·Clinical Research·

Effect of FFP2/N95 facemask wear on retinal and choroidal thickness profile in healthy subjects

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Abstract

● **AIM:** To investigate the influence of non-oil 95 (N95)/filtering facepiece 2 (FFP2) facemask wear on retinal thickness, choroidal thickness (CT), retinal nerve fiber layer thickness (RNFLT), and ganglion cell layer thickness (GCLT) in healthy subjects.

● **METHODS:** In this prospective study, 53 healthy participants who used FFP2/N95 facemask were enrolled. Participants underwent optical coherence tomography imaging before and at 1 and 4h following FFP2/N95 facemask wear. The last imaging session was performed 1h after FFP2/N95 removal. Retinal thickness, CT, RNFLT, and GCLT were assessed at each session. Vital parameters were also assessed.

● **RESULTS:** The pulse rate of the subjects significantly decreased at 1 and 4h compared to baseline values (P<0.05). No significant changes in retinal thickness, RNFLT, and GCLT were observed in the study. CT profile showed a significant increase at all measured locations except 1-mm temporal, 1-mm inferior and 2-mm inferior points following FFP2/N95 wear which turned to baseline values after FFP2/N95 removal. Pulse rate and CT changes at 4h were significantly correlated (P<0.05).

● **CONCLUSION:** Parasympathetic activation during FFP2/N95 facemask wear might have a role on elevated CT measurements in healthy individuals by virtue of increased choroidal blood flow.

● **KEYWORDS:** choroid; ganglion cell layer; FFP2/N95 facemask; retina; retinal nerve fiber layer; thickness

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INTRODUCTION

The non-oil 95 (N95)/filtering facepiece 2 (FFP2) masks, used as occupational safety equipment under normal conditions, have increased their frequency of use among healthcare workers and even for the normal population, as their protection is much higher with the pandemic[1-3]. There might be several adverse consequences due to extended use of these masks. It has been reported that respiratory compromise may occur due to hypoxia and hypercapnia after extended use of FFP-2 like masks[4].

Choroid is a vascularly active tissue supplying the outer retina. Its other essential functions include thermoregulation, arrangement of the position of the retina by choroidal thickness (CT) and producing several growth factors[5]. As choroid is one of the most highly vascularized tissue of the human body, investigators have shown alterations in CT based upon the hypoxia-related changes in several conditions[6-7]. Recently, a study has found a transient increase in CT after the use of FFP2/N95 facemask. Authors proposed hypercapnia as a possible reason for elevated CT following FFP2/N95 facemask wear[8].

In the present study, we aimed to assess the impact of FFP2/N95 facemask wear on retinal and CT profile of healthy subjects and relationship between vital parameters. For this purpose, retinal thickness, retinal nerve fiber layer thickness (RNFLT) and ganglion cell layer thickness (GCLT) was analysed by using spectral domain optical coherence tomography (SD-OCT). Enhanced depth imaging (EDI)-OCT was used to evaluate CT profile of the participants.

SUBJECTS AND METHODS

Ethical Approval This is a prospective observational study in full accord with the principles laid out in the Declaration of Helsinki. A local ethical approval was taken prior to the study with the reference number (2020-78). Informed consent explaining the methods of the study was obtained from all participants.

Eligibility Criteria Participants were recruited among healthcare workers in the same hospital. Inclusion criteria for the study were best corrected visual acuity of 20/20, no drug and/or alcohol intake, non-smoking, no history of glaucoma and/or any ocular pathology, no evidence of glaucomatous findings including glaucomatous optic disk alterations (*e.g.*, notching, excavation, focal neuroretinal rim thinning and peripapillary hemorrhage), glaucomatous visual field defects and retina nerve fiber layer loss, no history of any systemic disease, spherical refractive error ≤2 diopters (D) and/or cylindrical refractive error ≤1 D. Exclusion criteria were high intraocular pressure (IOP) levels (≥21 mm Hg), history of glaucoma, any history of ocular disease, history of ocular trauma and/or ocular intervention, history of any systemic disorder (*e.g.*, diabetes, hypertension, other cardiovascular pathology), topical and/or systemic medication use (*e.g.*, steroids) and axial length (AL) values ≥24 mm.

