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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

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Abstract (250/250)

At arterial sites of endothelial denudation and dysfunction, activated platelets contribute to vascular injury through the release of potent contracting factors such as serotonin (5-HT).

This study evaluated whether omega-3 polyunsaturated fatty acids (PUFAs), known to protect the vascular system, are able to prevent platelets-induced contractile responses in isolated arteries and, if so, to investigate the underlying mechanism and the importance of the omega-3 PUFAs formulation.

Porcine coronary arteries (PCA), human internal mammary arteries (IMA) and washed human platelets were prepared and vascular reactivity was studied in organ chambers.

In PCA rings, aggregating platelets caused concentration-dependent contractions that were significantly inhibited by the 5-HT_{2A} receptor antagonist ketanserin, and by EPA:DHA 6:1 but not EPA:DHA 1:1 at 0.4 % v/v. EPA:DHA 6:1 also prevented the 5-HT-induced contractions but affected only slightly those to the thromboxane A₂ analogue U46619. The inhibitory effect of EPA:DHA 6:1 on platelets-induced contractions was not observed in rings without endothelium, and prevented by an eNOS inhibitor but not by inhibitors of endothelium-dependent hyperpolarization. In IMA rings, EPA:DHA 6:1 but not EPA:DHA 1:1 at 0.4 % v/v significantly prevented the 5-HT-induced contraction, and induced greater endothelium-dependent relaxations than bradykinin and acetylcholine sensitive to an eNOS inhibitor.

EPA:DHA 6:1 strongly inhibits platelets- and 5-HT-induced contractions in PCA rings and those to 5-HT in IMA rings most likely through an increased endothelial formation of NO.

These findings suggest that the omega-3 PUFAs EPA:DHA 6:1 formulation may be of interest to prevent platelets-induced vascular injury at arterial sites of endothelial dysfunction.

Keywords Omega-3 polyunsaturated fatty acids; endothelium; nitric oxide; eNOS; platelets; serotonin.

1. Introduction

Several epidemiological studies and randomized control trials have indicated an inverse relationship between the dietary intake of long-chain omega-3 polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the primary and secondary prevention of cardiovascular diseases (Delgado-Lista et al., 2012; DiNicolantonio et al., 2014). Indeed, in 1999 the GISSI-P trial showed that dietary supplementation with omega-3 PUFAs (1 g/day) significantly decreased the risk of cardiovascular death (GISSI-Prevenzione Investigators, 1999). Similarly, EPA supplementation in Japanese patients with a history of coronary artery diseases is associated with a significant reduction of major coronary events, especially non-fatal events (Yokoyama et al., 2007). The beneficial cardiovascular effect of omega-3 PUFAs has been attributed, at least in part, to their ability to improve the blood lipid profile, particularly by decreasing the triglyceride level (Delgado-Lista et al., 2012; Shaikh et al., 2014). Additional mechanisms have also been suggested, including anti-atherosclerotic effects, anti-hypertensive effects, anti-inflammatory effects and inhibition of platelet aggregation (Calder, 2010; Stone, 1996). Moreover, the beneficial cardiovascular effect of omega-3 PUFAs may also be due to the improvement of the endothelial function. Indeed, our previous study demonstrated that amongst several omega-3 PUFAs formulations, EPA:DHA 6:1 was identified as a potent stimulator of the endothelial formation of nitric oxide (NO), and also, to a lesser extent, of endothelium-dependent hyperpolarization (EDH) in porcine coronary arteries (Zgheel et al., 2014). The increased endothelial NO formation is mediated by a redox-sensitive activation of Src/PI3-kinase/Akt and MAPKs pathways leading to endothelial NO synthase (eNOS) activation, which is dependent on both ratio and purity of the EPA:DHA formulation (Zgheel et al., 2014).

The spontaneous contraction of arteries, such as the coronary artery in Prinzmetal angina, has been described as an independent risk factor of cardiovascular events (MacAlpin, 2015).

Coronary artery spasms are generally more frequent at sites of atherosclerotic lesions, which are characterized by an endothelial dysfunction (Zaya et al., 2014). The local activation of platelets at sites of endothelial dysfunction has been suggested to contribute to vascular injury, in part, by releasing vasoactive factors including serotonin (5-HT) and by stimulating the formation of vasoconstrictor prostanoids (Kim et al., 1992; Komori et al., 1989; Shimokawa et al., 1988a, 1988b; Shimokawa and Vanhoutte, 1991).

The long-term consumption of fish oil has been shown to improve endothelium-dependent vasodilatations in patients with coronary artery diseases, and also endothelium-dependent relaxations of coronary artery rings from hypercholesterolemic and atherosclerotic pigs (Shimokawa and Vanhoutte, 1988; Tagawa et al., 2002). Moreover, EPA induced endothelium-dependent and -independent relaxations of isolated sheep pulmonary arteries by increasing the formation of NO and by blunting the calcium pathway leading to relaxation (Singh et al., 2010). Altogether, these findings suggest that omega-3 PUFAs could exert a beneficial effect against platelets-induced vasospasms. Therefore, the present study aims to determine the ability of different ratios of EPA and DHA to inhibit aggregating platelets-induced contractions in porcine coronary artery and human internal mammary artery rings and, if so, to determine the role of the endothelium and the underlying mechanism.

2. Material and method

2.1. Chemicals

Omega-3 PUFAs were provided by Pivotal Therapeutics, Inc. (Woodbridge, ON, Canada). Unless stated, all products were obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France). 5-HT and TRAM-34 (1-[(2-Chlorophenyl)diphenylmethyl]-1H-pyrazole) were from R&D Systems (Lille, France). All compounds were dissolved in distilled water except for EPA:DHA formulations (ethanol:water 50:50), indomethacin (5% sodium carbonate aqueous solution) and bradykinin (Krebs buffer).

2.2. Vascular reactivity studies

Vascular reactivity was assessed as indicated previously (Zgheel et al., 2014). Briefly, rings of porcine coronary arteries (4–5 mm in length) were suspended in organ baths containing oxygenated (95% O₂, 5% CO₂) Krebs bicarbonate solution (composition in mM: NaCl 119, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ 1.18, CaCl₂ 1.25, NaHCO₃ 25 and D-glucose 11, pH 7.4, 37°C) for the determination of changes in isometric tension (basal tension of porcine coronary artery and human IMA rings: 5 g). The reactivity of the vascular smooth muscle was assessed in response to Krebs buffer containing 80 mM of potassium. The integrity of the endothelium was checked with bradykinin (0.3 μM) after a sub-maximal contraction (about 70%) with 9,11-dideoxy-9α,11α-methanoepoxy-prosta-5Z,13E-dien-1-oic acid (U46619). Rings were incubated for 10 min with different ratios and volumes of omega-3 PUFAs before construction of a concentration-contraction curve in response to either aggregating platelets, 5-HT or U46619.

Human internal mammary artery (IMA) segments were obtained from the Department of Cardiac Surgery of the University Hospital of Strasbourg (see Table 1 for patients characteristics). **All patients provided informed written consent allowing the scientific use of**

internal mammary artery segments not used for bypass surgery. IMA were immediately collected and placed in chilled Krebs solution at 4°C, cleaned and cut in rings of 4-5 mm in length before the evaluation of vascular reactivity. The vasorelaxant effect of acetylcholine, bradykinin and EPA:DHA 6:1 was determined by constructing a concentration-relaxation curve following contraction of IMA rings to about 70% of the maximal contraction with U46619. In some experiments, rings were exposed to an inhibitor for 30 min before contraction. Neither the removal of the endothelium nor the different pharmacological tools affected the basal tension (data not shown).

2.3. Platelets

Platelets freshly isolated from blood of healthy human donors and suspended in Tyrode-albumin buffer were kindly provided by the local Blood Transfusion Center (Etablissement Français du Sang-Alsace, Strasbourg). The suspension of platelets was adjusted to 600 000 platelets/ μ l. Thereafter, increasing numbers of platelets were added to the Krebs solution in the organ chamber triggering their subsequent aggregation and degranulation. In some cases, rings were incubated in the presence of a pharmacological agent for 30 min before the addition of either platelets or 5-HT.

