Dietary ω-3 polyunsaturated fatty acids are protective for myopia

Miaozhen Pan[a,b,1], Fei Zhao[a,b,1], Bintao Xie[a,b], Hao Wu[a,b], Sen Zhang[a,b], Cong Ye[a,c], Zhenqi Guan[a,b], Lin Kang[a,b], Yuqing Zhang[a,b], Xuan Zhou[a,b], Yi Lei[a,b], Qi Wang[a,b], Li Wang[a,b], Fan Yang[a,b], Chenchen Zhao[a,b], Jia Qu[a,b,c,d,2], and Xiangtian Zhou[a,b,c,d,e,2]

[a]School of Ophthalmology and Optometry and Eye Hospital, Wenzhou Medical University, Wenzhou 325027, China; [b]State Key Laboratory of Optometry, Ophthalmology, and Vision Science, Wenzhou 325027, China; [c]National Clinical Research Center for Ocular Diseases, Wenzhou 325027, China; [d]Oujiang Laboratory, Zhejiang Lab for Regenerative Medicine, Vision and Brain Health, Wenzhou 325001, China; and [e]Research Unit of Myopia Basic Research and Clinical Prevention and Control, Chinese Academy of Medical Sciences, Wenzhou 325027, China

Edited by Frank Schaeffel, Ophthalmic Research Institute, University of Tübingen, Tübingen, Germany, and accepted by Editorial Board Member Jeremy Nathans September 3, 2021 (received for review March 13, 2021)

Myopia is a leading cause of visual impairment and blindness worldwide. However, a safe and accessible approach for myopia control and prevention is currently unavailable. Here, we investigated the therapeutic effect of dietary supplements of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) on myopia progression in animal models and on decreases in choroidal blood perfusion (ChBP) caused by near work, a risk factor for myopia in young adults. We demonstrated that daily gavage of ω-3 PUFAs (300 mg docosahexaenoic acid [DHA] plus 60 mg eicosapentaenoic acid [EPA]) significantly attenuated the development of form deprivation myopia in guinea pigs and mice, as well as of lens-induced myopia in guinea pigs. Peribulbar injections of DHA also inhibited myopia progression in form-deprived guinea pigs. The suppression of myopia in guinea pigs was accompanied by inhibition of the "ChBP reduction–scleral hypoxia cascade." Additionally, treatment with DHA or EPA antagonized hypoxia-induced myofibroblast transdifferentiation in cultured human scleral fibroblasts. In human subjects, oral administration of ω-3 PUFAs partially alleviated the near-work–induced decreases in ChBP. Therefore, evidence from these animal and human studies suggests ω-3 PUFAs are potential and readily available candidates for myopia control.

ω-3 PUFAs | choroidal blood perfusion | scleral hypoxia | myofibroblast transdifferentiation | myopia

The myopia epidemic is now becoming a significant public health concern to modern society (1, 2). The percentage of the global population having myopia was predicted to increase from 28.3% in 2010 to 49.8% in 2050 (3). In East Asia, this percentage could even reach 90%, with up to 20% of the cases potentially developing into high myopia (refraction ≤−6 diopters [D]) (4, 5), which is one of the leading causes of irreversible blindness (6). The dramatic increase in the incidence of myopia, due in part to COVID-19 home confinement and increased online-viewing time, further highlights the importance of identifying a safe and effective approach to myopia control (7, 8).

Behavioral, pharmacological, and optical interventions are the current approaches for myopia control, all of which have unique limitations. Behavioral intervention (such as increasing the time spent outdoors) retards the progression of myopia into high myopia (9). However, competitive educational systems, as well as lifestyles that incorporate increasingly more electronic products (the prevalence of which tends to reduce the amount of time spent outdoors), are hard to avoid. Pharmacological intervention, such as the use of atropine drops, is effective in limiting myopia progression. Its use, however, is off-label in most areas, and widespread acceptance is restricted because of the potential side effects such as chronic pupillary dilatation, loss of accommodation, and declining long-term effectiveness for sustained myopia control (10–12). Mori et al. reported that the dietary intake of crocetin, a naturally occurring apocarotenoid dicarboxylic acid found in crocus and other plants, could prevent myopia development in a mouse model and in children (13, 14), but more clinical studies are needed to prove the efficacy and safety of this agent. Optical corrections such as orthokeratology and peripheral defocusing lenses suffer from risks of infectious keratitis (15) and the requirement of professional support (16). Such limitations are critical, considering the implications of employing these approaches in a larger population, especially where the availability of medical services is limited.

Thus, targeting multiple signaling cascades that promote the development of myopia, from retinal image processing to scleral growth, may be an effective strategy for myopia control. Previous studies have highlighted the significance of the cascade of events wherein the reduction of choroidal blood perfusion (ChBP, refers to the amount of choroidal blood flow) induces scleral hypoxia and myopia (17–20). The cascade begins when visual stimulus–induced myopic blur causes a reduction of choroidal thickness (ChT) and ChBP (20). In turn, these physiological changes induce hypoxia in the sclera, which is dependent on oxygen delivered by the choroidal vasculature (21). Hypoxia activates the hypoxia-induced factor-1α (HIF-1α) signaling pathway, which subsequently promotes scleral fibroblast-myofibroblast transdifferentiation and extracellular matrix (ECM) remodeling and in turn the development and progression of myopia (17).

Significance

Myopia is a leading cause of visual impairment. However, a safe and accessible approach for myopia control is currently unavailable. In the present study, we demonstrated that dietary supplements of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) attenuated myopia development in animal models. ω-3 PUFAs also ameliorated near-work–induced decreases in choroidal blood perfusion in young adults. Hence, when safety and effectiveness have been confirmed in future clinical studies, ω-3 PUFAs should be attractive candidates for myopia control in humans.

Author contributions: J.Q. and Xiangtian Zhou designed research; M.P., F.Z., B.X., H.W., S.Z., C.Y., Z.G., L.K., Y.Z., Xuan Zhou, Y.L., Q.W., L.W., F.Y., and C.Z. performed research; M.P. and F.Z. analyzed data; and M.P. and F.Z. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission. F.S. is a guest editor invited by the Editorial Board.

[1]M.P. and F.Z. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: jqu@wz.zj.cn or zxt@mail.eye.ac.cn.

This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2104689118/-/DCSupplemental.

Published October 21, 2021.

In the absence of effective treatments, and given the additional safety considerations when administering therapeutic agents to children (who are most vulnerable to myopia development), investigation into a dietary supplement for a safe and accessible approach for myopia control and prevention becomes compelling. However, research in this area is still new. Because of the beneficial effect of supplemental dietary omega-3 polyunsaturated fatty acids (ω-3 PUFAs) on cardiovascular health, daily supplements have been recommended for intake by the Food and Agriculture Organization of the United Nations (2010), which endorses the use of two types of ω-3 PUFAs, videlicet, 250 mg/day each of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) for adults (22). Mammalian brains are invariably rich in DHA (23), and numerous studies have defined the importance of ω-3 PUFAs, particularly DHA, in human neuronal development in different developmental stages (24–26). Recently, an untargeted mass spectrometric assay reported that the amounts of serum fatty acid metabolites were reduced in myopic human subjects compared to nonmyopic subjects (27). In particular, the levels of serum DHA were significantly lower in the myopic group (27). DHA and EPA can promote relaxation of vascular smooth muscle cells and vasodilation (28–31), inhibit cancerous cell growth and survival by reducing HIF-1α expression (32–34), and suppress transforming growth factor (TGF)-β1–mediated myofibroblast transdifferentiation (35–37). These effects of DHA and EPA are also known to be associated with the inhibition of myopia development.

The effects of ω-3 PUFAs on systemic conditions and on myopic subjects, combined with their involvement in mediating blood perfusion, HIF-1α, and cellular proliferation, prompted us to investigate if ω-3 PUFAs could suppress myopia development through modulating ChBP and scleral hypoxia. Thus, in this study, we first assessed the ability of ω-3 PUFAs supplementation to suppress myopia development in different animal models. We then examined the effects of DHA and EPA on ChBP and scleral hypoxia. We also determined if DHA or EPA could antagonize the effects of hypoxia on cultured human scleral fibroblasts (HSFs). Finally, we administered ω-3 PUFAs supplements to humans and observed their influence on near-work–induced ChBP reduction.

Results

Daily Gavage of ω-3 PUFAs Reduced Myopia Progression in Guinea Pigs and Mice. To assess the effect of ω-3 PUFAs on myopia development, we first determined if oral gavage of ω-3 PUFAs (DHA 300 mg plus EPA 60 mg) could inhibit myopia development in guinea pigs and mice. Neither the olive oil control (with a low content of PUFAs) nor the ω-3 PUFAs had significant effects on refraction, anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), axial length (AL), or radius of the corneal curvature (RCC) during normal refractive development in guinea pigs (*SI Appendix*, Fig. S1 *A–F*). Additionally, olive oil had no significant effects on refraction, ACD, LT, VCD, AL, or body weight during form deprivation (FD) compared to the control guinea pig group (FD only, no supplements) (Fig. 1 *A–C* and *SI Appendix*, Fig. S2 *A–C*).

After 1 and 2 wk of FD, significant FD myopia (FDM) was induced in all the FD-only, olive oil–treated, and ω-3 PUFA–treated guinea pigs (Fig. 1*A*). However, after 2 wk of treatment with ω-3 PUFAs, the induced myopic refractive error was 31.9% lower in the ω-3 PUFAs–treated group than in the olive oil–treated group ($P < 0.01$; Fig. 1*A*). The VCD and AL elongations were significantly smaller in the ω-3 PUFA–treated than in the control and olive oil–treated eyes (Fig. 1 *B* and *C*). Guinea pigs subjected to lens-induced myopia (LIM) also presented with significant reductions in myopia and AL elongation, but not VCD increase, upon ω-3 PUFAs treatment (Fig. 1 *D–F*). Interocular differences in ACD, LT, and body weight were not significantly different between the ω-3 PUFAs–treated group and their counterparts treated with olive oil, in either FDM (*SI Appendix*, Fig. S2 *A–C*) or LIM (*SI Appendix*, Fig. S2 *D–F*) groups.

We then performed similar experiments using the FDM model in mice. After 1 wk of FD, the development of myopic refractive error was 28.1% lower in the ω-3 PUFAs–treated group than in the olive oil–treated group ($P < 0.05$, Fig. 1*G*). However, there were no significant differences in VCD and AL (Fig. 1 *H* and *I*), and the interocular differences in ACD and LT, and body weights, were also not significantly different between the two groups (*SI Appendix*, Fig. S2 *G–I*).