Examination Protocol All subjects underwent a complete ophthalmic examination, including best corrected visual acuity and refractive assessment, slit-lamp biomicroscopic evaluation, IOP measurement with applanation tonometry and dilated fundus examination. Moreover, AL and anterior chamber depth (ACD) measurements were performed by using a optical biometry device (LenStar LS 900, Haag Streit Diagnostics).

Each participant was invited to wear FFP2/N95 facemask (3M VFlex™ 9152E) following baseline examination at 8.00 *a.m.* Consecutive measurements were taken at 1 and 4h after FFP2/N95 facemask wear. Last measurements were done 1h following FFP2/N95 facemask removal. Study participants were instructed not to remove the mask during the study period. The participants in the study were asked not to consume any caffeine-containing drinks and not to engage in unusual heavy physical activity. Subjects were only allowed to take a light lunch with minimal water.

Optical Coherence Tomography Analysis Following pupil dilation, all participants were examined using spectral domain OCT (Spectralis OCT; Heidelberg Engineering, Heidelberg, Germany). One experienced technician performed OCT examinations. Images were obtained using horizontal raster pattern scans, which were obtained *via* a 30°×25° scan field. CT measurements were done using EDI-OCT mode based on previous descriptions[9]. Automatic real time (ART) function mode resamples multiple frames (B-scans) for noise reduction. Only scans with a high signal-to-noise ratio (minimum of 20 dB) were included and used for the CT evaluation. CT was determined manually with caliper vertical line perpendicular to retina from the outer surface of the hyper-reflective line corresponding to Bruch's mebrane to the line of the inner surface of the sclera. CT was measured at 9 locations, including subfoveal point and at 1-mm intervals to the nasal, temporal, superior and inferior from subfoveal point (Figure 1). Images were evaluated by two experienced investigators (Gunay BO and Esenulku CM) who were masked to the measurement time. Reliability of CT measurements was assessed with intraclass correlation analysis. First investigator's (Gunay BO) CT measurements were included for the statistical analysis. Retinal thickness was examined using the retinal thickness map analysis protocol with five Early Treatment Diabetic Retinopathy Study (ETDRS) subfields, including central macular thickness (CMT) and at 3-mm nasal, temporal, superior, and inferior locations. Mean RNFLT and supero-nasal (91°–135°), supero-temporal (46–90°), temporal (316°–45°), infero-temporal (271°–315°), infero-nasal (226°–270°) and nasal (136°–225°) RNFLT was provided by the software module in the device. Automatic segmentation of GCLT was performed in single horizontal foveal scans to measure central GCLT and at 3-mm intervals to the superior, temporal, inferior and nasal locations.

Statistical Analysis Statistical analysis was performed using Statistical Package for the Social Sciences programme (Version 22; SPSS, Chicago, IL, USA). The Shapiro-Wilk test was used to determine the normality of the data. Baseline characteristics were given as mean±standard deviation (SD). Other measured variables were presented using mean [95% confidence interval (CI), lower bound–upper bound]. General linear model repeated measures analysis of covariance adjusted by age was used to compare measured parameters before and at 1, 4h and last visit after FFP2/N95 facemask wear. Fisher's least significant difference was used to analyse pairwise comparisons. Pearson correlation test was performed to examine the possible associations among measured parameters. Intraclass correlation coefficients (ICCs) were calculated to assess reliability of the CT measurements. Significance was assessed at the levels of $P<0.05$.

RESULTS

Baseline Characteristics Totally 53 participants were present in the study. There were 32 females (60.4%) and 21 males (39.6%). The mean±SD age of the subjects was 40.21±8.87y (range, 24 to 62y). The mean±SD body mass index (BMI) was 25.02%±3.43% (range, 15.80% to 30.80%) and mean spherical equivalent (SE)±SD was -0.41±1.3 D (range, -3.37 to +3.37 D). Table 1 summarizes changes in vital parameters of the subjects in the study. Pulse rate was significantly lower at 1h (mean, 80.8) and 4h (mean, 79.5) compared to baseline values (mean, 85.5, P=0.002 for baseline *vs* 1h and P=0.005 for baseline *vs* 4h). No significant difference of pulse rate was observed at last visit (mean, 84.5) compared to baseline measurements (P=0.507). Pulse rate change demostrated in Figure 2B. Saturation, systolic and diastolic blood pressures did not show significant changes in the study ($P>0.05$ for all).

