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## The high-dose fish oil (FO) supplementation increased Mfsd2a expression in the retina of healthy mice

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Mfsd2a is expressed mainly in the endothelial cells and is an essential regulator of blood vessel transcytosis. Therefore, decrease in Mfsd2a expression can be a risk factor for developing leaky blood vessels. Mfsd2a is also the main docosahexaenoic acid (DHA, C22:6n3) transporter. DHA, an omega-3 fatty acid, is one of the main structural lipids of the neuronal and vascular retina, crucial for the normal functioning of photoreceptors (PRs). However, the capacity of the retina to synthesize DHA is limited, and the maintenance of retinal DHA content relies on the uptake from blood-borne lipids. The currently recommended FO doses yielded low PUFAs tissue bioavailability, and supplementation with higher doses has been increasingly recommended. Nevertheless, the effects of higher FO doses on retinal Mfsd2a expression and blood vessels coverage are unknown.

Western blot and qPCR analyses showed that high dose FO supplementation increased Mfsd2a expression in the retina. Immunohistochemical analyses of Mfsd2a expression on retinal blood vessels (labeled with 488-conjugated Lycopersicon esculentum, lectin) and subsequent ImageJ analyses revealed 1.32-fold increase in the Mfsd2a retinal blood vessel coverage. In the same time the pericyte blood vessel coverage (CD13+ cells) was not affected with FO supplementation, and the increase in Mfsd2a blood vessel expression is not the result of the increased pericyte coverage.

Therefore, the high-dose FO supplementation emerges as the prophylactic fortifier of the retinal blood vessels that can serve either as prophylaxis in the healthy eye or as an adjuvant in developing targeted manipulations of the barrier during diseases.

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