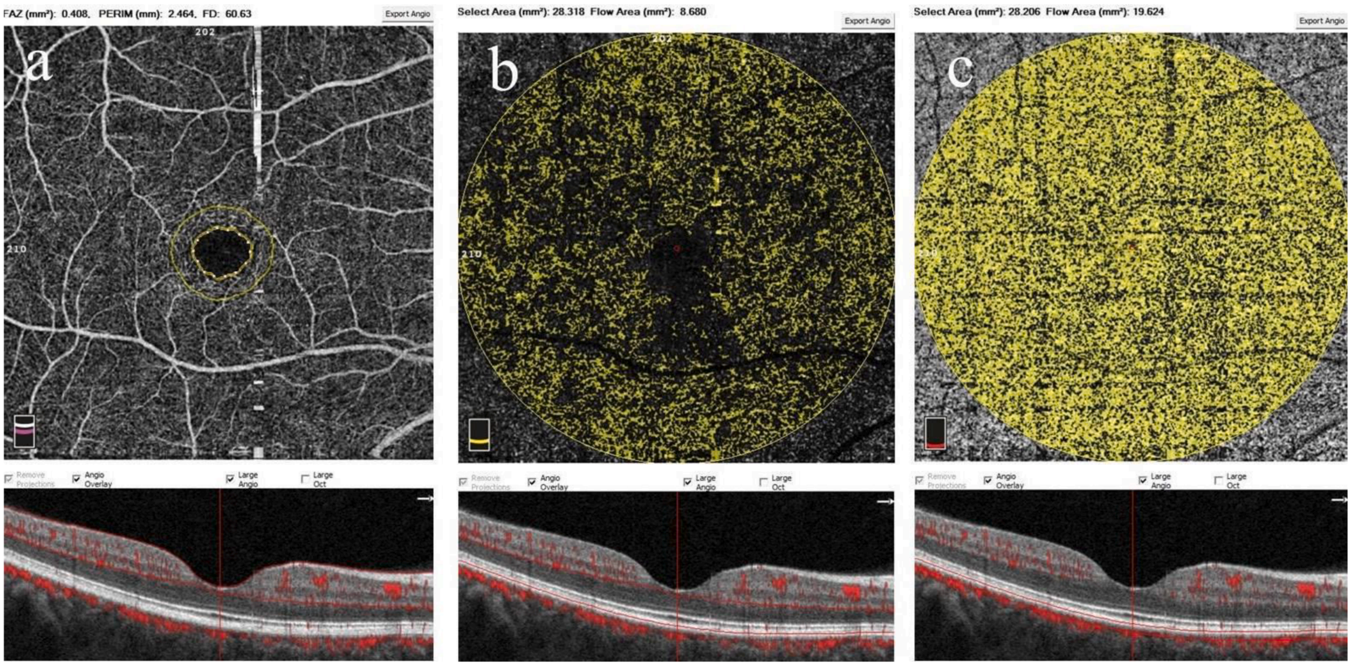

Fig. 3. The 6 × 6 mm^2 optical coherence tomography angiography en face slab of the right eye of a young healthy bodybuilder who has been consuming whey protein powder for at least 3 months depicting the quantification of foveal avascular zone parameters, including foveal avascular zone area and perimeter, as well as vessel densities 300 μm area around the foveal avascular zone (a), and capillary flow areas at the levels of (b) outer retina and (c) choriocapillaris. A cross-section through the macula center corresponding to the location of analysis is shown below. Scan quality index: 9/10.