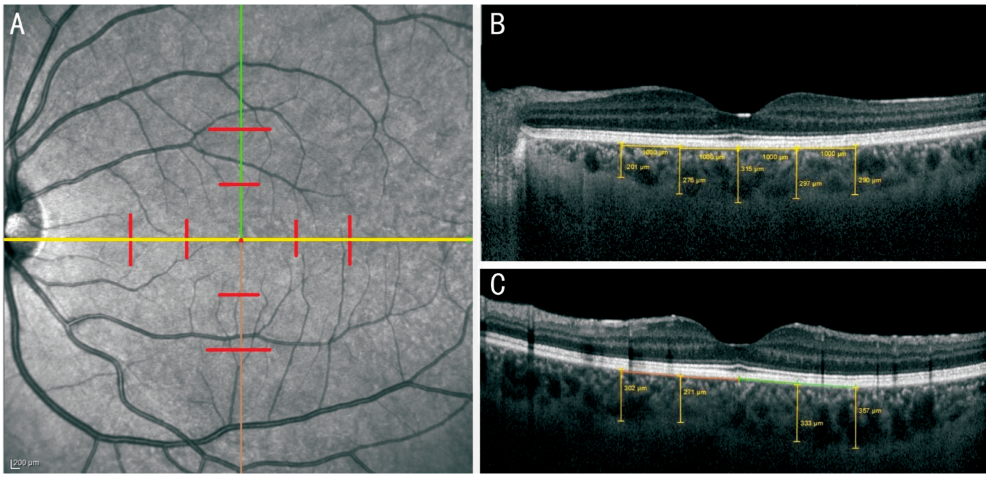

Figure 1 Measurement of choroidal thickness (CT) profile A: CT measured locations in infrared reflectance imaging; B: Subfoveal CT, 1- and 2-mm nasal and temporal CT measurements were performed along the horizontal yellow line; C: The 1- and 2-mm superior and inferior CT measurements were performed along the vertical green (superior) and orange line (inferior).

Table 1 Changes of vital signs in the study mean (95%CI)

Parameters	Saturation (%)	Pulse rate	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)
Baseline	97.04 (96.6–97.4)	85.5 (81.9–89.0)^{a}	105.0 (100.5–109.5)	70.1 (67.6–72.7)
1h	96.7 (96.2–97.3)	80.8 (77.9–83.8)^{b}	107.0 (103.1–110.8)	71.0 (68.1–73.9)
4h	96.5 (95.9–97.0)	79.5 (75.4–83.5)^{c}	106.0 (100.9–111.1)	71.7 (68.3–75.1)
Last	96.4 (95.4–97.3)	84.5 (80.8–88.2)^{d}	105.2 (100.8–109.7)	69.1 (66.1–71.4)
P	0.43	0.003^{e}	0.79	0.27

Pairwise comparisons, P=0.002$^{a\text{-}b}$, 0.005$^{a\text{-}c}$, 0.507$^{a\text{-}d}$, 0.485$^{b\text{-}c}$, 0.035$^{b\text{-}d}$, 0.013$^{c\text{-}d}$. ^{e}General linear model repeated measures, P<0.05.

Table 2 shows CMT profile, RNFLT, and GCLT obtained in the study. No significant changes of CMT, RNFLT and GCLT were observed (P>0.05 for all).

Table 3 and Figure 2A summarize the changes in CT profile in the study. CT profile significantly increased at all locations except 1-mm temporal, 1-mm inferior and 2-mm inferior at 1 and 4h compared to baseline measurements (P<0.05). Pairwise comparisons showed significant differences of CT profile between baseline-1h, baseline-4h, 1-4h, 1h-last, and 4h-last examination at measured points.

Table 4 summarizes changes in IOP, AL and ACD. IOP was significantly lower at last examination compared to baseline measurement (mean, 14.1 mm Hg *vs* mean, 12.7 mm Hg, P<0.001). AL and ACD exhibited no significant changes in the study (P>0.05 for both).