Additionally, we tested the effects of lower doses of ω-3 PUFAs on FDM development in guinea pigs. After 2 wk of treatment using a 10th of the original dose (1/10 ω-3 PUFAs), that is, DHA 30 mg plus EPA 6.0 mg, the differences in myopic refraction, as well as the inhibition of AL and VCD elongation, were similar to those seen in the original high-dose group (1/1 ω-3 PUFAs), that is, DHA 300 mg plus EPA 60 mg (*SI Appendix*, Fig. S3 *A–C*). However, at a 100th of the original ω-3 PUFAs dose (1/100 ω-3 PUFAs), that is, DHA 3.0 mg plus EPA 0.6 mg, there were no effects on these parameters. Furthermore, interocular differences in ACD, LT, and body weight were not significantly affected by either of the reduced-dose treatments (*SI Appendix*, Fig. S3 *D–F*).

Overall, these results demonstrate that daily gavage of ω-3 PUFAs, at the chosen doses, significantly suppressed FDM or LIM development in both guinea pigs and mice.

Peribulbar Injection of DHA-Attenuated Myopia Progression in Guinea Pigs. Having shown that orally administered ω-3 PUFAs reduced myopia progression, we then determined if specific types of ω-3 PUFAs (e.g., DHA and EPA) delivered as peribulbar injections (*SI Appendix*, *SI Materials and Methods*), had similar effects on FDM development in guinea pigs.

After 1 and 2 wk of treatment, FD induced significant myopic refractions in vehicle-treated eyes and in the low-dose (1 μg/day) and the high-dose (3 μg/day) DHA-treated eyes (Fig. 2*A*). However, after 2 wk of treatment, myopia progression in the high-dose group was 35.3% less than that in the vehicle-treated group ($P < 0.01$; Fig. 2*A*). This inhibitory effect was accompanied by significant inhibition of the induced increases in both VCD and AL (Fig. 2 *B* and *C*). For comparison with standard treatments, we also administered atropine as a positive control. Atropine (0.1%) reduced the myopic refractive error by 35.6% compared with the vehicle ($P < 0.01$; Fig. 2*A*), demonstrating that the inhibitory effect of high-dose DHA was similar to that of atropine. Interocular differences in ACD, LT, and RCC were not significantly different between the atropine-treated eyes and the vehicle controls (*SI Appendix*, Fig. S4 *A–C*).

Although the effect of 3.0 μg/d EPA followed a similar trend to that of 0.1% atropine in inhibiting FDM (Fig. 2*D*) and reducing the increases of VCD and AL (Fig. 2 *E* and *F*), the differences in myopia inhibition, VCD increase, and AL elongation between 3.0 μg/d EPA and the vehicle control were not significant ($P = 0.13$, $P = 0.08$, and $P = 0.13$, respectively; Fig. 2 *D–F*). Interocular differences in ACD, LT, and RCC between the EPA-treated eyes and vehicle controls were also not significant (*SI Appendix*, Fig. S4 *D–F*).

Taken together, these results indicate that in guinea pigs, DHA significantly suppressed FDM progression, but EPA had no significant inhibitory effect when given by peribulbar injection at the doses used.

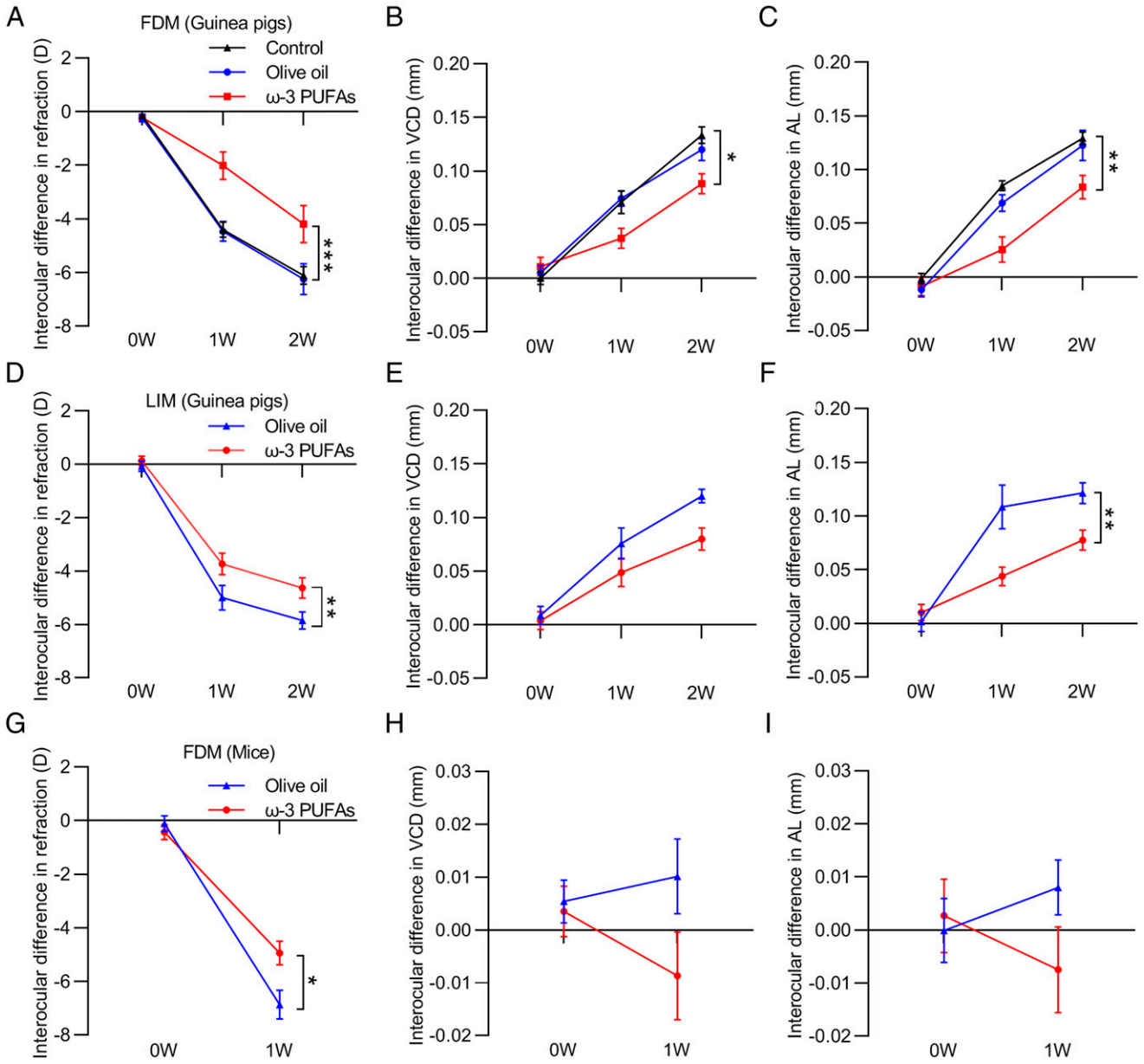

Fig. 1. Daily gavage of ω-3 PUFAs (DHA 300 mg plus EPA 60 mg) inhibited the myopic shift of refraction and elongation of VCD and AL in FDM- and LIM-treated guinea pigs and FDM-treated mice. (*A–C*) Interocular differences in refraction (*A*), VCD (*B*), and AL (*C*) in FDM guinea pigs. (*D–F*) Interocular differences in refraction (*D*), VCD (*E*), and AL (*F*) in LIM guinea pigs. (*G–I*) Interocular differences in refraction (*G*), VCD (*H*), and AL (*I*) in FDM mice. Results are presented as means ± SEs of the means. n = 12, 16, and 17 guinea pigs for FDM-only, olive oil, and ω-3 PUFAs groups, respectively, in *A–C*; n = 7 and 8 guinea pigs for olive oil control and ω-3 PUFAs, respectively, in *D–F*; n = 21 mice for both the olive oil control and ω-3 PUFAs groups in *G–I*. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$: significant differences between ω-3 PUFAs and olive oil–treated groups in two-way repeated measures ANOVA with Bonferroni post hoc tests. W, week(s). Values for each individual eye are given in *SI Appendix*, Table S1. The detailed statistical data, including the F-values and degrees of freedom, are given in *SI Appendix*, Table S2.

DHA and EPA Levels Were Dysregulated during Myopia Progression in Guinea Pigs. To further study the levels of DHA and EPA during FDM development, we measured DHA and EPA levels in serum, sclera, and retina in guinea pigs at different stages of myopia development. Lipid metabolism in humans follows a circadian rhythm (38); therefore, for the optimal times to measure the DHA and EPA levels in guinea pigs after 2 d of FD, we chose 10:00, which represents the initial stages of visual input during the day, and 18:00, which represents later stages of visual input. Thus, the notation "2D-10:00" implies that FD was begun at 10:00 and that the tissue samples were collected 2 d later at 10:00. Similarly, the notation "2D-18:00" implies that FD started at 18:00 and ended 2 d later at the same time. To measure the metabolic changes due to long-term FD, that is, after 1 wk, we sampled them only at 10:00.

In guinea pigs that were exposed to FD for 2 d, we observed decreases in serum DHA levels at 18:00 (Fig. 3*A*) and in serum EPA levels at 1000 (Fig. 3*B*), compared to the non-FD controls. The scleral DHA levels at 2D-10:00 were reduced compared to those in fellow eyes and in non-FD controls (Fig. 3*C*). Scleral EPA levels were reduced not only at 2D-10:00 but also at 2D-18:00 relative to those in non-FD controls or their fellow eyes (Fig. 3*D*). In contrast, retinal DHA levels were increased compared to those in the fellow eyes and/or non-FD controls at