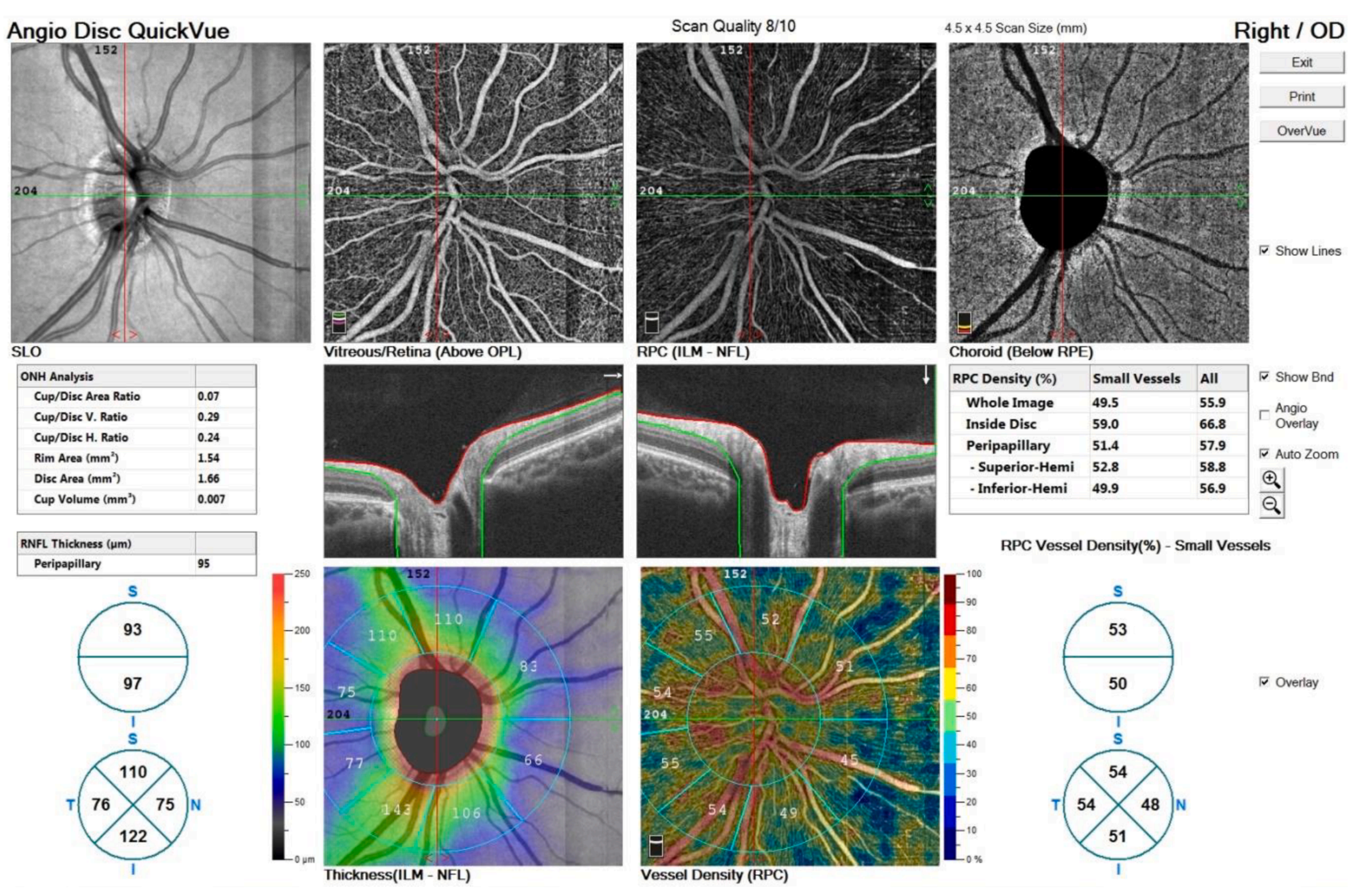

Fig. 4. The 4.5 × 4.5 mm^2 Angio Disc Quick View displaying assessment of retinal nerve fiber layer thickness and optic nerve head vessel density in the right eye of a young healthy male bodybuilder who has been using whey protein powder for at least 3 months. The Angio Disc function was used to quantify the optic nerve head vessel density in the whole image, inside disk and peripapillary region, while the retinal nerve fiber layer thickness was assessed globally.

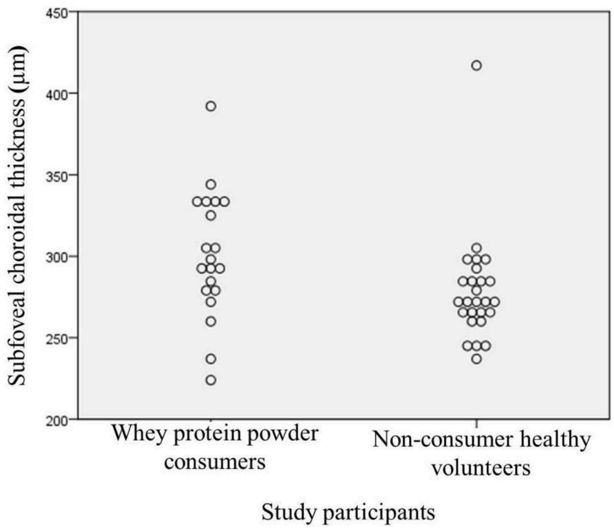

Fig. 5. A dot-plot depicting a significant difference in subfoveal choroidal thickness between whey protein powder consumers and non-consumer bodybuilders.