Table 5 shows correlation analyses between changes in CT profile at 4h and age, BMI, and IOP, saturation, pulse rate, systolic and diastolic blood pressures changes. There was a positive correlation between age and changes in subfoveal CT (r=0.386, P=0.004), 1-mm nasal CT (r=0.338, P=0.013), 1-mm superior CT (r=0.396, P=0.003) and 2-mm superior CT (r=0.341, P=0.013). Pulse rate changes were positively correlated with changes in 1-mm nasal CT (r=0.340, P=0.014), 2-mm nasal CT (r=0.314, P=0.024), 1-mm superior CT (r=0.283, P=0.042) and 2-mm superior CT (r=0.372, P=0.007). Table 6 presents ICCs of CT measurements in the study. The ICCs showed good reliability at all measured locations (ICCs>0.90).

DISCUSSION

Our study demonstrated significant changes in CT profile of healthy subjects due to FFP2/N95 facemask wear. We observed that choroid showed significant increase at subfoveal, 1-mm nasal, 2-mm nasal, 2-mm temporal, 1-mm superior and 2-mm superior points 1 and 4h after wearing FFP2/N95 facemask which then returned to baseline values 1h following facemask removal. Moreover, regarding vital signs of our subjects, pulse rate significantly reduced at 1 and 4h after FFP2/N95 facemask wear as compared to baseline measurements. Pulse rate then reached to baseline values 1h following facemask removal. Retinal thickness, RNFLT and GCLT did not show significant changes in the study.

Extended use of personal protective equipment has become routine of healthcare professionals during the current pandemic. FFP2/N95 facemasks are primary components of such equipments that effectiveness of them have already been shown during previous epidemics regarding their role in prevention of viral transmission[10]. However, FFP2/N95 facemask wear was also demonstrated to cause cardiac and respiratory changes along with subjective perception of discomfort[10]. Their extended use may result in significant

Table 2 Changes in retinal thickness, RNFLT, and GCLT in the study

µm, mean (95%CI)

Parameters	CMT	N-RT	T-RT	S-RT	I-RT	Mean RNFLT	SN-RNFLT	ST-RNFLT	T-RNFLT	IT-RNFLT	IN-RNFLT	N-RNFLT	Central GCLT	S-GCLT	T-GCLT	I-GCLT	N-GCLT
Baseline	269.4 (264.8–274.0)	349.4 (345.1–353.6)	336.1 (332.7–339.6)	351.1 (347.4–354.7)	347.0 (343.3–350.7)	96.6 (93.7–99.6)	102.1 (94.4–109.7)	133.0 (127.8–138.3)	76.1 (73.2–79.0)	146.4 (140.9–151.8)	107.9 (100.7–115.1)	69.4 (65.4–73.4)	15.1 (14.0–16.2)	55.1 (53.6–56.5)	50.0 (48.7–51.3)	55.1 (53.7–56.5)	53.6 (51.6–55.6)
1h	269.5 (264.9–274.2)	348.4 (344.1–352.8)	335.6 (332.1–339.1)	350.9 (347.3–354.5)	346.8 (343.1–350.6)	96.2 (93.1–99.3)	101.3 (93.8–108.8)	131.7 (125.9–137.5)	74.9 (71.9–77.9)	146.2 (140.2–152.2)	109.4 (101.1–117.7)	70.8 (66.7–74.9)	15.1 (14.0–16.1)	55.4 (54.0–56.8)	50.2 (48.7–51.6)	54.8 (53.3–56.2)	53.5 (51.6–55.3)
4h	270.0 (265.6–274.5)	349.4 (345.1–353.7)	335.8 (332.2–339.3)	351.2 (347.6–354.7)	346.9 (343.2–350.7)	96.6 (93.5–99.6)	103.8 (95.9–111.6)	133.0 (127.0–138.9)	74.6 (72.0–77.2)	144.4 (139.4–149.4)	111.5 (103.8–119.2)	71.5 (67.6–75.5)	15.0 (14.0–16.0)	55.4 (54.1–56.8)	50.2 (48.8–51.6)	54.7 (53.3–56.1)	53.7 (51.8–55.6)
Last	270.2 (265.5–274.9)	348.2 (343.8–353.6)	335.5 (332.0–339.0)	350.7 (347.0–354.3)	346.6 (342.7–350.5)	96.8 (93.7–100.0)	100.3 (93.7–107.0)	131.8 (125.8–137.7)	74.6 (71.2–77.6)	146.7 (140.4–153.0)	111.6 (103.2–119.9)	72.5 (67.7–77.3)	14.9 (13.9–15.9)	55.1 (53.7–56.5)	49.8 (48.5–51.1)	54.6 (53.1–56.1)	53.3 (51.3–55.3)
P	0.16	0.50	0.47	0.43	0.59	0.18	0.21	0.68	0.16	0.54	0.23	0.10	0.65	0.057	0.44	0.04	0.41