2.4. Statistical analysis

All values are expressed as means \pm S.E.M. of n different experiments. Statistical analysis was performed using Student's paired *t*-test or an analysis of variance test followed by Bonferroni's post-hoc test as appropriate using Graphpad Prism software (version 5.04 for Windows, GraphPad Software, INC, CA, USA). *P* < 0.05 was considered as statistically significant.

3. Results

3.1. EPA:DHA 6:1 inhibits platelets-induced contractions in coronary artery rings: role of 5-HT and endothelial NO

Addition of increasing numbers of washed human platelets to organ baths induced concentration-dependent contractions in coronary artery rings, which were similar in rings with or without endothelium (Fig. 1A). The platelets-induced contractions were abolished in the presence of ketanserin, a 5-HT₂ receptor antagonist, but not in the presence of the cyclooxygenase inhibitor indomethacin, indicating a major role of platelet-derived 5-HT (Fig. 1A and B). Although the EPA:DHA 1:1 formulation at 0.4 % v/v did not affect platelet-induced contractile responses in rings either with or without endothelium, EPA:DHA 6:1 at 0.4 % v/v abolished the contractile response in rings with endothelium but not in those without endothelium (Fig. 1C and D). The inhibitory effect of EPA:DHA 6:1 was prevented in the presence of the eNOS inhibitor N^G-nitro-L-arginine (NLA) whereas it was not affected by inhibitors of EDH (TRAM-34 plus apamin, inhibitors of intermediate (IK_{Ca}) and small (SK_{Ca}) conductance calcium-activated potassium channels, respectively) or cyclooxygenases (indomethacin, Fig. 1E). These findings indicate that EPA:DHA 6:1 is a superior omega-3 PUFAs formulation reducing the activated platelets-induced 5-HT-mediated contractile responses in coronary artery rings **most likely** through an increased endothelial formation of NO.

3.2. EPA:DHA 6:1 inhibits 5-HT-induced contractions in coronary artery rings by increasing endothelial NO

Next, experiments were performed to determine whether omega-3 PUFAs attenuate contractions induced by 5-HT in coronary artery rings, and to clarify the role of the EPA:DHA ratio and the endothelium. In rings with endothelium, the EPA:DHA 1:1

formulation (0.4 % v/v) slightly but significantly attenuated the 5-HT-induced contractions only at the highest concentration (10^{-5} M, Fig. 2A). In contrast, EPA:DHA 6:1 strongly inhibited 5-HT-induced contractions in a concentration-dependent manner (Fig. 2B). Indeed, EPA:DHA 6:1 at concentrations of 0.2 and 0.4 % v/v significantly reduced the contractile responses assessed at 10^{-5} M of 5-HT by 57.5 ± 2.1 and 93.2 ± 0.5 %, respectively (Fig. 2B). Although, EPA:DHA 6:1 markedly reduced 5-HT-induced contractile responses in rings with endothelium, no such effect was observed in those without endothelium (Fig. 2C). In addition, EPA:DHA formulations at a ratio of either 1:1 or 6:1 did not affect contractions induced by the thromboxane A₂ analogue U46619 (Fig. 3). These findings indicate that EPA:DHA 6:1 is a superior omega-3 PUFAs formulation that preferentially attenuates the 5-HT-induced contractile responses in intact coronary artery rings.

Since EPA:DHA 6:1 is a superior stimulator than EPA:DHA 1:1 of the endothelial formation of NO and also, to some extent, of EDH (Zgheel et al., 2014), the role of NO and EDH in the ability of EPA:DHA 6:1 to inhibit the 5-HT-induced contraction was investigated. In the presence of indomethacin, the inhibitory effect of EPA:DHA 6:1 on 5-HT-induced contractile responses was abolished in the presence of the eNOS inhibitor NLA and not affected by inhibitors of EDH (TRAM-34 plus apamin, inhibitors of IK_{Ca} and SK_{Ca}, respectively), indicating a major role of NO (Fig. 4A and B). To determine the sensitivity of the 5-HT-induced contractile response to the inhibitory effect of NO, rings without endothelium were exposed to a low concentration of the NO donor sodium nitroprusside (SNP, 100 nM) prior to the construction of a concentration-contraction curve. Preliminary studies have indicated that SNP at 100 nM induced about 20-30 % relaxation of porcine coronary artery rings (data not shown). SNP (100 nM) markedly attenuated 5-HT-induced contractile responses, whereas those to U46619 were reduced only slightly but significantly at concentrations greater than 1 μ M (Fig. 5A and B). Altogether, these findings indicate that EPA:DHA 6:1 effectively

inhibits 5-HT-induced contractile responses mainly by increasing the endothelial formation of NO.

3.3. EPA:DHA 6:1 is a superior inhibitor of 5-HT-induced contractile responses in human internal mammary artery rings

To determine the clinical relevance of the present findings, experiments were performed with human internal mammary artery (IMA) rings obtained from patients undergoing bypass surgery. Like with porcine coronary artery rings, EPA:DHA 1:1 did not affect the 5-HT-induced contractile responses in IMA rings, whereas they were abolished by EPA:DHA 6:1 at 0.4 % v/v (Fig. 6A and B). Moreover, the inhibitory effect of EPA:DHA 6:1 was abolished by NLA and not affected by inhibitors of EDH, indicating a pivotal role of the NO pathway (Fig. 6C). In contrast to contractions induced by 5-HT, those induced by U46619 were not affected by EPA:DHA 6:1 at 0.4 % v/v (Fig. 6D). In addition, EPA:DHA 6:1 as well as bradykinin and acetylcholine, two physiological agonists, induced concentration-dependent relaxations in human IMA rings (Fig. 7). These relaxations were abolished by NLA and not affected by inhibitors of EDH (Fig. 7A-C). Moreover, the relaxation induced by EPA:DHA 6:1 was significantly more pronounced than that to either bradykinin or acetylcholine (Fig. 7 A-D). Altogether, these findings indicate that EPA:DHA 6:1 is a potent inhibitor of 5-HT-induced contractile responses in IMA rings most likely due to its ability to stimulate the endothelial formation of NO.

4. Discussion

The major novel findings of this study indicate that EPA:DHA 6:1, an omega-3 PUFAs formulation, effectively prevents activated platelets-induced 5-HT-mediated contractions in porcine coronary artery and 5-HT-mediated contractions in human internal mammary artery rings most likely by stimulating the endothelial formation of NO.

The inhibition of the activated platelets- and 5-HT-induced contractions in coronary arteries is dependent on the EPA:DHA ratio, with the 6:1 ratio being more potent than the 1:1 ratio.

These results are in line with our previous study indicating that the EPA:DHA 6:1 is a superior omega-3 PUFAs formulation for the induction of endothelium-dependent relaxations mediated by NO, and, to a lesser extent, EDH (Zgheel et al., 2014). Indeed, although EPA and DHA alone evoked similar endothelium-dependent relaxations, their combination EPA:DHA 6:1 and 9:1 demonstrated superiority to the formulation EPA:DHA 1:1, 3:1, 1:3, 1:6 and 1:9.

Although the mechanism explaining such a synergistic effect remains unclear, it might involve differences in the integration of omega-3 PUFAs into membrane lipids and/or that the stability of the omega-3 PUFAs requires an optimal EPA:DHA ratio. EPA but not DHA has also been shown to cause cerebral microvascular vasodilatation involving arachidonic acid metabolites (Ellis et al., 1990), whereas DHA but not EPA supplementation improved forearm microvascular reactivity in overweight hyperlipidemic men (Mori et al., 2000).

The inhibitory effect of EPA:DHA 6:1 requires the presence of a functional endothelium and since it is prevented by an inhibitor of eNOS, it implies a major role of the endothelial formation of NO. Similarly, EPA:DHA 6:1 also effectively inhibited the 5-HT-induced contractions in human internal mammary artery rings by an endothelium-dependent NO-mediated mechanism. Previous studies have also shown that chronic ingestion of omega-3 PUFAs, in particular EPA, improved endothelium-dependent NO-mediated relaxations in

porcine coronary artery rings as well as the forearm endothelium-dependent vasodilatation in humans (Shimokawa et al., 1987; Tagawa et al., 2002).