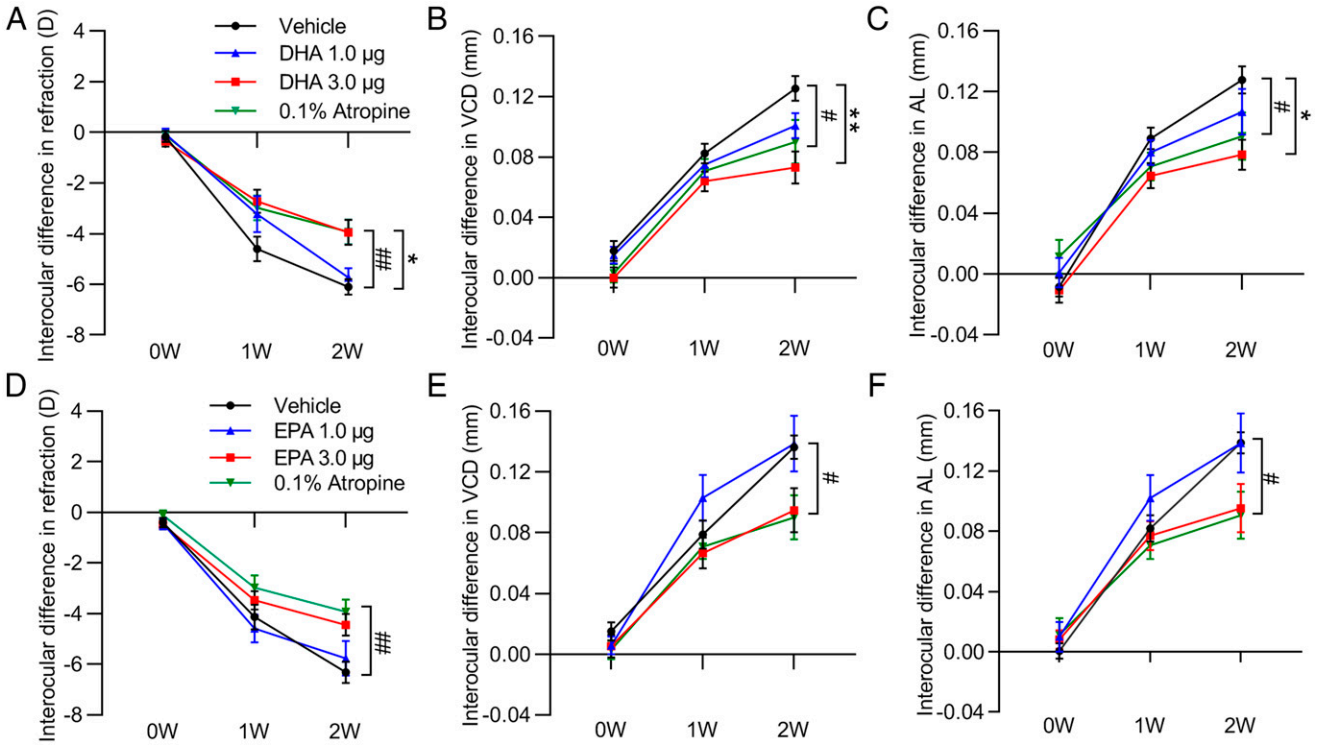

Fig. 2. Daily peribulbar injection of DHA and EPA inhibited the FD-induced myopic shift and elongation of VCD and AL in guinea pigs. (*A–C*) Interocular differences in refraction (*A*), VCD (*B*), and AL (*C*) in DHA-injected FDM guinea pigs. (*D–F*) Interocular differences in refraction (*D*), VCD (*E*), and AL (*F*) in EPA-injected FDM guinea pigs. Results are presented as means ± SEs of the means. n = 13, 12, 13, and 14 guinea pigs for vehicle control, 1 μg DHA, 3 μg DHA, and 0.1% atropine in *A–C*; n = 16, 14, 17, and 14 guinea pigs for vehicle control, 1 μg EPA, 3 μg EPA, and 0.1% atropine in *D–F*. #$P < 0.05$ and ##$P < 0.01$: significant differences between vehicle- and 0.1% atropine–treated groups; *$P < 0.05$, **$P < 0.01$: significant differences between vehicle- and DHA- or EPA-treated groups in two-way repeated measures ANOVA with Bonferroni post hoc test. W, week(s). Values for each individual eye are given in *SI Appendix*, Table S3. The detailed statistical data, including the F-values and degrees of freedom, are given in *SI Appendix*, Table S4.

both 2D-10:00 and 2D-18:00 (Fig. 3*E*). The retinal EPA levels were also increased compared to those in non-FD controls at 2D-18:00 (Fig. 3*F*). In general, the levels of DHA and EPA were decreased in serum and sclera and increased in retina after 2 d of FD. However, after 1 wk of FD, the differences in DHA and EPA levels in the serum, sclera, and retina were no longer significant (Fig. 3 *A–F*)

Thus, the DHA and EPA levels in serum, sclera, and retina were dysregulated during the early stage of FD in guinea pigs.

Gavage of ω-3 PUFAs Selectively Increased Serum and Scleral DHA and EPA. We then measured the levels of DHA and EPA in guinea pig sera, scleras, and retinas after giving ω-3 PUFAs or olive oil by gavage. Olive oil had no significant effect on the levels of DHA and EPA in the three tissues (Fig. 4 *A–F*). However, serum DHA and EPA levels were increased 3 h after ω-3 PUFAs gavage and were sustained for 12 h after gavage (Fig. 4 *A* and *B*). Scleral DHA content was significantly increased 3 and 6 h after ω-3 PUFA supplementation (Fig. 4*C*), but scleral EPA remained unchanged at all intervals (Fig. 4*D*). After DHA gavage, retinal DHA levels were unaltered for 6 h but were lower than those in the olive oil group after 12 h ($P < 0.01$; Fig. 4*E*). Retinal EPA levels remained unchanged at all intervals (Fig. 4*F*). These data show that gavage of ω-3 PUFAs selectively elevated the serum DHA and EPA levels and scleral DHA levels.

ω-3 PUFAs Inhibited the ChBP Reduction–Scleral Hypoxia Cascade during Myopia Development. To investigate the mechanisms underlying the protective effect of ω-3 PUFAs on myopia development, we determined if gavage of ω-3 PUFAs could modulate ChT and ChBP during the development of FDM in guinea pigs. Earlier, we reported that an increase in ChT and ChBP was accompanied by inhibition of scleral hypoxia and slowing of myopia progression (19). ChT and ChBP signal points were imaged by optical coherence tomography (OCT; Fig. 5*A*) and OCT angiography (OCTA; Fig. 5*B*; details of OCT and OCTA are provided in *SI Appendix, SI Materials and Methods*). After 2 wk of FD, both the ChT and ChBP were significantly decreased in the olive oil–treated group (Fig. 5 *C* and *D*), but the changes in ChT and ChBP were significantly reduced in the ω-3 PUFAs group compared to the olive oil group ($P < 0.01$; Fig. 5 *C* and *D*). We also tested the effects of using a tenth of the original ω-3 PUFAs dose (1/10 ω-3 PUFAs), that is, DHA 30 mg plus EPA 6.0 mg, on ChT and ChBP. For both parameters, the outcomes were like those of the original high dose (1/1 ω-3 PUFAs), that is, DHA 300 mg plus EPA 60 mg (*SI Appendix*, Fig. S5 *A* and *B*). Finally, ω-3 PUFAs at the original high dose also inhibited the reduction of ChT and ChBP in the LIM guinea pigs (Fig. 5 *E* and *F*).

We then determined the effects of peribulbar injections of DHA and EPA on ChT and ChBP. Earlier reports showed that inhibition of human myopia development by atropine is accompanied by suppression of the correlated decline in ChT (39, 40); therefore, we used atropine treatment as a positive control. Compared with the vehicle injection, 2 wk of DHA injections (both low and high doses) significantly inhibited FD-induced declines in ChT (Fig. 5*G*). Only the high dose of DHA significantly inhibited the decline in ChBP (Fig. 5*H*). However, EPA injections did not significantly inhibit the FD-induced decreases in ChT and ChBP (Fig. 5 *I* and *J*), which is consistent with the lack of myopia and ocular growth inhibitions by EPA (Fig. 2 *D–F*).

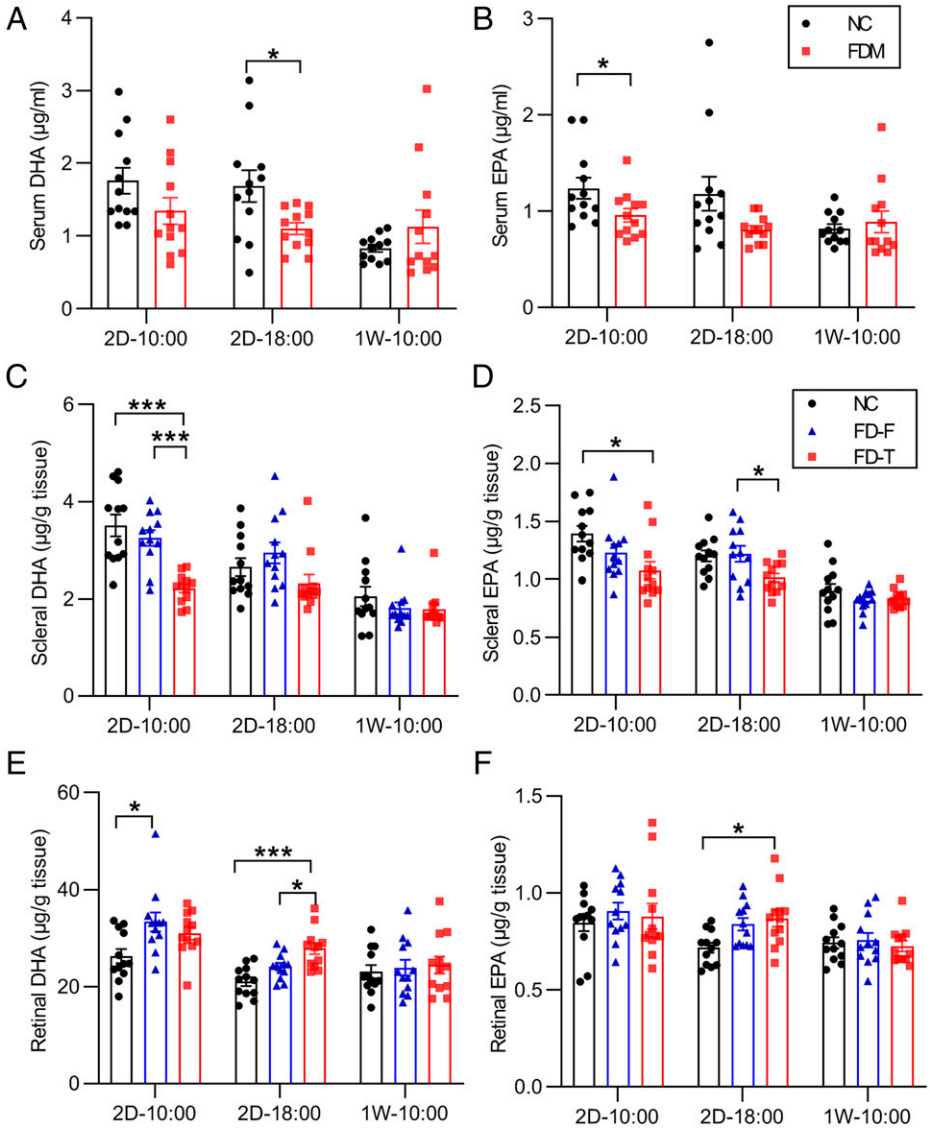

Fig. 3. Alterations in DHA and EPA levels in serum, sclera, and retina of FD guinea pigs and non-FD controls that received no other treatment: serum DHA and EPA levels (*A* and *B*), scleral DHA and EPA levels (*C* and *D*), and retinal DHA and EPA levels (*E* and *F*) in non-FD controls and FD guinea pigs. Each data point represents one individual guinea pig. Results are presented as means ± SEs of the means. $n = 12$ guinea pigs for each group in *A–F*. $^{*}P < 0.05$ and $^{***}P < 0.001$; data in *A* and *B* were assessed by unpaired two-tailed *t* tests; data in *C–F* were analyzed by two-way ANOVA with Bonferroni's post hoc tests. W, week(s); NC, non-FD control guinea pigs; FD-F, form-deprived fellow eyes; FD-T, form-deprived treated eyes. The detailed statistical data, including the F-values and degrees of freedom, are given in *SI Appendix*, Table S5.