Non-protein components are mostly eliminated by *"isolation"* in the next concentration procedure, resulting in a premium protein that is up to 95% pure. In addition, the human body is incapable of producing all nine essential AAs found in so-called *"complete proteins,"* whereas *"incomplete proteins"* contain only a subset, if any, of the required AAs [14].

Typically, protein supplements are frequently advertised as muscle-building and performance-enhancing items to athletes and other physically active people. These supplements, which can be ready-to-drink or powders to mix with water, can also benefit persons suffering from illness, travma, or sarcopenia of old age [15], as well as those attempting to lose weight by reducing muscle loss (through the protein-sparing modified fast) [16]. The protein recommended dietary allowance for adults in the United States and Canada is 0.8 g/kg body weight, which is especially important for people who are only moderately active [17]. A high protein diet paired with exercise results in increased muscle mass [18]. Protein consumption of 1.2–1.8 g/kg body mass per day, as suggested by the International Olympic Committee, benefits both strength and endurance athletes [19]. Indeed, protein supplements are frequently taken in an effort to meet this level. For energy demands, roughly 25% of daily protein should be consumed, i.e., around 2.0 to 2.5 g/kg [20]. Matter of fact, protein supplement popularity, especially among bodybuilders, has been largely impacted by promises of enhanced muscle mass, higher fat loss, improved performance, and improved recovery markers [21].

There have been quite different perspectives on the nutritional worth and effects of dietary protein and sport supplement products. This is notably true in terms of individual sports activity levels, as well as overall diet and metabolic condition [4]. Essentially, protein is a vital nutrient in the human diet throughout life. This is fundamentally because it secures growth in infancy, promotes muscle and bone metabolism, assures the maintenance and development of a proper neurological system, and aids in the maintenance of muscle mass and physical performance in later ages [22]. Athletes, like bodybuilders, may have heightened physiological protein requirements in multi-stress settings of goal-directed, frequent, intense, and/or protracted exercise routines to maintain adequate protein synthesis and energy generation, as well as proper immunological function [1].

Regarding protein supplements, whey PPs is the most popular ingredient [15], as it was in our study; however, products may also include casein, soy, hemp, pea, or rice protein. Whey PPs are soluble proteins that account for around 20% of the protein content of cow's

Table 1
Comparative analysis of microstructural and microvascular morphological variations between whey protein powder consumer and non-consumer bodybuilders.

Parameters		Whey protein powder consumers (n = 23) Mean±Standard deviation	Non-consumers (n = 21)	p value
Enhanced depth imaging optical coherence tomography				
Central macular thickness (µm)		270.55 ± 24.60	265.85 ± 12.44	0.402**
Subfoveal choroidal thickness (µm)		301.40 ± 38.91	278.12 ± 33.58	0.035**
Optical coherence tomography angiography				
SCP VD (%)	Whole	52.320 ± 2.777	53.104 ± 1.796	0.252**
	Foveal	24.607 ± 6.683	25.738 ± 5.497	0.532*
	Parafoveal	55.670 ± 3.659	56.035 ± 1.749	0.657**
	Perifoveal	52.775 ± 2.936	53.488 ± 1.851	0.319**
DCP VD (%)	Whole	57.980 ± 3.489	59.031 ± 3.728	0.335**
	Foveal	42.850 ± 8.671	42.950 ± 7.477	0.967*
	Parafoveal	59.555 ± 2.388	60.281 ± 2.435	0.318**
	Perifoveal	59.780 ± 3.559	60.688 ± 3.838	0.416**
FAZ parameters	FAZ area (mm^2)	0.249 ± 0.124	0.249 ± 0.105	0.989*
	FAZ perimeter (mm)	1.855 ± 0.477	1.894 ± 0.418	0.774*
	FD-300 (%)	57.916 ± 4.510	57.632 ± 3.516	0.812**
Capillary flow areas (mm^2)	Outer retinal	7.860 ± 1.549	7.931 ± 1.358	0.869**
	Choriocapillaris	20.294 ± 0.900	21.569 ± 5.948	0.349*

SCP=Superficial capillary plexus, DCP=Deep capillary plexus, VD=Vessel density, FAZ=Foveal avascular zone, FD-300=Foveal VD in a 300-µm wide region surrounding the FAZ, n=Number of participants, mm=millimeter, mm^2=Millimeter square µm=Micrometer,%=Percentage, *=Mann Whitney U, **=Student t-test.