CMT: Central macular thickness; RT: Retinal thickness; RNFLT: Retinal nerve fiber layer thickness; GCLT: Ganglion cell layer thickness; N: Nasal; T: Temporal; S: Superior; I: Inferior; SN: Superonasal; ST: Superotemporal; IT: Inferotemporal; IN: Inferonasal.

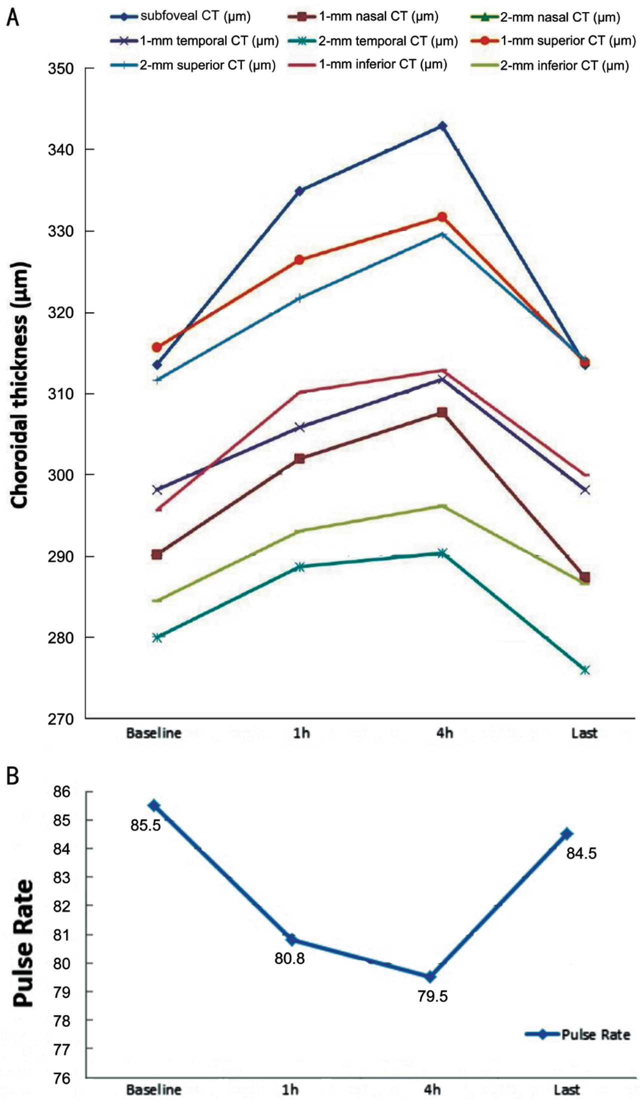

Figure 2 Changes of choroidal thickness (CT) profile (A) and the pulse rate (B) at 1, 4h, and last examination in the study.

systemic physiologic compromise in healthy individuals leading to hypoxia, hypercapnia, dizziness, headache and thermal discomfort[4].

Choroid plays a major role in the normal physiology of the eye. Choroidal blood flow is affected in various systemic conditions. Investigators have demonstrated that local tissue hypoxia might influence the choroidal vasculature. Choroidal blood flow was shown to be increased in response to hypoxia[6,11]. The CT has been considered as a surrogate marker for choroidal blood flow[12-13]. Correlation between CT and choroidal blood flow has been investigated and it has been shown that higher choroidal blood flow was associated with increased CT[14]. Supportively, we identified significantly increased CT following FFP2/N95 facemask wear at 1 and 4h at SFCT and 1-mm and 2-mm nasal, superior and 2-mm temporal CT. CT values returned to baseline levels 1h after removal of FFP2/N95 facemask. FFP2/N95 facemasks can induce flow resistance and cause a discernable increase in