Atropine also significantly inhibited the FD-induced declines in ChT and ChBP (Fig. 5 *G–J*). The above results demonstrate that DHA, but not EPA, inhibits myopia development by limiting the declines in ChT and ChBP, and they show that the anti-myopia actions of DHA are similar to those of atropine at a clinically relevant dose.

Declines in ChBP are associated with scleral hypoxia and an increase in scleral HIF-1α protein content (18). These changes can trigger scleral myofibroblast transdifferentiation and ECM remodeling, leading to myopia-associated expansion of the posterior sclera (18). To test for a role of ω-3 PUFAs in this process, we determined the effect of ω-3 PUFA gavage on scleral HIF-1α protein levels. In the control group fed with olive oil, the levels of scleral HIF-1α in the FD-treated eyes (FD-T) were higher than those in the fellow eyes (FD-F) ($P < 0.05$; Fig. 5 *K* and *L*). However, this increase was suppressed in FD-T eyes of guinea pigs fed with ω-3 PUFAs, compared to the FD-F eyes (Fig. 5 *K* and *L*).

Similar inhibition of the FD-induced increase in HIF-1α protein levels was also present after peribulbar injection of DHA or EPA. In the vehicle control group, HIF-1α levels were higher in the scleras of FD-T eyes than of FD-F eyes ($P < 0.01$; Fig. 5 *M* and *N*). However, DHA or EPA treatment suppressed this increase in FD-T eyes compared with FD-F eyes (Fig. 5 *M* and *N*).

The results of these experiments are consistent with the hypothesis that ω-3 PUFAs, especially DHA, inhibit the ChBP reduction–scleral hypoxia cascade during myopia development.

Both DHA and EPA Antagonized Hypoxia-Induced Transdifferentiation of HSFs. Scleral fibroblasts are involved in scleral ECM remodeling and myopia progression (41). To explore the potential effects of DHA and EPA on scleral ECM remodeling, we studied the effects on the in vitro hypoxic response of HSFs. HSFs were treated with different doses of DHA or EPA for 24 h and then incubated under 21% O_2 (normoxia) or 5% O_2

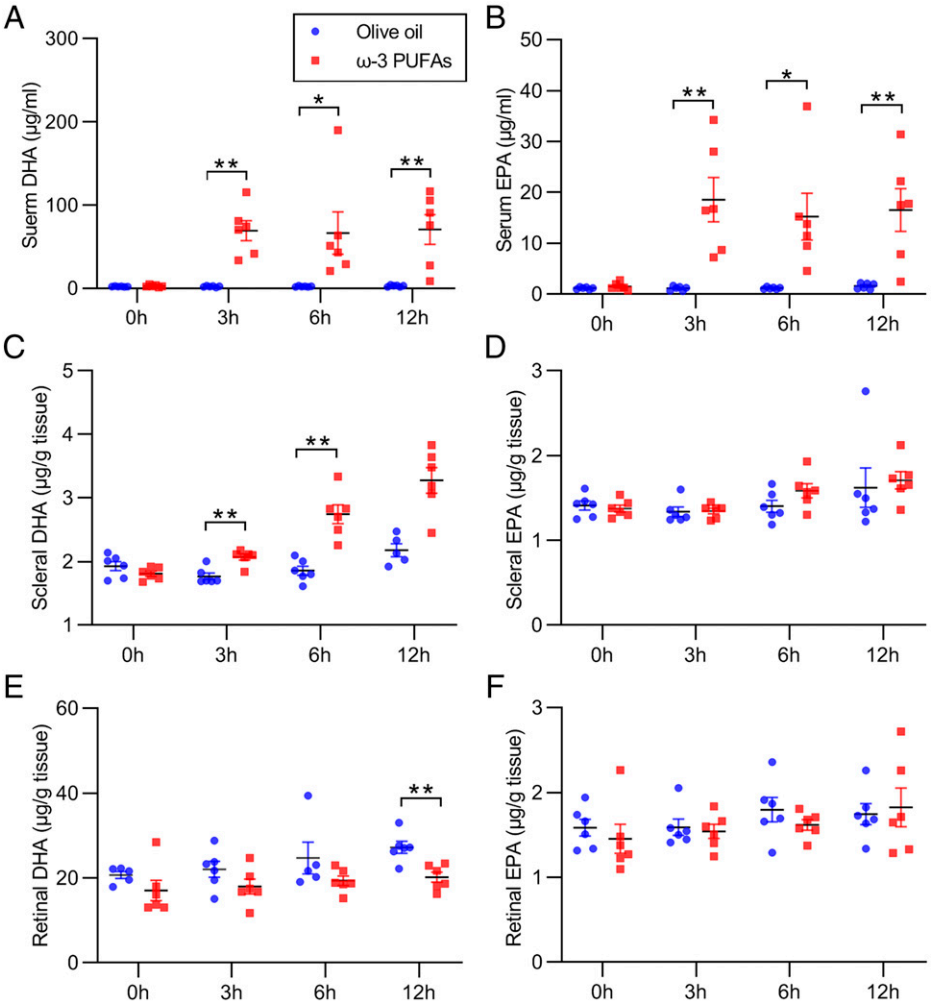

Fig. 4. Distribution of DHA and EPA in serum, retina, and sclera at intervals after gavage of ω-3 PUFAs (DHA 300 mg plus EPA 60 mg) in guinea pigs. (*A* and *B*) Changes in DHA and EPA levels in serum. (*C* and *D*) Changes in DHA and EPA levels in sclera. (*E* and *F*) Changes in DHA and EPA levels in retina. Results are presented as means ± SEs of the means. $n = 6$ for each group in *A–F*; $^{*}P < 0.05$, $^{**}P < 0.01$: significant differences between olive oil– and ω-3 PUFAs–treated groups at each designated interval; unpaired two-tailed *t* tests.

(hypoxia) for another 48 h. Hypoxia significantly increased HIF-1α protein content in the HSFs (Fig. 6 *A* and *B*). Consistent with the results of our previous studies (18), in the normal control (NC) group, hypoxia reduced the levels of collagen type 1α1 (Fig. 6 *A* and *C*), whereas it increased the levels of myofibroblast marker α-smooth muscle actin (α-SMA; Fig. 6 *A* and *D*). In the hypoxic environment, neither DHA nor EPA had a significant effect on HIF-1α content, compared to that in the NC group (Fig. 6 *A* and *B*). However, DHA (20 μM) and EPA (5 μM) suppressed the hypoxia-induced declines in collagen levels (Fig. 6 *A* and *C*), and both doses of DHA and EPA significantly suppressed the hypoxia-induced increases in α-SMA levels (Fig. 6 *A* and *D*).

Overall, these results suggest that both DHA and EPA suppressed myofibroblast transdifferentiation and restored the protein levels of collagen in HSFs in a hypoxic environment.

Oral Administration of ω-3 PUFAs Partially Inhibited Near-Work–Induced Declines in ChBP in Human Subjects. Excessive near work has been proposed as a risk factor for human myopia (5, 42), and it induces declines in ChBP that may be responsible for this condition (18, 43). Therefore, we studied the effect of ω-3 PUFAs (DHA 600 mg plus EPA 120 mg) on near-work–induced declines in ChBP in human young adults (Fig. 7*A*). Prior to delivery of the supplements, near work had no significant effect on ChT (Fig. 7*B* and *SI Appendix*, Fig. S6*B*) or extravascular stromal area (Fig. 7*C* and *SI Appendix*, Fig. S6*C*). This indicates that near work had little effect on overall ChT or on the area of the choroidal stroma not occupied by blood vessels. However, near work led to significant decreases in the choroidal vascularity index (Fig. 7*D* and *SI Appendix*, Fig. S6*D*) and vascular luminal area (Fig. 7*E* and *SI Appendix*, Fig. S4*E*). Simultaneously, near work significantly increased the choriocapillaris flow deficit, that is, regions having no blood flow signals (Fig. 7*F* and *SI Appendix*, Fig. S4*F*), indicating that near work induced declines in ChBP in humans. On the other hand, ω-3 PUFAs supplements significantly attenuated the declines in choroidal vascularity index (Fig. 7*D*) but had no significant effect on luminal area (Fig. 7*E*) or the choriocapillaris flow deficit (Fig. 7*F*).

Therefore, these results suggest that ω-3 PUFAs partially improve the near-work–induced declines in ChBP in human subjects.

Discussion

This study provides evidence that ω-3 PUFAs can play a protective role in myopia. Here, we demonstrated that the metabolism of ω-3 PUFAs was dysregulated in experimental myopia