Table 2
The optic nerve head parameter analysis between whey protein powder consumer and non-consumer bodybuilders.

Parameters		Whey protein powder consumers (n = 23) Mean±Standard deviation	Non-consumers (n = 21)	p value
Cup-disk ratio		0.105 ± 0.090	0.130 ± 0.096	0.371*
Global RNFL thickness (µm)		116.75 ± 10.41	114.50 ± 11.70	0.502**
VD (%)	wiVD	49.615 ± 2.539	48.754 ± 1.918	0.196**
	Inside disk	54.435 ± 3.290	53.965 ± 4.008	0.673**
	Peripapillary	51.365 ± 3.037	50.681 ± 2.519	0.408**

RNFL=Retinal nerve fiber layer, VD=Vessel density, wiVD=Whole image VD, n=Number of participants, µm=Micrometer,%=Percentage, *=Mann Whitney U, **=Student t-test.

milk. They are often separated from the liquid portion of milk during the cheese-making process, which is then followed by filtration and drying. The whey PP fraction contains a variety of proteins such as β-lactoglobulin, α-lactalbumin, immunoglobulins, and glycomacropeptides, all of which have different properties. They are high-quality proteins that contain all required and non-essential AAs, as well as a high concentration of BCAAs (*leucine, isoleucine,* and *valine*). In addition to the indirect effect on glycemic response, whey PPs improve vascular function and lower other cardiometabolic risks by reducing ambulatory blood pressure (BP) and thereby improving elements of hemodynamics [23]. This occurs when it is ingested with carbs, which induces fast digestion and consequently the release of AAs. Hence, whey PPs promote muscle building and fat loss, and its rapid absorption by the body aids not just in post-workout recovery but also in the prevention of cardiovascular diseases and the maintenance of a healthy metabolism. Despite its good properties, whey PP is indigestible for certain people since milk (lactose) contains sugar, which is a common allergy [24], though this was not the case in our study.

Considering the growing evidence that dairy proteins may play a role in improved microvascular function, whey PPs have been investigated in different studies to assess the short- and long-term effects on the microvascular system overall. However, there has been very little, if any, attention paid to the PP-related ocular microstructural and microcirculation changes. In our study, the duration of whey PP consumption ranged from 6 to 12 months, with a median of 9.5 months indicating chronic consumption ($>$ 4 weeks), which was associated with PP-related changes in both microstructure and microvascular morphology of the retina, choroid, and ONH, albeit with varying statistical significance. Most importantly, whey PP consumers were associated with increased SFCT and CMT, with the former being statistically significant compared to non-consumers. These changes were accompanied by markedly increased whole, inner, and peripapillary VDs in the ONH, despite the absence of statistical significance. Also, a lower cup-disk ratio among whey PP consumers was associated with a thicker RNFL. Unlike these findings, all macular regions had comparable decreased VDs; however, changes in the SCP and DCP were not statistically significant. Variable statistical significance in relation to investigated parameters could potentially be linked to a small study population, for which it is considered that enlarging the study population could provide clinically and statistically relevant outcomes.

Matter of fact, whey PP processing yields a high concentration of bioactive peptides and physiologically active AAs. These nutrients have different qualities that may influence many aspects of endothelial function and vascular health. These features include promoting the synthesis and bioavailability of nitric oxide (NO), lowering inflammation, and, most importantly, inhibiting the angiotensin-converting enzyme (ACE), a critical component of the renin-angiotensin system (RAS). The anti-hypertensive activities of whey PP-derived bioactive peptides are assumed to be mediated primarily via the RAS, an important BP regulator. It has long been recognized that peptides derived from milk proteins can suppress ACE effectively [25]. Furthermore, ACE destroys bradykinin, a 9-AA residue peptide generated by the kinin-kallikrein system in various cell types and tissues that acts as a vasodilator. While much attention so far has been on whey-derived peptides limiting angiotensin II production from angiotensin I, new research suggests that ACE inhibition, which inhibits bradykinin breakdown, may be equally essential. This causes the concentration of bradykinin to rise, resulting in a larger vasodilatory impact [26]. This could also explain why bodybuilders who had been consuming whey PPs for a long period had comparatively increased microstructural and microcirculation in the ocular posterior segment in our study. Even if all participants were non-obese and systemically normotensive, the effect of whey PP-derived peptides on the RAS system, specifically the prevention of vasoconstriction and promotion of vasodilation, may be an important factor in long-term BP reduction. This, in turn, could be related with less mechanical stress on the endothelium and, as a result, an overall vascular functional improvement.