In addition to the direct activation of the endothelial formation of NO, studies have suggested that omega-3 PUFAs might also exert beneficial effects by inhibiting the aggregation and activation of platelets (Abeywardena and Head, 2001). Indeed, dietary supplementation of human volunteers with fish oil rich in omega-3 PUFAs is associated *ex vivo* with a reduced susceptibility of platelets to aggregation (von Schacky et al., 1985). Moreover, EPA and DHA inhibited platelet reactivity by a direct inhibition of the thromboxane receptor (Swann et al., 1989). In the present study, platelets-induced contractions in porcine coronary artery rings involved mainly a 5-HT-mediated mechanism, as indicated by the prevention of the contractions in the presence of ketanserin, an antagonist of 5-HT₂ receptors. Moreover, the contraction induced by aggregating platelets in coronary artery rings was not affected by indomethacin, ruling out the involvement of prostanoids.

Previous studies have indicated that in the rat mesenteric artery and aorta, EPA and DHA inhibited contractions in response to both a thromboxane A₂ analogue and prostaglandin F_{2α} involving TP and FP receptors, respectively (Shimokawa and Vanhoutte, 1988; Zellers et al., 1991). However, the present findings indicate that although EPA:DHA 6:1 effectively inhibited the 5-HT-induced contraction in porcine coronary artery rings, the contraction induced by the thromboxane A₂ analogue was not affected. Moreover, contractions induced by activated platelets in coronary artery rings, and the inhibitory effect of EPA:DHA 6:1 on platelet-induced contractions were both not affected by indomethacin, indicating that vasoactive prostanoids are not involved. Altogether, the present findings and the previous ones suggest that the role of prostanoids on the vascular effects of omega-3 PUFAs might depend on the species and/or the vascular bed.

To determine whether the observations in porcine coronary artery rings have a potential relevance for human blood vessels, the effect of EPA:DHA on 5-HT-induced contractions was assessed in human IMA rings. In cardiac surgery, the IMA is often used for coronary artery bypass graft, and is known to have a similar reactivity as the coronary artery (Shimokawa et al., 1988a). In addition, previous studies have indicated that the endothelium of the IMA expresses eNOS, releases NO and is subjected to potent NO-mediated relaxations in response to acetylcholine (He et al., 2011; Shapira et al., 1999). Moreover, the presence of 5-HT receptors has been demonstrated in IMA, and their activation by 5-HT has been suggested to contribute to vasospasms (Rosenfeldt et al., 1999), in particular in arteries with an endothelial dysfunction (Komori et al., 1989). In the present study, 5-HT induced potent concentration-dependent contractions of IMA rings, which were significantly inhibited by EPA:DHA 6:1, but not by 1:1 at 0.4% v/v. In addition, EPA:DHA 6:1 also induced concentration-dependent NO-mediated relaxations in IMA rings and inhibited the 5-HT-induced contractions by a NO-dependent mechanism. Of interest, the ability of EPA:DHA 6:1 to induce endothelium-dependent NO-mediated relaxation in IMA rings was greater than that induced by physiological agonists including acetylcholine and bradykinin. Indeed, both physiological agonists induced only partial relaxations of IMA rings (less than 55%) indicating that IMAs obtained from patients undergoing coronary artery bypass graft surgery have an endothelial dysfunction. Taken together, these findings suggest that EPA:DHA 6:1 is a potent stimulator of the endothelial formation of NO, and, hence, may help to prevent platelet activation which can contribute to local vasospasms and possibly also to the arterial response to injury. Indeed, previous studies have indicated that platelets contribute to vascular remodeling via different mechanisms including recruitment of immune cells into the arterial wall, formation of inflammatory mediators, shedding of deleterious microparticles, chemokines release, and direct cell-cell interaction (Lannan et al., 2014; Nording et al., 2015).

In addition, the platelets-induced perivascular recruitment of T lymphocytes contributed to coronary artery remodeling in a model of left ventricular hypertrophy (Yang et al., 2012). Moreover, platelets promoted arterial remodeling through the GPIIb/IIIa-mediated interaction with endothelial cells and the release of platelet dense granules (Chandraratne et al., 2015; King et al., 2009).

In conclusion, the present findings indicate that EPA:DHA 6:1 is able to effectively prevent the platelets-induced 5-HT-mediated contractions in porcine coronary and human internal mammary artery rings **most likely** through an increased endothelial formation of NO, and, hence, may help to protect the cardiovascular system. The present findings using the human internal mammary artery further highlight the potential protective effect of omega-3 PUFAs on the human vasculature.

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6. Conflict of interest

The authors report no conflict of interest.

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Figure Legends

Figure 1. The platelets-induced 5-HT-mediated contraction of porcine coronary artery rings is inhibited by EPA:DHA and is dependent on the EPA:DHA ratio and formation of NO. (A, B) Coronary artery rings with or without endothelium were incubated for 30 min in the presence or absence of either (A) ketanserin (5-HT_{2A} serotonin receptor antagonist, 10 μM) or (B) indomethacin (cyclooxygenases inhibitor, 10 μM) before being subjected to increasing concentrations of washed human platelets to construct a concentration-contraction curve. (C, D) Coronary artery rings with or without endothelium were incubated for 10 min with an EPA:DHA ratio of either of 1:1 (C) or 6:1 (D) at 0.4 % v/v before being subjected to increasing concentrations of washed human platelets. (E) Coronary artery rings were incubated with either NLA (N^G-L-nitro-arginine, a NO synthase inhibitor, 300 μM), the combination of TRAM + APA (TRAM-34 + apamin, inhibitors of the endothelium-dependent hyperpolarization, both at 100 nM), or indomethacin (10 μM) for 30 min before addition of EPA:DHA 6:1 at 0.4 % v/v and subsequent contraction with increasing concentrations of washed human platelets. Results are expressed as mean ± **S.E.M.** of 3 different experiments. (A) * $P < 0.05$ vs. Control. (E) * $P < 0.05$ vs. EPA:DHA 6:1.

Figure 2. Effects of EPA:DHA ratio and concentration on contractile responses to 5-HT in porcine coronary artery rings. Coronary artery rings with (A, B) or without endothelium (C) were incubated for 10 min with an EPA:DHA ratio of either of 1:1 (A) or 6:1 (B, C) at indicated concentrations before being subjected to increasing concentrations of 5-HT. Results are expressed as mean ± **S.E.M.** of 5 different experiments. * $P < 0.05$ vs. Control.

Figure 3. Effects of EPA:DHA ratio and concentration on contractile responses to a thromboxane A₂ analogue in porcine coronary artery rings. Coronary artery rings with

endothelium were incubated for 10 min with EPA:DHA ratio of either of 1:1 (A) or 6:1 (B) at the indicated concentration before being subjected to increasing concentrations of U46619. Results are expressed as mean \pm **S.E.M.** of 4 different experiments.

Figure 4. Characterization of the role of endothelium-derived relaxing factors in the inhibitory effect of EPA:DHA 6:1 on the 5-HT-induced contraction in porcine coronary artery rings.