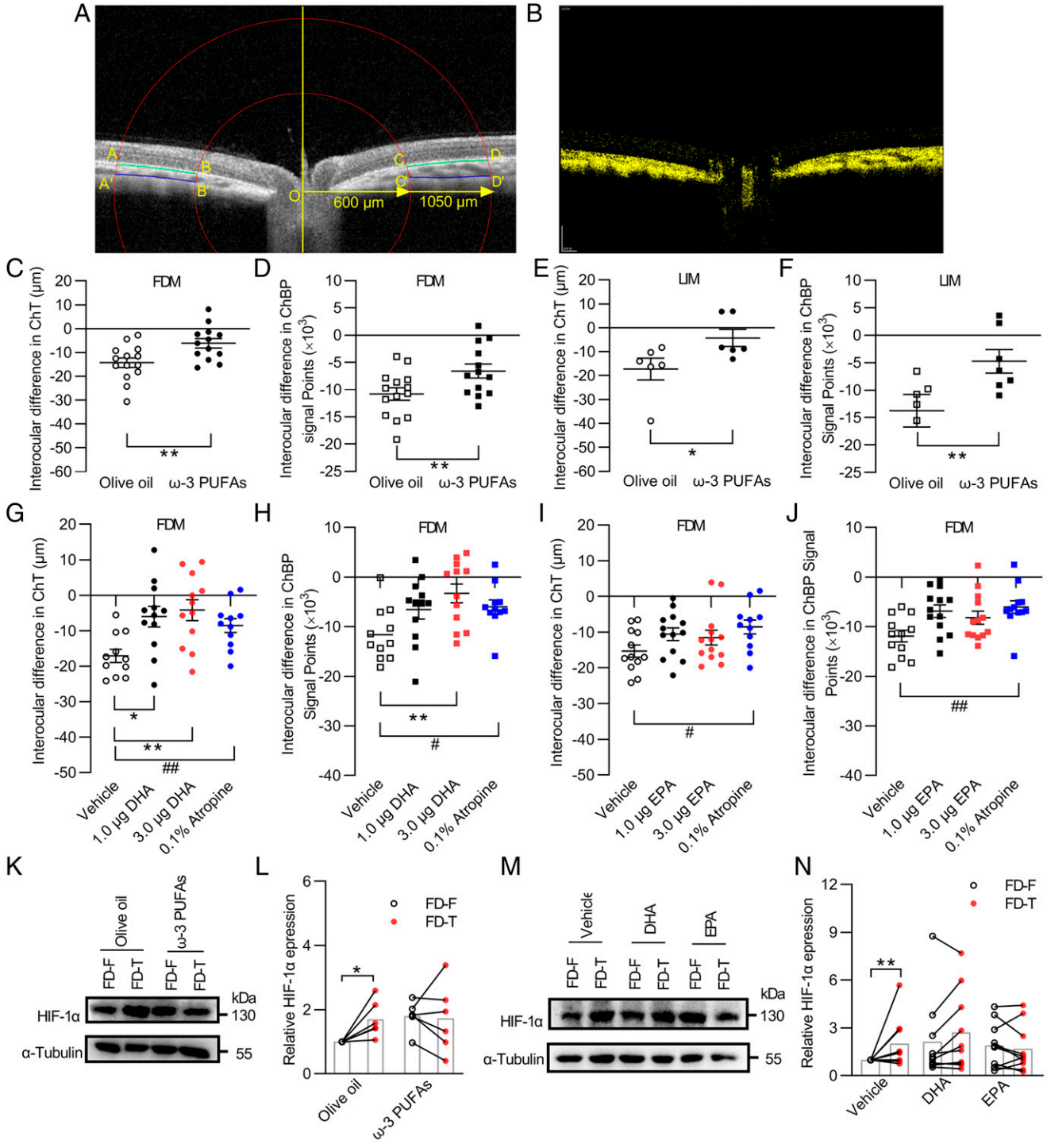

Fig. 5. Effects of ω-3 PUFAs (DHA 300 mg plus EPA 60 mg) on ChT, ChBP, and scleral HIF-1α protein levels during myopia development in guinea pigs. (*A*) OCT B-scan (lateral scan) image. Green line shows the interior surface of the choroid and blue the exterior surface. O, optic disk; ChT, nasal: A, A', B, B'; ChT, temporal: C, C', D, D'. (*B*) OCTA image. Yellow points in the defined regions of interest represent the ChBP signal. ChT (*C*) and ChBP (*D*) after 2 wk of FD plus olive oil or ω-3 PUFAs administration. ChT (*E*) and ChBP (*F*) after 2 wk of LIM and olive oil or ω-3 PUFAs administration. ChT (*G*) and ChBP (*H*) after 2 wk of vehicle control, 1.0 μg DHA, 3.0 μg DHA, or 0.1% atropine injection in FD eyes. ChT (*I*) and ChBP (*J*) after 2 wk of vehicle control, 1.0 μg EPA, 3.0 μg EPA, or 0.1% atropine injection in FD eyes. (*K* and *L*) Levels of HIF-1α content as determined by Western blot analysis after 2 wk of FD and daily gavage of ω-3 PUFAs. (*M* and *N*) Levels of HIF-1α protein as determined by Western blot analysis, after 2 wk of FD and vehicle, 3.0 μg DHA, or EPA injection. Results are presented as means ± SEs of the means. #$P < 0.05$ and ##$P < 0.01$: significant differences between vehicle- and 0.1% atropine–treated groups; *$P < 0.05$ and **$P < 0.01$: significant differences between vehicle- and DHA- or EPA-treated groups. Data in *C*–*F* were assessed by unpaired two-tailed *t* tests; $n = 15$ and 13 guinea pigs for olive oil and ω-3 PUFAs gavage FD groups in *C* and *D*; $n = 6$ and 7 guinea pigs for olive oil and ω-3 PUFAs gavage in LIM groups in *E* and *F*. Data in *G*–*J* were assessed by one-way ANOVA with Bonferroni's post hoc tests; $n = 11$, 12, 12, and 11 guinea pigs for vehicle, 1 μg DHA, 3 μg DHA, and 0.1% atropine in *G* and *H*, respectively; $n = 12$, 13, 13, and 11 guinea pigs for vehicle, 1 μg EPA, 3 μg EPA, and 0.1% atropine in *I* and *J*, respectively; data in *K*–*N* were assessed by two-way repeated measures ANOVA with Bonferroni post hoc tests; $n = 6$ for olive oil and ω-3 PUFAs gavage groups in *L*; $n = 10$ for DHA- and EPA-injected groups in *N*. FD-F: FD fellow eye; FD-T: FD-treated eye. Values for each individual eye are given in *SI Appendix*, Table S6. The detailed statistical data, including the F-values and degrees of freedom, are given in *SI Appendix*, Table S7.

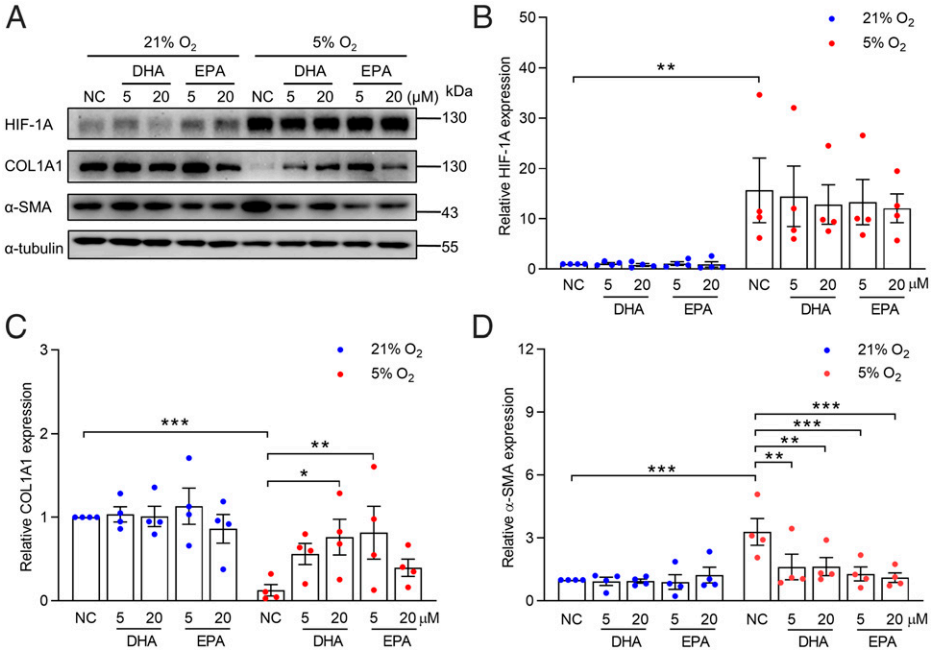

Fig. 6. The effect of DHA and EPA on hypoxia-dependent events in HSFs. (*A*) Western blot analysis showing the protein levels of HIF-1A, COL1A1, and α-SMA in HSFs treated with DHA/EPA and under 21% (normoxic) or 5% (hypoxic) oxygen levels. (*B–D*) The bar graph represents protein levels of HIF-1A (*B*), COL1A1 (*C*), and α-SMA (*D*) ($n = 4$), relative to NC (cells treated with ethanol); α-tubulin was used as the loading control. Results are presented as means ± SEs of the means. $*P < 0.05$, $**P < 0.001$, and $***P < 0.001$; two-way ANOVA with Bonferroni's post hoc tests. The detailed statistical data, including the F-values and degrees of freedom, are given in *SI Appendix*, Table S8.

models. ω-3 PUFAs, especially DHA, suppressed myopia development, inhibited myopic ChBP decreases, and attenuated scleral hypoxia. Furthermore, in human young adults, the decrease in ChBP induced by near work (a proposed myopia risk factor) was partially alleviated by dietary ω-3 PUFAs.

ω-3 PUFAs Attenuated Myopia Progression in Different Experimental Models. Mixtures of ω-3 PUFAs containing DHA and EPA have been reported to provide health benefits in human subjects such as hepatoprotection (44) and antifibrosis (45). In the current study, we found that scleral DHA and EPA levels were decreased in myopic guinea pigs. Furthermore, we showed that oral administration of ω-3 PUFAs significantly suppressed FDM and LIM development in guinea pigs, as well as FDM in mice. In addition, peribulbar injections of DHA inhibited myopia development in guinea pigs. Previous studies showed that combined usage of DHA and EPA has significant human health benefits (46, 47). However, we did not assess the combined effects of EPA and DHA in different proportions on myopia development, and thus, further studies are required to explore the optimal dosage and ratio of DHA and EPA for human myopia control.

Our study showed that scleral DHA and EPA levels were reduced after 2 d of FD and returned to baseline after 1 wk of FD in guinea pigs. This suggests that the metabolic changes in ω-3 PUFAs occur during the initial stages of myopia development, so that an early intervention targeting the PUFAs could ameliorate the onset and progression myopia. Such initial changes in DHA and EPA levels are comparable to those seen with intense light, wherein increasing the amount of time spent outdoors (exposure to sunlight) reduces the onset of myopia in children (48, 49). Early intervention is particularly important because children who have developed myopia and are continually exposed to myopic stimuli such as increased near work are more likely to progress toward sight-threatening, high myopia (50).

Interestingly, in animals with FDM, the levels of DHA and EPA were increased in the serum and sclera but decreased in the retina. DHA is abundant in the retina, in which it accounts for ~50 to 60% of the total fatty acid content within the outer segments of rod photoreceptors. There, it plays important roles in the synthesis of disk membranes where it provides an appropriate environment for conformational changes in rhodopsin and where it modifies the activity of retinal enzymes (51). A deficiency of DHA in the retina disturbs membrane fluidity and function and could alter the process of outer segment renewal (51). In guinea pigs, it also leads to changes in retinal function, resulting in low ERG amplitudes (52). The retina has a complex and effective recycling system that ensures conservation of retinal DHA levels even during periods of prolonged dietary ω-3 PUFAs deficiency (53). It may be that the decrease of serum DHA level enhances adaptation of the retina to metabolic stress. In any case, the metabolism and functions of ω-3 PUFAs in the retina are very complex and well beyond the scope of the present study. Further investigations are needed to understand the roles of ω-3 PUFAs in the regulation of eye growth and myopia progression and to identify the mechanisms of action, thus increasing our understanding of the function of ω-3 PUFAs while laying solid foundations for therapeutic use in human myopia.

ω-3 PUFAs Might Suppress Myopia Progression by Inhibiting the ChBP Reduction–Scleral Hypoxia Cascade. ω-3 PUFAs have been reported to regulate hypertension through vasodilatory effects (54), and dietary ω-3 PUFAs retarded the development of hypertension in rat models (55). Limbu et al. demonstrated that DHA and EPA induced vasodilation of aortic and mesenteric resistance arteries in rats (28), and ω-3 PUFAs improved coronary vasodilation in heart transplant recipients (31). These effects of DHA or EPA on vasodilation may be related to their regulation of nitric oxide and angiotensin-II production (56, 57).