Moreover, whey PPs promote insulin production and action [27]. Insulin is thought to be a two-edged sword when it comes to NO production and bioavailability, with insulin-mediated pathways influencing both vasorelaxation and vasoconstriction. Insulin stimulation of the ERK1/2 pathway results in an increase in endothelin synthesis, a vasoconstrictive polypeptide. However, insulin signaling also promotes the PI3K/AKT pathway, which leads to eNOS phosphorylation and increased NO generation, resulting in vasorelaxation [28]. In this context, an individual's level of insulin sensitivity or resistance may potentially be a modifying factor [29]. Animal studies demonstrate a comparable imbalance in insulin signaling in obese, glucose-intolerant mice versus lean mice, suggesting the idea that this contributes to a decreased NO production seen in cardio-metabolic disease [30]. Although our study included generally healthy non-obese young male bodybuilders, additional research on factors other than ocular microstructural and microvascular morphology, as well as correlation analyses, could aid in determining the precise effects of protein supplements, in this case whey PPs, both in the short- and long-term.

Our results should be interpreted cautiously, given the study's inherent limitations. It was a single-centered cross-sectional study with a relatively small sample size. This could have hampered our understanding of the processes underlying the link between protein supplementation and prospective posterior segment microstructural and microvascular morphological changes. Another limitation was that blood protein levels were not measured, which would have allowed for a more objective assessment of how much protein supplementation led to retinal, choroidal, and ONH microstructural and microvascular morphological changes. The inclusion of exclusively male healthy bodybuilders could potentially be viewed as a limitation. However, females, particularly those on oral contraceptives, may experience a dose-dependent effect in plasma protein levels [31]. Besides, despite the lack of a significant change in choroidal thickness in healthy females on oral contraceptives, there have been changes in choroidal microvasculature [32], as has changes in retinal DCP VDs [33]. Obviously, analysis of only male bodybuilders appears to have contributed to unbiased study outcomes that could have resulted in different clinically and statistically irrelevant outcomes if female bodybuilders, particularly those on oral contraceptives, had been included for analysis. In addition, residual confounding factors could have resulted in unexplained bias in the data analysis. Long-term large-scale longitudinal research incorporating diverse genders, protein supplements, and a wide age range could improve the efficacy of our study results.

Despite its limitations, to the best of our knowledge, our study is the first of its kind, and with its rigorous exclusion criteria to ensure maximal homogeneity of the participants, this could be considered as a positive element, as could the duration with which participants consumed whey PPs. Additionally, compared to older adults, a relatively lower average age of study participants is likely to influence microstructural and microvascular morphological results. Luckily, both study groups were roughly the same age, which could have mitigated the inherent bias in our results to some extent.

5. Conclusions

Protein supplements, particularly whey PPs, appear to be connected to a range of changes in the central retina and subfoveal choroid, as well as ONH microstructural and microvascular morphology. This suggests that paying attention to these clinical facts when doing ocular tests on patients attending gyms for bodybuilding and taking nutritional supplements, such as protein supplements, could be crucial.

Consent to participate

Informed consent was obtained from all study participants.

Ethics

Institutional review board approval was obtained from the local ethics committee (Afyonkarahisar Health Sciences University Ethic Committee, Approval code: 2011-KAEK-2: 2022/16-603).

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CRediT authorship contribution statement

Hamidu Hamisi Gobeka: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mustafa Doğan:** Conceptualization, Data curation, Formal analysis, Methodology, Resources, Software, Supervision, Writing – original draft, Writing – review & editing. **İbrahim Ethem Ay:** Conceptualization, Data curation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Eda Erdal:** Formal analysis, Investigation, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors report no conflict of interest for this research. The authors alone are responsible for the content and writing of this article.

Data availability

The data supporting our study findings are available from the corresponding author upon reasonable request.

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