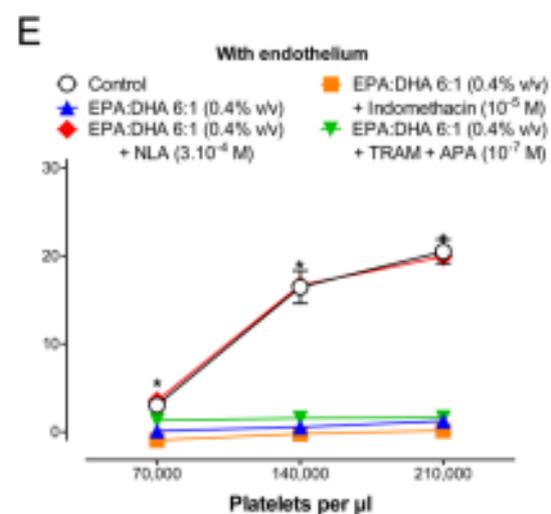
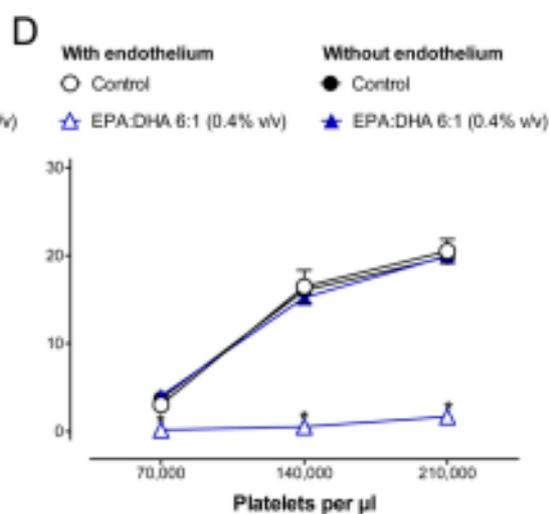
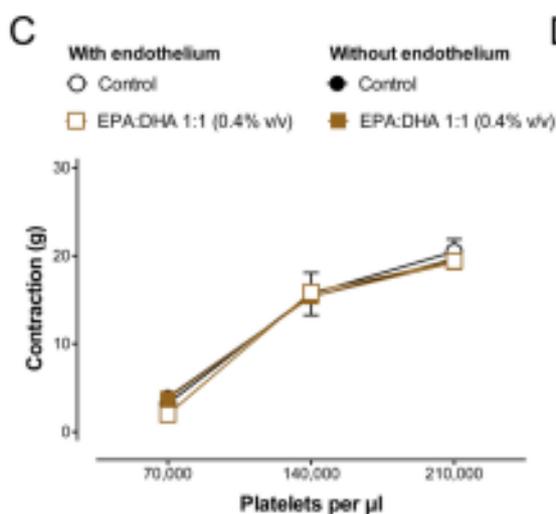
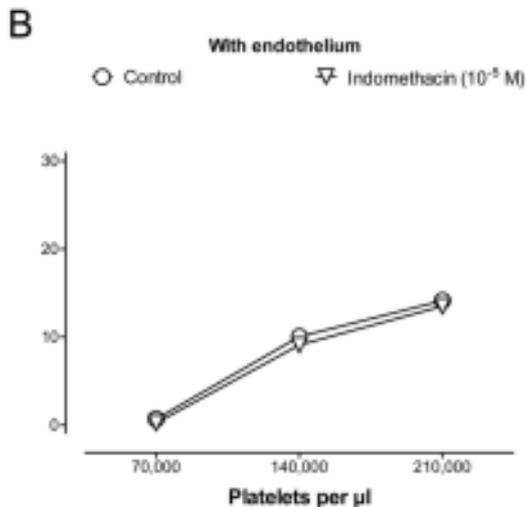
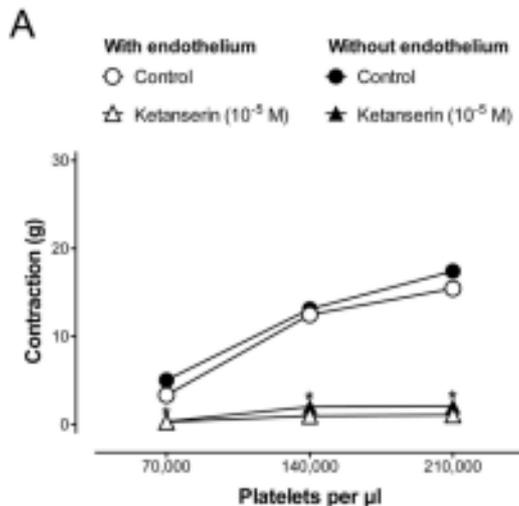
Coronary artery rings with endothelium were incubated with 0.4 % v/v of EPA:DHA 6:1 for 10 min before being subjected to increasing concentrations of 5-HT. Some rings were incubated with either (A) NLA (N^o-L-nitro-arginine, 300 μ M) or (B) the combination of TRAM + APA (TRAM-34 + apamin, both at 100 nM) for 30 min before the addition of EPA:DHA 6:1. All experiments were performed in the presence of indomethacin (10 μ M). Results are expressed as mean \pm **S.E.M.** of 4-5 different experiments. * $P < 0.05$ vs. Control. # $P < 0.05$ vs. EPA:DHA 6:1.