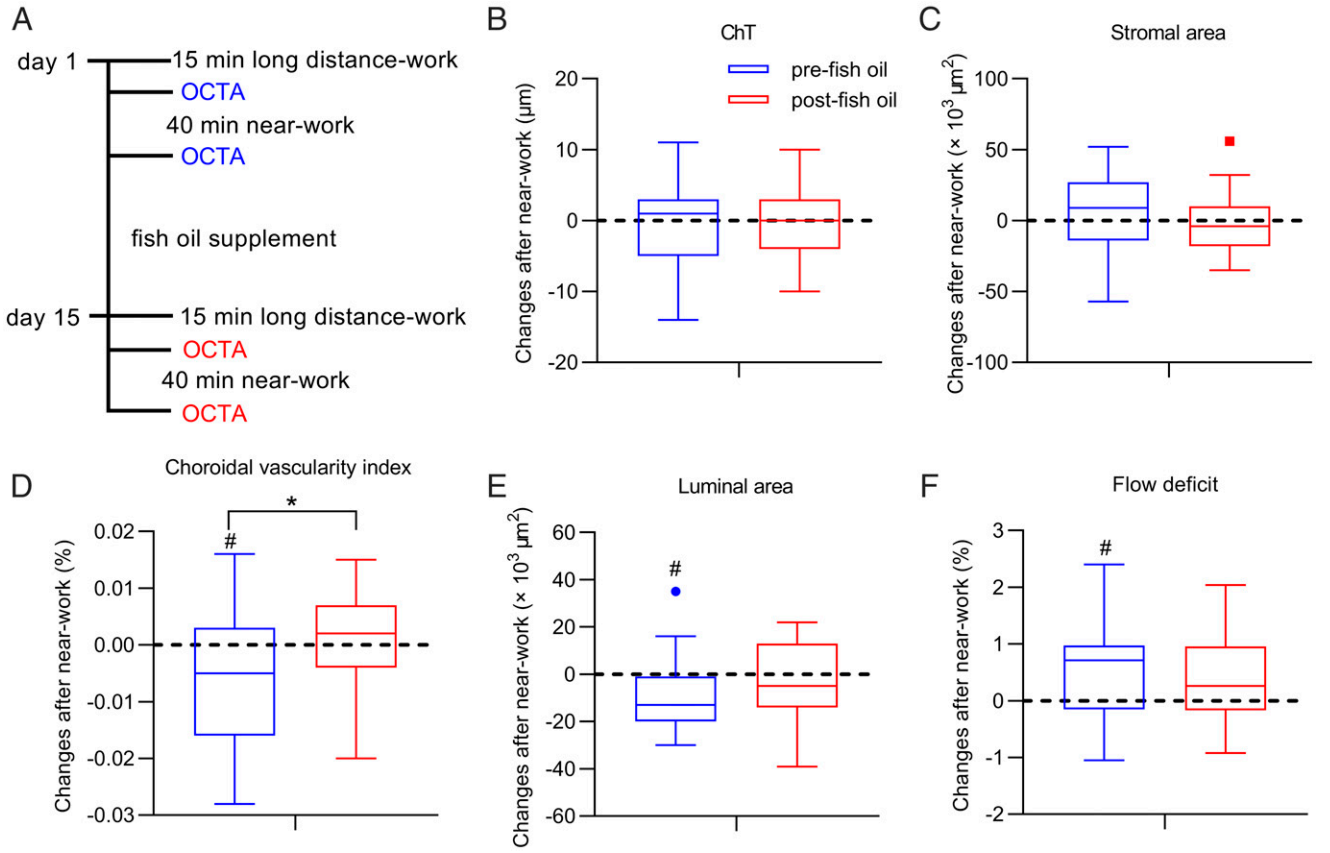

Fig. 7. Effects of ω-3 PUFAs (DHA 600 mg plus EPA 120 mg) on choroidal perfusion after near work in humans. (*A*) Schematic diagram of near-work experimental flow. (*B–F*) Effects of ω-3 PUFAs on ChT (*B*), stromal area (*C*), choroidal vascularity index (*D*), luminal area (*E*), and flow deficit (*F*) after 40 min of reading. $n = 31$; box-plot diagrams showing data distribution; lines within the boxes indicate medians; bars indicate range; and blue and red dots indicate outliers. *$P < 0.05$, significant differences between pre- and post–fish oil supplements, unpaired two-tailed *t* tests; #$P < 0.05$, significant differences between after and before 40 min near work, paired two-tailed *t* tests.

In the ocular system, multiple studies have documented significant declines in ChT and ChBP during myopia induction in various experimental animal models (19, 20, 58) as well as humans (43, 59), and these changes might subsequently trigger scleral hypoxia and the ensuing myopia (18). In line with the vasodilation role of ω-3 PUFAs in the cardiovascular system and with choroidal changes during myopia, the present study showed that gavage of ω-3 PUFAs or peribulbar injections of DHA ameliorated the FD- and lens-induced declines in ChT and ChBP, followed by the inhibition of FDM-associated increases in scleral HIF-1α protein levels. These results in rodent models suggest that DHA mitigates ChBP reduction and scleral hypoxia and consequently suppresses myopia. Another intervention that was found to retard myopia development and inhibit choroidal thinning in children was crocetin intake (14); however, the mechanism by which this apocarotenoid dicarboxylic acid works is as yet unknown.

Our in vitro studies showed that both DHA and EPA suppressed hypoxia-induced myofibroblast transdifferentiation and offset declines in collagen levels in cultured HSFs without affecting HIF-1α up-regulation. This HIF-1α–independent effect is supported by previous studies in liver and kidney, demonstrating that DHA directly relieves fibrosis by suppressing TGF-β1–mediated myofibroblast transdifferentiation (35–37). In addition, the current study showed that the scleral DHA levels in guinea pigs were decreased after 2 d of FD, whereas the experimental elevation of DHA levels resulted in inhibition of myopia. This implies that the suppression of myopia by DHA might be achieved by directly inhibiting scleral fibroblast–myofibroblast transdifferentiation. Further studies are needed to determine if DHA could alleviate myopia by modulating the scleral myofibroblast transdifferentiation in a TGF-β1–dependent pathway.

It is noteworthy that administration of ω-3 PUFAs induced elevation of the DHA level in sclera but not in the retina. This difference might be due to the avascularity of guinea pig retinas or the slowing of drug delivery from choroid to retina by the blood–retinal barrier function of the retinal pigment epithelium (60). These results are consistent with the idea that DHA might suppress myopia progression by specifically targeting choroid–sclera interactions. At the same time, EPA diminished the increases in scleral HIF-1α protein levels, but it did not induce significant ChBP recovery or myopia inhibition, suggesting the involvement of additional underlying mechanisms. The above evidence for choroidal and scleral involvement further supports the idea that ω-3 PUFAs suppressed myopia development. Whether or not there are other targets such as the retina, and what the detailed molecular mechanisms might be, will need further investigation.

ω-3 PUFAs: A Candidate for Myopia Control? The current study demonstrated that ω-3 PUFAs significantly inhibit the progression of experimental myopia in animal models and that oral administration of ω-3 PUFAs partially suppressed the near-work–induced declines of ChBP in human subjects. Of the available myopia control measures, atropine has been studied extensively (61, 62); however, because of the side effects, lack of complete effectiveness, incomplete clarity regarding the mechanism of action, and the long-term implications of its use

(10, 63), it is necessary to find alternative means of myopia control. Accordingly, we compared the protective effects of 0.1% atropine and DHA on the declines in ChBP and myopia development. DHA (3.0 μg) was as effective as atropine in inhibiting experimentally induced myopia in guinea pigs, thus providing a potential alternative agent for limiting the progression of experimental myopia. ω-3 PUFAs are easily accessible as oral supplements and are safe even for pregnant women and babies (64). In fact, the Institute of Medicine, Food and Nutrition Board of the United Nations recommends intake levels of 0.5 to 0.9 g/day for children under 10 y old and 1.0 to 1.6 g/day for adults (65). The American Heart Association recommends a regular intake of either ω-PUFAs rich foods or oral supplements (in consultation with a physician) for improvement of cardiovascular health (66); however, these recommended dosages of ω-3 PUFAs are determined for the safe development of the brain and other neurological systems, and comparable data are not available for ocular safety and efficacy (67). In our study, the daily intakes of DHA and EPA for guinea pigs were 1.5 and 0.3 g/kg, respectively. According to the K_m factors between guinea pigs and humans (68), the corresponding dosage for humans would reach 300 mg/kg DHA, which is high by clinical standards. Therefore, we lowered the oral dosages in guinea pigs to a 10th and a 100th of the original dosage (i.e., to DHA 30 mg plus EPA 6 mg and to DHA 3 mg plus EPA 0.6 mg, respectively) and studied the myopic ocular changes. The 10-fold dose of ω-3 PUFAs resulted in similar inhibitions of myopia and ocular growth, along with amelioration of ChT and ChBP decreases, as seen with the original high dose. This shows that the high dose and the 1/10 dose of the ω-3 PUFAs were saturating and that a still lower dose might be effective. In any case, the lower-dosage data provides only guidance for human studies, as a basis for determining the safe and effective doses of DHA and EPA for controlling human myopia. A recent systematic metanalysis of 38 randomized controlled trials highlighted the safety and the inhibitory effects of ω-3 PUFAs on cardiovascular mortality and other cardiovascular outcomes (69). One of the trials reported ω-3 PUFAs was associated with a low risk of atrial fibrillation (5.4 incident cases per 1,000 people/y) (70). Furthermore, compared with oral administration, delivery of ω-3 PUFAs through eye drops might provide a better choice for human myopia control in the future.

In the present study, we induced myopia and measured choroidal parameters in juvenile guinea pigs during their stage of most active eye growth. While it is generally recognized that myopia progresses more rapidly in human children than in adults (71), such a rapid ocular growth phase cannot be clearly defined in humans during short-term recruitment. The concept of ω-3 PUFAs administration and its marked effects in the guinea pig model makes it a viable candidate for testing in humans in longitudinal clinical studies. Ideal and safe drug dosages need to be estimated before administering them to children, and this will require further ethical and parental approval. In any event, randomized controlled trials will be required to test the hypothesis that administration of ω-3 PUFAs is an effective and safe approach to controlling human myopia.

In conclusion, ω-3 PUFAs inhibited myopia progression in guinea pigs and mice. The ω-3 PUFAs also inhibited FD-induced changes in choroidal structure and vasculature, as well as in scleral HIF-1α protein content. Such ω-3 PUFAs–induced changes could be critical for suppressing scleral myofibroblast transdifferentiation and maintaining the normal scleral ECM turnover that inhibits myopia development. The partial attenuation of near-work–induced declines in the choroidal vascularity index by ω-3 PUFAs supplements suggests that ω-3 PUFAs may have a protective effect against human myopia.

Materials and Methods

Animals. Animal treatment and care were approved by the Animal Care and Ethics Committee at Wenzhou Medical University (wydw2020-0071). The research protocols were conducted according to the Association for Research in Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Guinea pigs and mice were used as animal models in this study. All animals were reared in 12-h light (400 to 500 lx) and 12-h dark (0 lx) cycles (incandescent lights on at 08:00 and off at 20:00). The room temperature was maintained at 25 °C, and the animals had ad libitum access to food and water throughout the experiment.