Table 3 CT profile changes in the study μm, mean, 95%CI

Parameters	Baseline	1h	4h	Last	*P*
SFCT	313.5 (296.9–330.2)	334.9 (317.8–352.1)	342.9 (324.8–361.0)	313.5 (298.0–329.0)	0.018
1-mm nasal CT	290.1 (273.0–307.2)	301.9 (283.3–320.5)	307.6 (288.4–326.8)	287.3 (269.7–304.8)	0.001
2-mm nasal CT	240.3 (221.2–259.4)	246.7 (227.5–266.0)	247.6 (227.2–267.9)	232.9 (214.4–251.4)	0.017
1-mm temporal CT	298.1 (282.7–313.4)	305.8 (289.8–321.8)	311.7 (295.9–327.6)	298.1 (283.6–312.7)	0.133
2-mm temporal CT	279.9 (264.7–295.1)	288.6 (273.0–304.3)	290.3 (276.0–304.7)	275.9 (260.8–290.9)	0.028
1-mm superior CT	315.6 (298.4–332.7)	326.4 (309.0–343.7)	331.7 (314.4–349.0)	313.8 (297.0–330.7)	0.001
2-mm superior CT	311.6 (294.5–328.6)	321.7 (305.2–338.1)	329.6 (311.8–347.5)	314.1 (297.0–331.2)	0.015
1-mm inferior CT	295.6 (279.1–312.1)	310.1 (291.9–328.2)	312.8 (293.0–332.7)	299.9 (281.0–318.7)	0.250
2-mm inferior CT	284.4 (267.9–300.9)	293.0 (276.8–309.3)	296.1 (278.6–313.6)	286.5 (269.7–303.2)	0.702

SFCT: Subfoveal choroidal thickness; CT: Choroidal thickness. Pairwise comparisons: For SFCT, baseline-1h and baseline-4h; $P<0.001$, 1h-4h; $P=0.002$, 4h-last; $P<0.001$, baseline-last; $P=0.99$. For 1-mm nasal CT, Baseline-1h; $P=0.001$, Baseline-4h; $P<0.001$, 1h-4h; $P=0.09$, 4h-last; $P<0.001$, Baseline-last; $P=0.50$. For 2-mm nasal CT, Baseline-1h; $P=0.003$, Baseline-4h; $P=0.04$, 1h-4h; $P=0.78$, 4h-last; $P=0.001$, Baseline-last; $P=0.006$. For 2-mm temporal CT, Baseline-1h; $P=0.04$, Baseline-4h; $P=0.01$, 1h-4h; $P=0.61$, 4h-last; $P=0.001$, Baseline-last, $P=0.28$. For 1-mm superiorCT, Baseline-1h; $P=0.01$, Baseline-4h; $P=0.005$, 1h-4h; $P=0.22$, 4h-last; $P<0.001$, Baseline-last; $P=0.71$. For 2-mm superior CT, Baseline-1h; $P=0.04$, Baseline-4h; $P=0.01$, 1h-4h; $P=0.08$, 4h-last; $P=0.01$, Baseline-last; $P=0.52$.

inhaled carbon dioxide with reduced inspired oxygen resulting in breathing discomfort[15]. Furthermore, increased resistance to inspiratory and expiratory flow, especially when continues beyond 10min, has been shown to result in respiratory alkalosis, fatigue and decreased physical work capacity[16]. The effect of increased carbon dioxide levels on choroidal blood flow has been found in experimental investigations. It was stated that hypercarbia leads to significantly increased choroidal blood flow[17]. In addition, it has been suggested that slowing of breathing during FFP2/N95 facemask wear might induce an autonomic cardiovascular regulation with reduced sympathetic and increased parasympathetic responses, resulting a shift toward vagal activities as suggested previously[18]. Significantly reduced pulse rate in the present study may propose a parasympathetic activation during FFP2/N95 facemask wear. We also observed significant relationships between 4h changes of CT and pulse rate in our study as revealed by the correlation analysis. Because parasympathetic innervation has been shown to vasodilate and increase choroidal blood flow, increased CT might be due to this parasympathetic response rather than hypoxia in the present study[19-20].