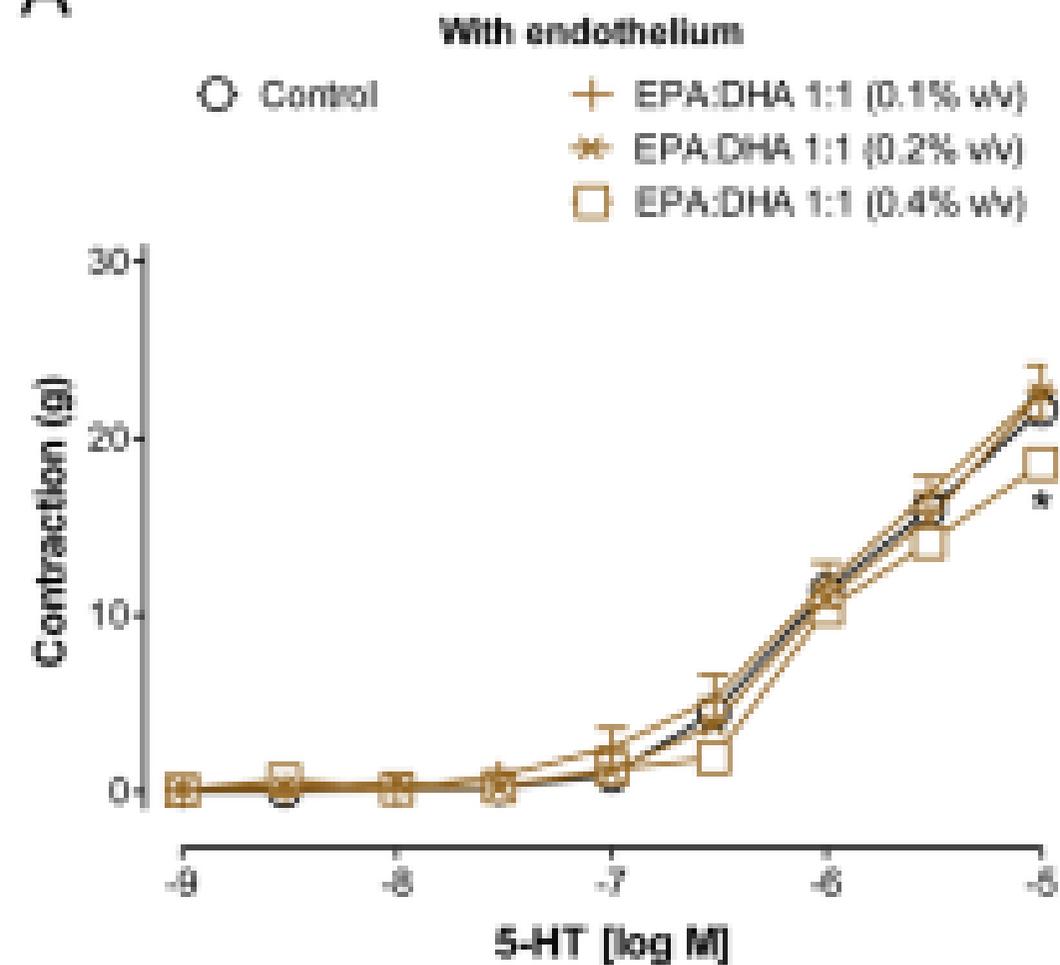
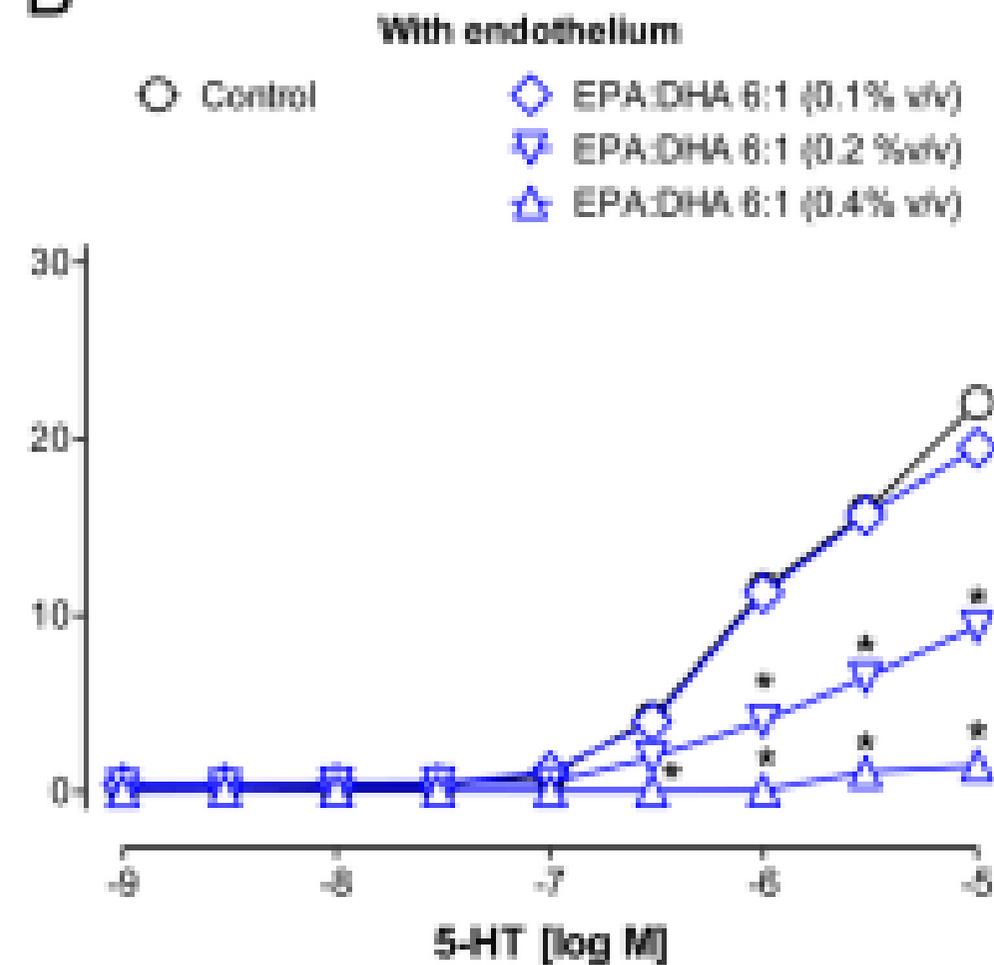
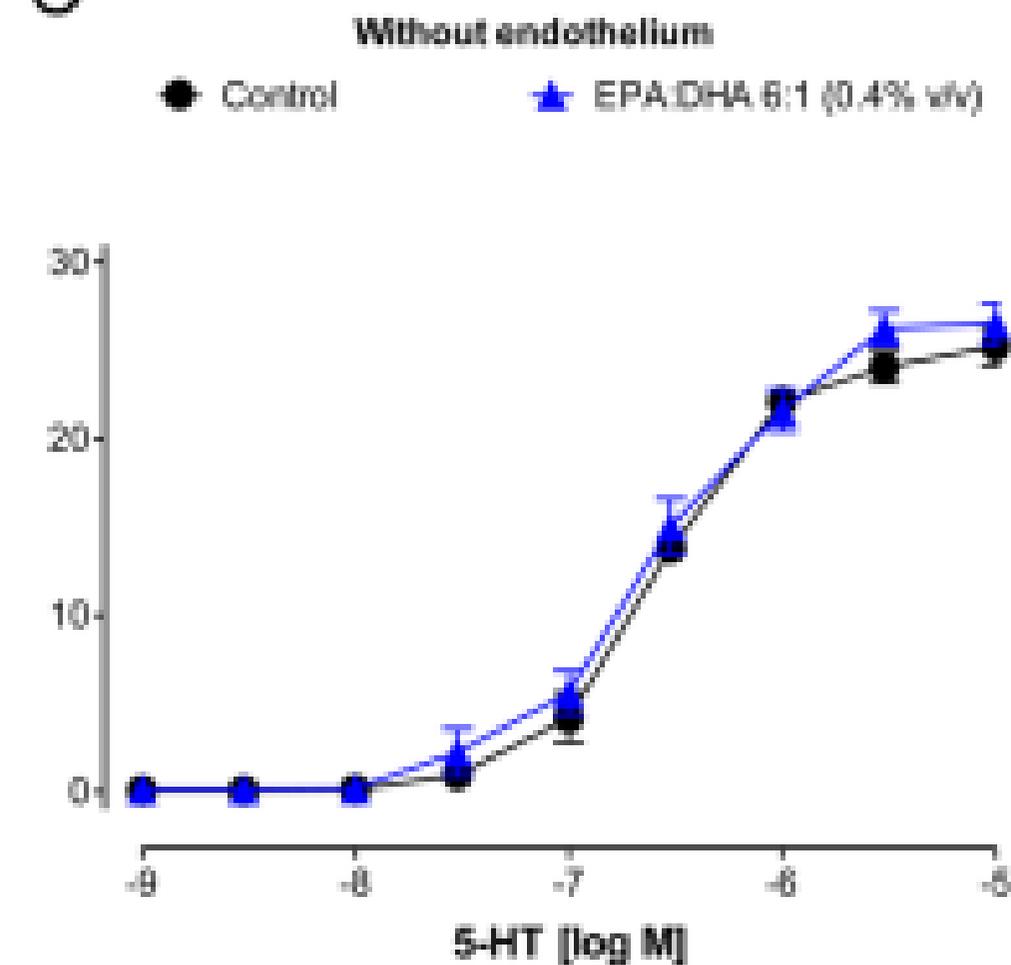
Figure 5. The presence of a low concentration of the NO donor sodium nitroprusside markedly inhibits contractile responses to 5-HT, but only slightly those to U44619 in porcine coronary artery rings. Coronary artery rings without endothelium were incubated with sodium nitroprusside (SNP, 100 nM) before being subjected to increasing concentrations of either (A) 5-HT or (B) U46619. Results are expressed as mean \pm **S.E.M.** of 5 different experiments. * $P < 0.05$ vs. Control.

Figure 6. EPA:DHA 6:1, but not 1:1 ratio, inhibits the 5-HT-induced contractions in human internal mammary artery rings through the eNOS-derived NO pathway. (A, B) Rings of human internal mammary artery (IMA) collected after coronary artery bypass graft surgery were incubated with EPA:DHA ratio of either 1:1 (A) or 6:1 (B) at the indicated concentration for 10 min before being subjected to increasing concentrations of 5-HT. (C) Rings of IMA were

incubated for 30 min with either NLA (300 μ M) or the combination of TRAM + APA (TRAM-34 + apamin, both at 100 nM) in the presence or absence of EPA:DHA 6:1 at 0.4 % v/v before addition of increasing concentrations of 5-HT. (D) Rings of IMA were incubated for 10 min with EPA:DHA 6:1 at the indicated concentration before addition of increasing concentrations of U46619. All experiments were performed in the presence of indomethacin (10 μ M). Results are expressed as mean \pm S.E.M. of 4-5 different experiments. * $P < 0.05$ vs. Control. # $P < 0.05$ vs. EPA:DHA 6:1.