Animal Experimental Designs. We employed four experimental designs using guinea pigs and mice:

1) Daily gavage of ω-3 PUFAs in 3-wk-old guinea pigs and mice. The guinea pigs were randomly divided into five groups: 1) FD-only controls; 2) FDM vehicle controls that received daily gavage of olive oil; 3) LIM vehicle controls that received daily gavage of olive oil; 4) guinea pigs with FDM that received daily gavage of ω-3 PUFAs, including ω-3 PUFAs containing DHA 300 mg plus EPA 60 mg (1/1 ω-3 PUFAs), ω-3 PUFAs containing DHA 30 mg plus EPA 6.0 mg (1/10 ω-3 PUFAs), or ω-3 PUFAs containing DHA 3.0 mg plus EPA 0.6 mg (1/100 ω-3 PUFAs); and 5) guinea pigs with LIM that received daily gavage of ω-3 PUFAs containing DHA 300 mg plus EPA 60 mg. FDM mice, in contrast, were assigned to only two groups, which received daily gavage of either the olive oil vehicle or the ω-3 PUFAs containing DHA 112.5 mg plus EPA 22.5 mg.
2) Peribulbar injections in 3-wk-old FDM guinea pigs. The FDM guinea pigs were randomly assigned to receive one of the following peribulbar injections: 1) vehicle, 2) low-dose DHA (1.0 μg), 3) high-dose DHA (3.0 μg), 4) low-dose EPA (1.0 μg), 5) high-dose EPA (3.0 μg), or 6) 0.1% atropine.
3) Analysis of DHA and EPA levels in FDM in 3-wk-old guinea pigs. The guinea pigs were randomly divided into three groups: 1) 2 d of FD starting and ending at 10:00; 2) 2 d of FD starting and ending at 18:00; and 3) 1 wk of FD starting and ending at 1000. DHA and EPA levels were measured in the serum, sclera, and retina.
4) Analysis of DHA and EPA distribution after ω-3 PUFAs gavage in 3-wk-old guinea pigs. The guinea pigs were randomly divided into eight groups, and DHA and EPA distributions were determined at 0, 3, 6, or 12 h after gavage with either olive oil or ω-3 PUFAs. DHA and EPA levels were measured in the serum, sclera, and retina.

Reagents. The source of ω-3 PUFAs was fish oil purchased from Nature's Way, Kids Smart. DHA and EPA (MedChemExpress) were dissolved separately in 100% ethanol and stored at −80 °C. The stock solutions were diluted in 0.9% NaCl to reach the final concentration immediately before use. The concentration of ethanol in all final solutions was less than 0.1%. Atropine sulfate monohydrate (≥97%) (Stanford Chemicals) was dissolved in 0.9% NaCl solution.

Drug Preparation and In Vivo Injection, Gas Chromatography–Mass Spectrometry, ChT and ChBP Measurement, In Vitro HSFs, and Western Blot Analysis. Details of drug preparation and in vivo injection, gas chromatography–mass spectrometry analysis, measurement of guinea pig ChT and ChBP, experimental hypoxia of in vitro HSFs, and Western blot analysis are provided in *SI Appendix, SI Materials and Methods*.

Clinical Study. The clinical study was approved by the Ethics Committee of the Eye Hospital of Wenzhou Medical University (2020-207-K-189-01) and was conducted according to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants. The study was registered as a clinical trial (ChiCTR2000041340) on the Chinese Clinical Trial Registry (https://www.chictr.org.cn/).

Participants were recruited from the Year-1 students at Wenzhou Medical University. Those without histories of ocular surgeries, ocular diseases, diabetes, hypertension, hematological diseases, with refraction <−1 D and >−6 D, astigmatism <1 D in both eyes, and anisometropia <1 D, were invited to participate in this clinical study. Besides measurements of body height and weight as well as blood pressure, participants also received a comprehensive ocular examination, including measurements of refraction, AL (IOLMaster 700, Carl Zeiss Meditec), and intraocular pressure (TX-20, Canon), as well as fundus photography (VisuCam 224, Carl Zeiss Meditec), OCT (Cirrus 5000, Carl Zeiss Meditec), and slit-lamp examination (BQ900, Haag-Streit Diagnostics). All the examinations were performed by a group of certified clinicians. These parameters are presented in *SI Appendix*, Table S9.

Participants watched 15 min of television at a "long" distance (3 m). Then, ChT and choroidal capillary vessel density were estimated using OCTA (VG200S, SVision) as previously described (72). Both horizontal and vertical planes were scanned. Only the vertical data were presented in the results because the trends in horizontal and vertical change were similar, as previously described (72). Following that, the subjects spent 40 min reading (Chinese, fifth font in Microsoft YaHei) on an electronic display at "near" distance (33 cm) (Matepad, Huawei), after which the OCTA was repeated. After the measurements, each participant ingested two capsules of fish oil per day for 14 consecutive days (Nature's Way), providing a daily dose of 600 mg DHA plus 120 mg EPA. Choroidal parameters were measured again, before and after 40 min of reading, on day 15 (Fig. 7*A*). All data were expressed as the difference in values measured after the 40 min of reading minus the values before the 40 min of reading, that is, Difference = $value_{postreading} - value_{prereading}$.

Statistics. All data were analyzed using SPSS software 16.0 (IBM, Armonk). The *Shapiro–Wilk* normality test was used to analyze the distribution of all data sets. Independent data sets were compared by unpaired two-tailed *t* tests or nonparametric *u* tests. For multiple comparisons, one-way ANOVA or two-way ANOVA with Bonferroni's post hoc tests were used. For multiple comparisons over time, two-way repeated-measure ANOVA with Bonferroni's post hoc tests were used. $P < 0.05$ was considered statistically significant. Other descriptive statistics and tests are provided in the figure legends or tables. For statistical differences that were close to $P = 0.05$, an original false discovery rate (FDR) method of Benjamini and Hochberg was used to correct for multiple comparisons by controlling the FDR.

Data Availability. All study data are included in the article and/or *SI Appendix*.

ACKNOWLEDGMENTS. We thank Nethrajeith Srinivasalu (The University of Newcastle, NSW, Australia), Jiangfan Chen (Wenzhou Medical University, China), Yongsheng Chang (Tianjin Medical University, China), Jichun Yang (Beijing University, China), and William K. Stell (Cumming School of Medicine, University of Calgary, Canada) for critical comments and editorial support in manuscript preparation. We also thank Britt Bromberg, PhD, English Language Service, of Xenofile Editing (https://xenofileediting.com) for editing assistance in the final preparation of the manuscript. This work was supported by the National Natural Science Foundation of China Grants 81830027, U20A20364, 82025009, 81670886, and 81700868 and the Natural Science Foundation of Zhejiang Province (Zhejiang Provincial Natural Science Foundation of China) Grant LQ20H120001.

