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Characterization of DHA an n-3 long chain (LC) Polyunsaturated fatty acid (PUFA) on vasomotor responses in the rat small mesenteric arteries.

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Background: Diets high in n-3 LC PUFAs ("fish oils") improve cardiovascular health, effects range from reduced inflammation and atherosclerosis to reductions in blood pressure[1]. Supplementation studies have shown that fish oils improve vasomotor responses and indicate improvement in endothelial cell function underlies the improvement in vasomotor parameters[2]. However a recent postprandial study provided evidence that n-3 fish oils also improve endothelium independent relaxation, suggesting a direct action on vascular smooth muscle[3]. We aimed to characterise the vasodilator mechanisms of a major constituent of fish oils, docosahexaenoic acid (DHA) in an ex-vivo model of vascular function the isolated rat mesenteric artery. The ultimate aim is to use the knowledge gained to inform the design of further studies with human volunteers.

Methods: Mesenteric arteries (diameter ~300 μm) from male WKY rats were mounted in a wire myograph in Krebs solution. Arteries were pre-constricted with the thromboxane mimetic U46619 and cumulative concentration response curves were constructed for the vasodilator responses to DHA (100 nM-30 μM). Blockade of vasodilator pathways namely NO (L-NAME, 300 μM), PGI_2 (indomethacin,10 μM), CYP450 (clotrimazole 1 μM) and endothelium dependent hyperpolarization (EDH; blockade of KCa2.3 (apamin 50 nM), $K_{Ca}3.1$ (TRAM-34 1 μM) and KCa1.1 (paxilline 1μM)) on DHA induced relation. In some experiments the endothelium was removed to assess the endothelium independent effects of DHA. Data is presented as mean ± SEM from *n* animals. Any difference in effect was considered statistically significant if $P<0.05$ as determined by one-way ANOVA with Bonferroni's post-test.

Results: DHA caused concentration dependent relaxation of the mesenteric artery(max relaxation 91±3%, 30μM, n=7). This relaxation was not reduced by the inhibition of NO synthase, cyclooxygenase, or CYP450 ($P>0.05$, n=7). In experiments that assessed the involment of the EDH pathway, block of KCa2.3 had no effect, but block of either of $K_{Ca}3.1$ (67±6% Vs. 43±6%, 1 μM DHA, $P<0.05$) or $K_{Ca}1.1$ (78±6 Vs. 30±11%, 1μM DHA, n=5 $P<0.05$) reduced DHA mediated relaxation. Combined blockade of $K_{Ca}1.1$ and $K_{Ca}3.1$ caused a further reduction in relaxation. Removal of the endothelium partially inhibited DHA mediated relaxation (86±6 Vs 41.8%, 1μM DHA, n=4, $P<0.05$)

Conclusions: DHA produces powerful vasodilator responses at concentrations relevant to the postprandial concentrations found in human plasma (1-30 μM). This vasodilation was not affected by inhibition of three major endothelium dependent vasodilator pathways (NO and PGI_2 or metabolites of CYP450) but block of components of EDH, specifically KCa3.1 and 1.1 significantly blocked vasodilation. DHA relaxation was partially inhibited upon removal of the endothelium. Thus relaxation produced by DHA is mediated in part by stimulation an endothelium dependent pathway. However the mechanisms underlying the large residual relaxation remain to be elucidated. Our results indicate a previously unreported action of DHA on the EDH component of endothelium dependent relaxation.

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2. Hall, WL. Nutrition Research Reviews **22**:18, 2009

3. Armah CK et al. Clinical Science **114**:679, 2008.