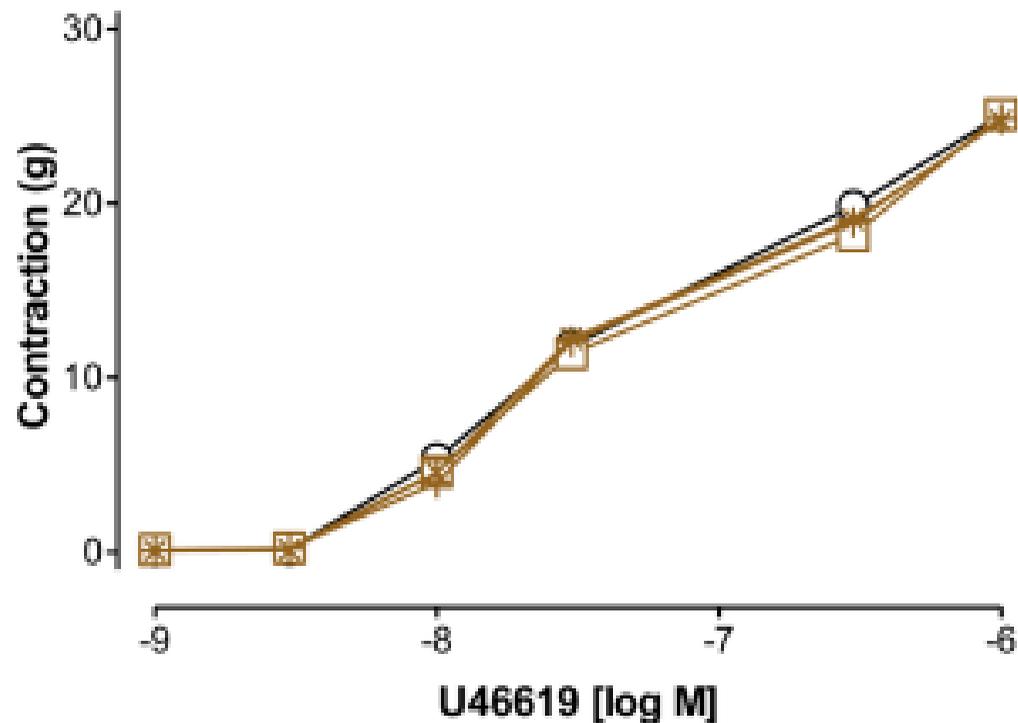
Figure 7. EPA:DHA 6:1 induces greater NO-mediated relaxations than physiological agonists in human IMA rings. Rings of human IMA were incubated for 30 min with either NLA (300 μ M), the combination of TRAM + APA (TRAM-34 + apamin, both at 100 nM), or both before addition of increasing concentrations of either bradykinin (A), acetylcholine (B) or EPA:DHA 6:1 (C). All experiments were performed in the presence of indomethacin (10 μ M). Results are expressed as mean \pm S.E.M. of 4-5 different experiments. Maximal values of relaxation (E_{max}) to either bradykinin, acetylcholine or EPA:DHA 6:1 are shown in D. * $P < 0.05$ vs. Control.



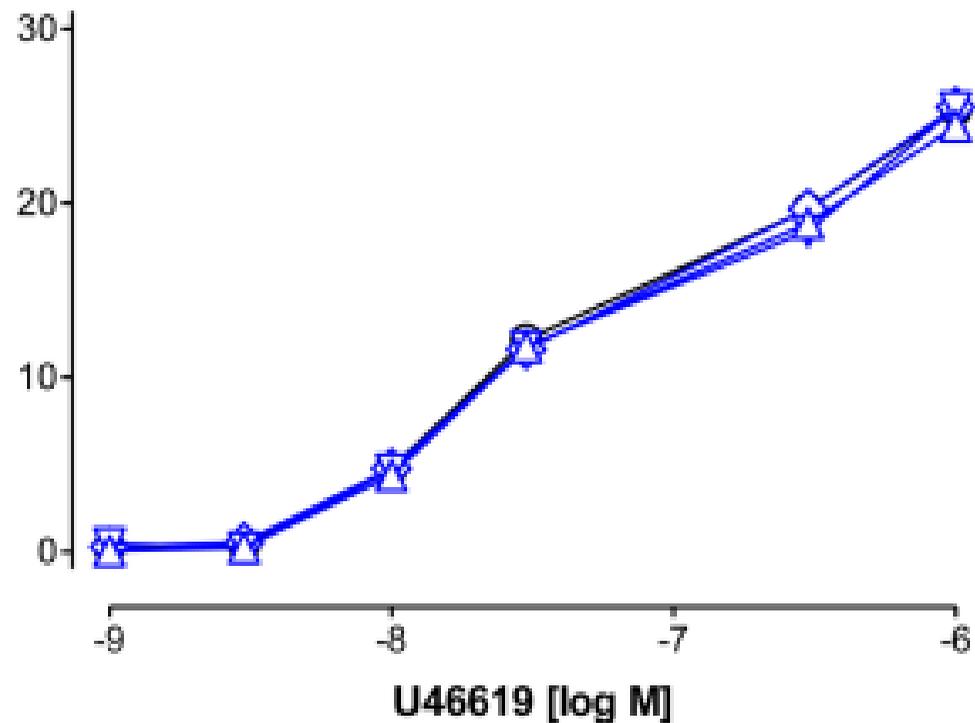
A**B****C**

A**With endothelium**

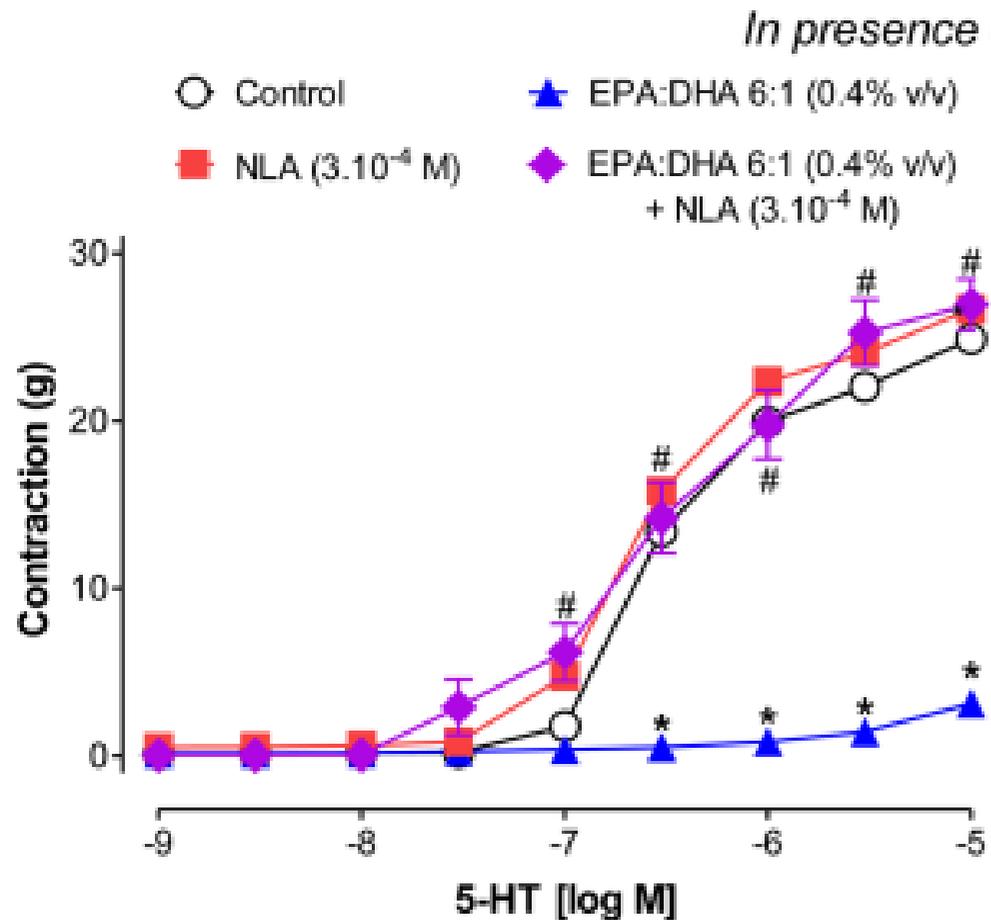
- Control
- + EPA:DHA 1:1 (0.1% v/v)
- * EPA:DHA 1:1 (0.2% v/v)
- EPA:DHA 1:1 (0.4% v/v)