1. E. Dolgin, The myopia boom. *Nature* **519**, 276–278 (2015).
2. P. N. Baird *et al.*, Myopia. *Nat. Rev. Dis. Primers* **6**, 99 (2020).
3. B. A. Holden *et al.*, Global prevalence of myopia and high myopia and temporal trends from 2000 through 2050. *Ophthalmology* **123**, 1036–1042 (2016).
4. S. K. Jung, J. H. Lee, H. Kakizaki, D. Jee, Prevalence of myopia and its association with body stature and educational level in 19-year-old male conscripts in Seoul, South Korea. *Invest. Ophthalmol. Vis. Sci.* **53**, 5579–5583 (2012).
5. I. Morgan, K. Rose, How genetic is school myopia? *Prog. Retin. Eye Res.* **24**, 1–38 (2005).
6. J. Cooper, A. V. Tkatchenko, A review of current concepts of the etiology and treatment of myopia. *Eye Contact Lens* **44**, 231–247 (2018).
7. V. Navel, S. Beze, F. Dutheil, COVID-19, sweat, tears … and myopia? *Clin. Exp. Optom.* **103**, 555 (2020).
8. C. W. Wong *et al.*, Digital screen time during the COVID-19 pandemic: Risk for a further myopia boom? *Am. J. Ophthalmol.* **223**, 333–337 (2021).
9. M. He *et al.*, Effect of time spent outdoors at school on the development of myopia among children in China: A randomized clinical trial. *JAMA* **314**, 1142–1148 (2015).
10. K. L. Loh, Q. Lu, D. Tan, A. Chia, Risk factors for progressive myopia in the atropine therapy for myopia study. *Am. J. Ophthalmol.* **159**, 945–949 (2015).
11. A. Chia *et al.*, Atropine for the treatment of childhood myopia: Safety and efficacy of 0.5%, 0.1%, and 0.01% doses (atropine for the treatment of myopia 2). *Ophthalmology* **119**, 347–354 (2012).
12. J. C. Yam *et al.*, Low-concentration atropine for myopia progression (LAMP) study: A randomized, double-blinded, placebo-controlled trial of 0.05%, 0.025%, and 0.01% atropine eye drops in myopia control. *Ophthalmology* **126**, 113–124 (2019).
13. K. Mori *et al.*, Oral crocetin administration suppressed refractive shift and axial elongation in a murine model of lens-induced myopia. *Sci. Rep.* **9**, 295 (2019).
14. K. Mori *et al.*, The effect of dietary supplementation of crocetin for myopia control in children: A randomized clinical trial. *J. Clin. Med.* **8**, 1179 (2019).
15. W. S. Van Meter *et al.*; American Academy of Ophthalmology, Safety of overnight orthokeratology for myopia: A report by the American Academy of Ophthalmology. *Ophthalmology* **115**, 2301–2313.e1 (2008).
16. K. E. Moore, J. S. Benoit, D. A. Berntsen, Spherical soft contact lens designs and peripheral defocus in myopic eyes. *Optom. Vis. Sci.* **94**, 370–379 (2017).
17. H. Wu *et al.*, Scleral hypoxia is a target for myopia control. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E7091–E7100 (2018).
18. F. Zhao *et al.*, Scleral HIF-1α is a prominent regulatory candidate for genetic and environmental interactions in human myopia pathogenesis. *EBioMedicine* **57**, 102878 (2020).
19. X. Zhou *et al.*, Increased choroidal blood perfusion can inhibit form deprivation myopia in guinea pigs. *Invest. Ophthalmol. Vis. Sci.* **61**, 25 (2020).
20. S. Zhang *et al.*, Changes in choroidal thickness and choroidal blood perfusion in guinea pig myopia. *Invest. Ophthalmol. Vis. Sci.* **60**, 3074–3083 (2019).
21. J. A. Summers, The choroid as a sclera growth regulator. *Exp. Eye Res.* **114**, 120–127 (2013).
22. Anonymous, Fats and fatty acids in human nutrition. Report of an expert consultation. *FAO Food Nutr. Pap.* **91**, 1–166 (2010).
23. M. A. Crawford, R. P. Bazinet, A. J. Sinclair, Fat intake and CNS functioning: Ageing and disease. *Ann. Nutr. Metab.* **55**, 202–228 (2009).
24. S. E. Carlson *et al.*, DHA supplementation and pregnancy outcomes. *Am. J. Clin. Nutr.* **97**, 808–815 (2013).
25. K. A. Mulder, R. Elango, S. M. Innis, Fetal DHA inadequacy and the impact on child neurodevelopment: A follow-up of a randomised trial of maternal DHA supplementation in pregnancy. *Br. J. Nutr.* **119**, 271–279 (2018).
26. C. Cardoso, C. Afonso, N. M. Bandarra, D. H. A. Dietary, Dietary DHA, bioaccessibility, and neurobehavioral development in children. *Crit. Rev. Food Sci. Nutr.* **58**, 2617–2631 (2018).
27. B. Du *et al.*, A prospective study of serum metabolomic and lipidomic changes in myopic children and adolescents. *Exp. Eye Res.* **199**, 108182 (2020).
28. R. Limbu, G. S. Cottrell, A. J. McNeish, Characterisation of the vasodilation effects of DHA and EPA, n-3 PUFAs (fish oils), in rat aorta and mesenteric resistance arteries. *PLoS One* **13**, e0192484 (2018).
29. D. Ye *et al.*, Cytochrome p-450 epoxygenase metabolites of docosahexaenoate potently dilate coronary arterioles by activating large-conductance calcium-activated potassium channels. *J. Pharmacol. Exp. Ther.* **303**, 768–776 (2002).
30. K. R. Zehr, M. K. Walker, Omega-3 polyunsaturated fatty acids improve endothelial function in humans at risk for atherosclerosis: A review. *Prostaglandins Other Lipid Mediat.* **134**, 131–140 (2018).
31. F. J. Fleischhauer, W. D. Yan, T. A. Fischell, Fish oil improves endothelium-dependent coronary vasodilation in heart transplant recipients. *J. Am. Coll. Cardiol.* **21**, 982–989 (1993).
32. M. Mouradian *et al.*, Docosahexaenoic acid attenuates breast cancer cell metabolism and the Warburg phenotype by targeting bioenergetic function. *Mol. Carcinog.* **54**, 810–820 (2015).
33. L. Manzi, L. Costantini, R. Molinari, N. Merendino, Effect of dietary ω-3 polyunsaturated fatty acid DHA on glycolytic enzymes and warburg phenotypes in cancer. *BioMed Res. Int.* **2015**, 137097 (2015).
34. M. R. Alzoubi, H. Aldomi Al-Domi, Could omega-3 fatty acids a therapeutic treatment of the immune-metabolic consequence of intermittent hypoxia in obstructive sleep apnea? *Diabetes Metab. Syndr.* **11**, 297–304 (2017).
35. Z. Zeng *et al.*, Omega-3 polyunsaturated fatty acids attenuate fibroblast activation and kidney fibrosis involving MTORC2 signaling suppression. *Sci. Rep.* **7**, 46146 (2017).
36. J. He, K. Bai, B. Hong, F. Zhang, S. Zheng, Docosahexaenoic acid attenuates carbon tetrachloride-induced hepatic fibrosis in rats. *Int. Immunopharmacol.* **53**, 56–62 (2017).
37. K. A. Lytle, C. M. Depner, C. P. Wong, D. B. Jump, Docosahexaenoic acid attenuates Western diet-induced hepatic fibrosis in Ldlr-/- mice by targeting the TGFβ-Smad3 pathway. *J. Lipid Res.* **56**, 1936–1946 (2015).
38. E. Poggiogalle, H. Jamshed, C. M. Peterson, Circadian regulation of glucose, lipid, and energy metabolism in humans. *Metabolism* **84**, 11–27 (2018).
39. B. P. Sander, M. J. Collins, S. A. Read, Short-term effect of low-dose atropine and hyperopic defocus on choroidal thickness and axial length in young myopic adults. *J. Ophthalmol.* **2019**, 4782536 (2019).
40. L. Ye *et al.*, Comparisons of atropine versus cyclopentolate cycloplegia in myopic children. *Clin. Exp. Optom.* **104**, 143–150 (2020).
41. J. A. Rada, S. Shelton, T. T. Norton, The sclera and myopia. *Exp. Eye Res.* **82**, 185–200 (2006).
42. I. F. Hepsen, C. Evereklioglu, H. Bayramlar, The effect of reading and near-work on the development of myopia in emmetropic boys: A prospective, controlled, three-year follow-up study. *Vision Res.* **41**, 2511–2520 (2001).
43. Y. S. Yang, J. W. Koh, Choroidal blood flow change in eyes with high myopia. *Korean J. Ophthalmol.* **29**, 309–314 (2015).
44. D. M. Isaac *et al.*, Mixed lipid, fish oil, and soybean oil parenteral lipids impact cholestasis, hepatic phytosterol, and lipid composition. *J. Pediatr. Gastroenterol. Nutr.* **68**, 861–867 (2019).
45. Y. Yang *et al.*, Omega-3 polyunsaturated fatty acids prevent progression of liver fibrosis and promote liver regeneration after partial hepatectomy in cirrhotic rats. *Eur. Rev. Med. Pharmacol. Sci.* **23**, 10151–10160 (2019).
46. J. H. Liu *et al.*, Acute EPA-induced learning and memory impairment in mice is prevented by DHA. *Nat. Commun.* **11**, 5465 (2020).

47. L. Liu *et al.*, Dietary DHA/EPA ratio changes fatty acid composition and attenuates diet-induced accumulation of lipid in the liver of ApoE$^{-/-}$ mice. *Oxid. Med. Cell. Longev.* **2018**, 6256802 (2018).
48. J. X. Jin *et al.*, Effect of outdoor activity on myopia onset and progression in school-aged children in northeast China: The Sujiatun Eye Care Study. *BMC Ophthalmol.* **15**, 73 (2015).
49. P. C. Wu, C. L. Tsai, H. L. Wu, Y. H. Yang, H. K. Kuo, Outdoor activity during class recess reduces myopia onset and progression in school children. *Ophthalmology* **120**, 1080–1085 (2013).
50. J. Vongphanit, P. Mitchell, J. J. Wang, Prevalence and progression of myopic retinopathy in an older population. *Ophthalmology* **109**, 704–711 (2002).
51. G. Querques, R. Forte, E. H. Souied, Retina and omega-3. *J. Nutr. Metab.* **2011**, 748361 (2011).
52. H. S. Weisinger, A. J. Vingrys, A. J. Sinclair, The effect of docosahexaenoic acid on the electroretinogram of the guinea pig. *Lipids* **31**, 65–70 (1996).
53. B. G. Jeffrey, H. S. Weisinger, M. Neuringer, D. C. Mitchell, The role of docosahexaenoic acid in retinal function. *Lipids* **36**, 859–871 (2001).
54. C. I. Bercea, G. S. Cottrell, F. Tamagnini, A. J. McNeish, Omega-3 polyunsaturated fatty acids and hypertension: A review of vasodilatory mechanisms of docosahexaenoic acid and eicosapentaenoic acid. *Br. J. Pharmacol.* **178**, 860–877 (2021).
55. J. M. Frenoux, E. D. Prost, J. L. Belleville, J. L. Prost, A polyunsaturated fatty acid diet lowers blood pressure and improves antioxidant status in spontaneously hypertensive rats. *J. Nutr.* **131**, 39–45 (2001).
56. F. Zgheel *et al.*, EPA:DHA 6:1 is a superior omega-3 PUFAs formulation attenuating platelets-induced contractile responses in porcine coronary and human internal mammary artery by targeting the serotonin pathway via an increased endothelial formation of nitric oxide. *Eur. J. Pharmacol.* **853**, 41–48 (2019).
57. A. Ulu *et al.*, An omega-3 epoxide of docosahexaenoic acid lowers blood pressure in angiotensin-II-dependent hypertension. *J. Cardiovasc. Pharmacol.* **64**, 87–99 (2014).
58. Y. F. Shih *et al.*, Reduction in choroidal blood flow occurs in chicks wearing goggles that induce eye growth toward myopia. *Curr. Eye Res.* **12**, 219–227 (1993).
59. S. Wang, Y. Wang, X. Gao, N. Qian, Y. Zhuo, Choroidal thickness and high myopia: A cross-sectional study and meta-analysis. *BMC Ophthalmol.* **15**, 70 (2015).
60. D. L. Nickla, J. Wallman, The multifunctional choroid. *Prog. Retin. Eye Res.* **29**, 144–168 (2010).
61. R. H. Bedrossian, The effect of atropine on myopia. *Ophthalmology* **86**, 713–719 (1979).
62. T. V. Tkatchenko, A. V. Tkatchenko, Pharmacogenomic approach to antimyopia drug development: Pathways lead the way. *Trends Pharmacol. Sci.* **40**, 833–852 (2019).
63. H. D. M. Tran *et al.*, A review of myopia control with atropine. *J. Ocul. Pharmacol. Ther.* **34**, 374–379 (2018).
64. J. A. Dunstan, K. Simmer, G. Dixon, S. L. Prescott, Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: A randomised controlled trial. *Arch. Dis. Child. Fetal Neonatal Ed.* **93**, F45–F50 (2008).
65. P. Trumbo, S. Schlicker, A. A. Yates, M. Poos, Food and Nutrition Board of the Institute of Medicine, The National Academies, Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J. Am. Diet. Assoc.* **102**, 1621–1630 (2002).
66. E. B. Rimm *et al.*, Seafood long-chain n-3 polyunsaturated fatty acids and cardiovascular disease: A science advisory from the american heart association. *Circulation* **138**, e35–e47 (2018).
67. E. C. Rizos, E. E. Ntzani, M. S. Elisaf, Omega-3 fatty acid supplementation and cardiovascular disease events–reply. *JAMA* **309**, 29 (2013).
68. A. B. Nair, S. Jacob, A simple practice guide for dose conversion between animals and human. *J. Basic Clin. Pharm.* **7**, 27–31 (2016).
69. S. U. Khan *et al.*, Effect of omega-3 fatty acids on cardiovascular outcomes: A systematic review and meta-analysis. *EClinicalMedicine* **38**, 100997 (2021).
70. D. L. Bhatt *et al.*; REDUCE-IT Investigators, Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N. Engl. J. Med.* **380**, 11–22 (2019).
71. K. A. Rose, A. N. French, I. G. Morgan, Environmental factors and myopia: Paradoxes and prospects for prevention. *Asia Pac. J. Ophthalmol. (Phila.)* **5**, 403–410 (2016).
72. H. Wu *et al.*, Assessment of choroidal vascularity and choriocapillaris blood perfusion in anisomyopic adults by SS-OCT/OCTA. *Invest. Ophthalmol. Vis. Sci.* **62**, 8 (2021).