In support of our findings, Karatas Durusoy *et al*[8] recently published their results regarding CT increase after FFP2/N95 facemask wear. They measured CT at the center of the fovea and 1 mm nasal and temporal to the fovea 2h after wearing the FFP2/N95 facemask and 15min after removing the facemask. But, authors of that study analysed CT after FFP2/N95 use without assessing baseline measurements and vital parameters. They measure only subfoveal, 1 mm temporal and nasal point, we exhibited CT increase at multiple locations after FFP2/N95 facemask wear compared to the baseline CT measurements in our study and CT values also returned to almost baseline levels 1h following FFP2/N95 facemask removal. Moreover FFP2/N95 masks may contain valve or not depending on the manifacture. This information was not given in the abovementioned study. We have also revealed the regional differences of the change in the CT after FFP2/N95 facemask wear. Furthermore, we also detected significant changes in pulse rate. As we noted earlier, significant correlation between pulse rate and CT in our subjects may indicate the influence of parasympathetic response on CT alterations rather than hypoxia.

Table 4 Changes in IOP, AL, and ACD in the study mean (95%CI)

Parameters	IOP (mm Hg)	AL (mm)	ACD (mm)
Baseline	14.1 (13.4–14.8)^a	23.6 (23.3–23.8)	3.43 (3.34–3.53)
1h	13.6 (13.0–14.3)^b	23.61 (23.3–23.8)	3.43 (3.33–3.53)
4h	13.4 (12.8–14.0)^c	23.61 (23.3–23.8)	3.42 (3.33–3.52)
Last	12.7 (12.0–13.5)^d	23.61 (23.3–23.8)	3.43 (3.33–3.53)
P	<0.001	0.54	0.59

IOP: Intraocular pressure; AL: Axial length; ACD: Anterior chamber depth. Pairwise comparisons for IOP changes: $P=0.077^{a\text{-}b}$, $0.016^{a\text{-}c}$, $<0.001^{a\text{-}d}$, $0.380^{b\text{-}c}$, $0.011^{b\text{-}d}$, $0.019^{c\text{-}d}$.

We did not observe significant changes regarding retinal thickness, RNFLT and GCLT following FFP2/N95 facemask wear. Studies have shown favorable effect of hyperoxia on retinal thickness in ischemic retinal vascular conditions. This supports the idea that retinal thickness can show alterations due to an hypoxia-induced mechanism[21-22]. Increased RNFLT has been shown secondary to local hypoxia and hypercapnia-induced dilatation of the retinal vessels in healthy participants[23]. Experimental studies have also demonstrated functional retinal cell protection in the excess of carbon dioxide in the blood in ischemia-reperfusion models[24]. We

Table 5 Correlation results between choroidal thickness changes at 4h and other clinical factors in the study

Parameters	SFCT	1-mm nasal CT	2-mm nasal CT	1-mm temporal CT	2-mm temporal CT	1-mm superior CT	2-mm superior CT	1-mm inferior CT	2-mm inferior CT
Age									
r	0.386	0.338	0.195	0.249	0.266	0.396	0.341	0.118	-0.060
P	0.004	0.013	0.163	0.073	0.054	0.003	0.013	0.401	0.668
BMI									
r	0.121	0.154	-0.019	-0.028	0.120	0.144	0.098	0.120	-0.002
P	0.390	0.272	0.891	0.841	0.392	0.304	0.485	0.393	0.988
IOP									
r	-0.166	0.024	-0.001	-0.229	-0.039	0.196	0.222	0.233	0.126
P	0.234	0.867	0.993	0.100	0.783	0.159	0.111	0.093	0.367
Saturation									
r	-0.128	-0.097	0.065	-0.030	-0.034	-0.246	-0.083	-0.264	-0.018
P	0.366	0.495	0.647	0.835	0.812	0.079	0.560	-0.059	0.901
Pulse rate									
r	0.127	0.340	0.314	0.134	0.130	0.283	0.372	0.152	0.044
P	0.371	0.014	0.024	0.343	0.358	0.042	0.007	0.283	0.755
SBP									
r	0.072	0.021	-0.088	0.102	0.127	-0.044	0.006	0.092	-0.055
P	0.611	0.882	0.534	0.471	0.371	0.757	0.965	0.514	0.697
DBP									
r	0.093	0.068	-0.043	-0.062	0.187	-0.040	-0.006	0.138	-0.039
P	0.511	0.631	0.761	0.665	0.183	0.781	0.964	0.328	0.781

r: Pearson correlation analysis, $P<0.05$. CT: Choroidal thickness; SFCT: Subfoveal choroidal thickness; BMI: Body mass index; IOP: Intraocular pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