**B****With endothelium**

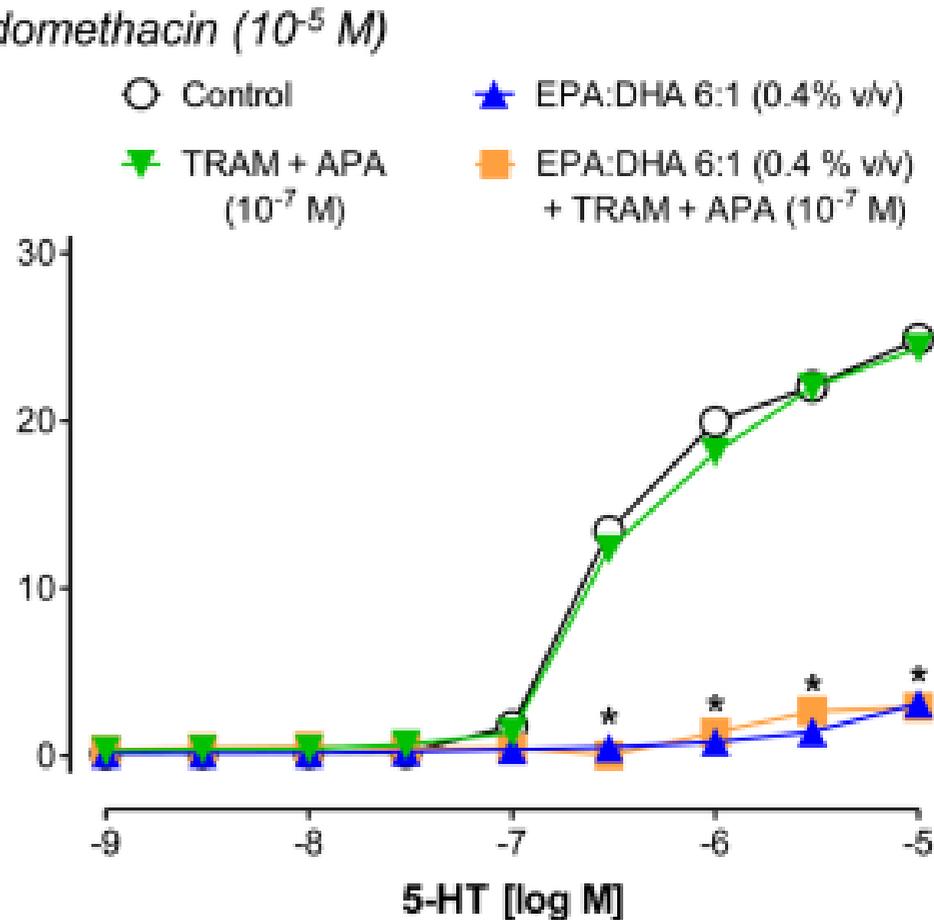
- Control
- ◇ EPA:DHA 6:1 (0.1% v/v)
- ▽ EPA:DHA 6:1 (0.2% v/v)
- △ EPA:DHA 6:1 (0.4% v/v)

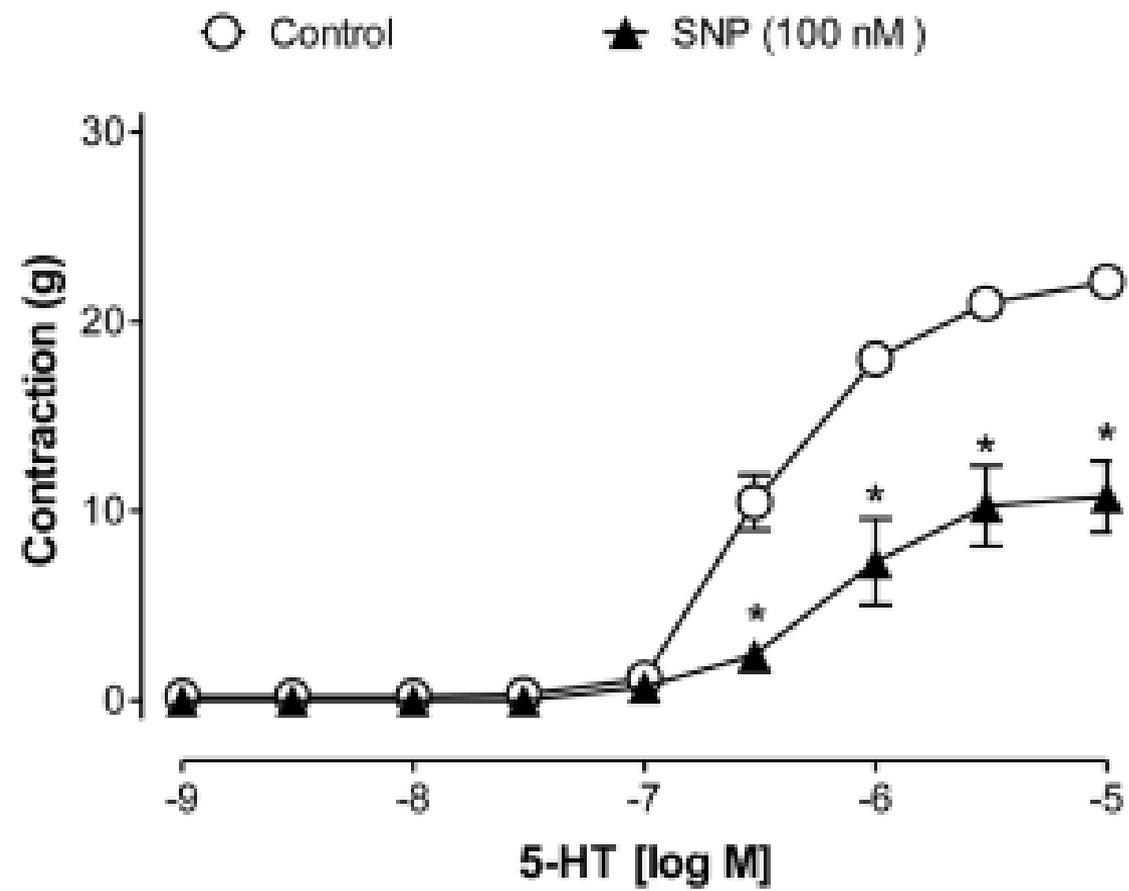
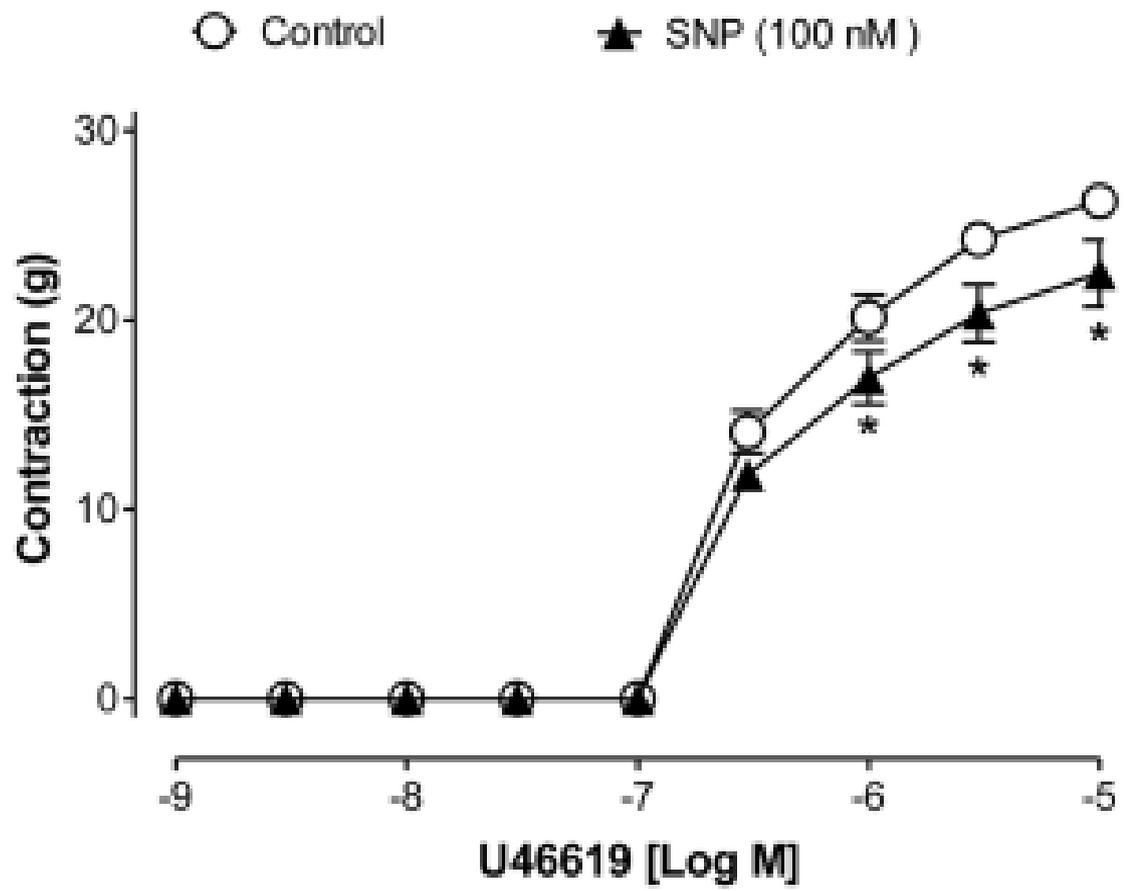