Table 6 Repeatability and reliability of CT measurements in the study

Parameters	Baseline ICC (95%CI)	1h ICC (95%CI)	4h ICC (95%CI)	Last visit ICC (95%CI)
SFCT	0.994 (0.989–0.996)	0.994 (0.990–0.997)	0.995 (0.991–0.997)	0.992 (0.987–0.996)
1-mm nasal CT	0.993 (0.988–0.996)	0.995 (0.991–0.997)	0.995 (0.992–0.997)	0.973 (0.954–0.985)
2-mm nasal CT	0.993 (0.988–0.996)	0.988 (0.978–0.993)	0.988 (0.979–0.993)	0.992 (0.986–0.995)
1-mm temporal CT	0.984 (0.973–0.991)	0.988 (0.979–0.993)	0.990 (0.983–0.994)	0.963 (0.937–0.979)
2-mm temporal CT	0.984 (0.972–0.991)	0.994 (0.990–0.997)	0.988 (0.980–0.993)	0.983 (0.971–0.991)
1-mm superior CT	0.983 (0.970–0.991)	0.962 (0.932–0.979)	0.985 (0.974–0.992)	0.995 (0.991–0.997)
2-mm superior CT	0.952 (0.915–0.973)	0.963 (0.934–0.979)	0.967 (0.942–0.982)	0.949 (0.910–0.971)
1-mm inferior CT	0.987 (0.976–0.993)	0.990 (0.982–0.994)	0.984 (0.971–0.991)	0.993 (0.987–0.996)
2-mm inferior CT	0.993 (0.987–0.996)	0.995 (0.991–0.997)	0.995 (0.992–0.997)	0.992 (0.986–0.996)

CT: Choroidal thickness; SFCT: Subfoveal choroidal thickness; ICC: Intraclass correlation.

believe that FFP2/N95 facemask may not alter retinal thickness profile at least in the acute period after wearing it.

There are several factors that should be taken into account when CT is assessed. Studies have shown that age, IOP and AL might have an impact on CT measurements in an healthy individual. Investigators have shown an inverse association between CT and increasing age and AL[25-26]. Furthermore, some authors have found decreased CT as a result of increased IOP, while some others could not reveal a relationship between CT and IOP values[27-28]. Although we did not find significant correlation between changes in CT profile and IOP in our study, IOP had a tendency to reduce during the study period. We believe that IOP decrease in our study could be due to the normal diurnal variation of IOP[29].

Investigators have demonstrated significant diurnal changes of CT in normal population with higher CT early in the morning and a decreasing trend during the day to at about 5.00 *p.m.*[30]. However, CT patterns in our study differed between before and after FFP2/N95 facemask wear. While an increasing CT trend was observed during FFP2/N95 wear in the study, CT decreased and returned to almost baseline values following FFP2/N95 removal. This incongruence of CT variation pattern in the present study, in fact, may confirm the effect of FFP2/N95 facemask wear on CT profile of our subjects.

The current study has some limitations. First, we performed manual segmentation of retinal pigment epithelium and chorioscleral border to measure CT during EDI-OCT analyis. This might influence the accuracy of CT measurements. Previous studies have exhibited that EDI-OCT assessment of CT is a highly reliable and reproducible method in healthy subjects[31-32]. Consistently, we observed good reliability of CT measurements based on ICC analysis in our study. Second, we did not have data to show hypercarbia in the study. Furthermore, no control group has been included in the present study in order to compare CT changes after FFP2/N95 facemask wear with those after a standard surgical mask wear. In our opinion, this will clearly manifest the impact of FFP2/N95 facemask on CT profile in healthy people.

In conclusion, our study showed that FFP2/N95 facemask wear results in significantly increased CT profile of healthy subjects without any changes in retinal thickness, RNFLT and GCLT. This effect diminished after FFP2/N95 facemask removal. Parasympathetic activation during FFP2/N95 facemask wear might have a role on elevated CT measurements in healthy individuals by virtue of increased choroidal blood flow. One can analyse choroidal hemodynamics to better expose the impact of FFP2/N95 facemask wear on choroid. We believe that physicians should be aware of this possible result while evaluating CT in an individual wearing a FFP2/N95 facemask.

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