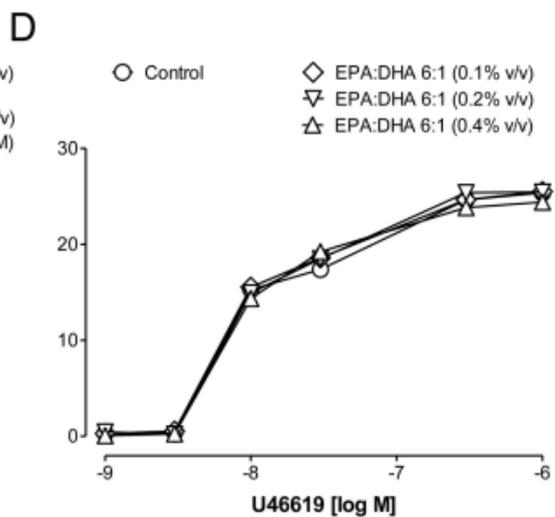
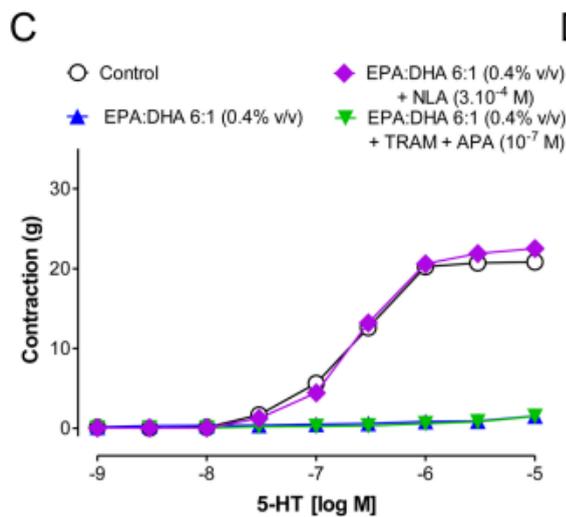
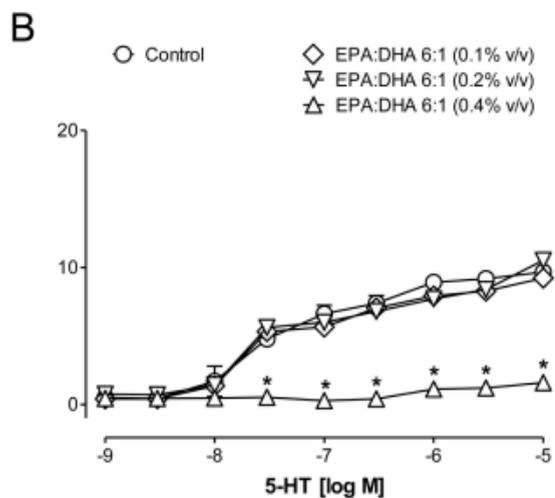
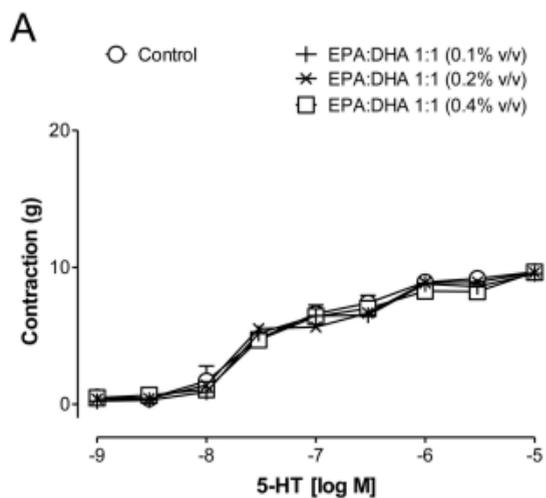
A



B



A**B**



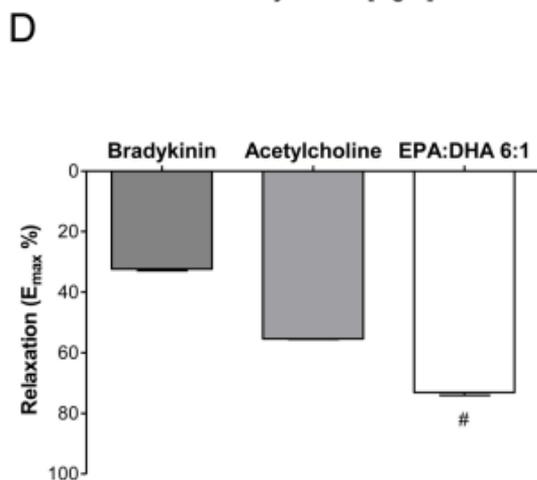
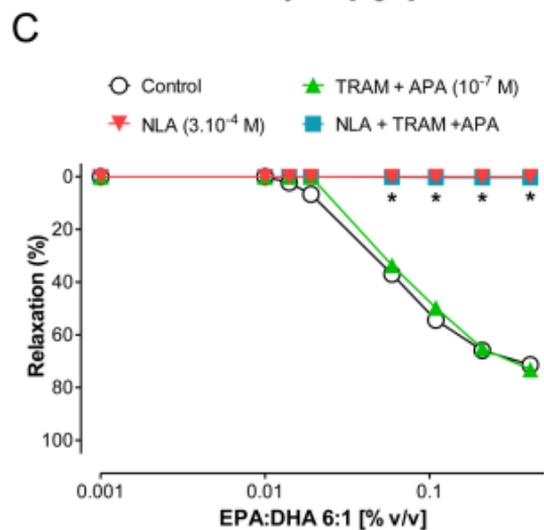
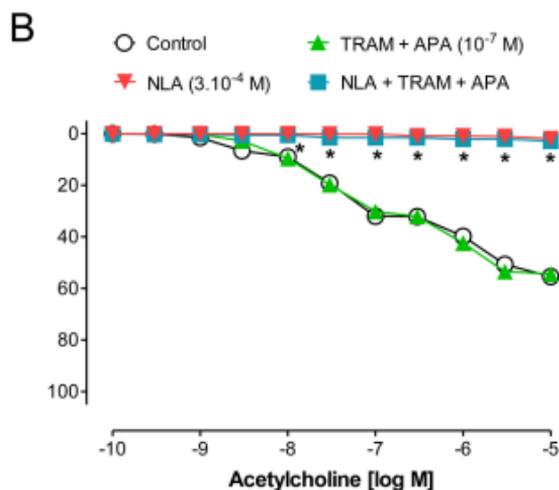
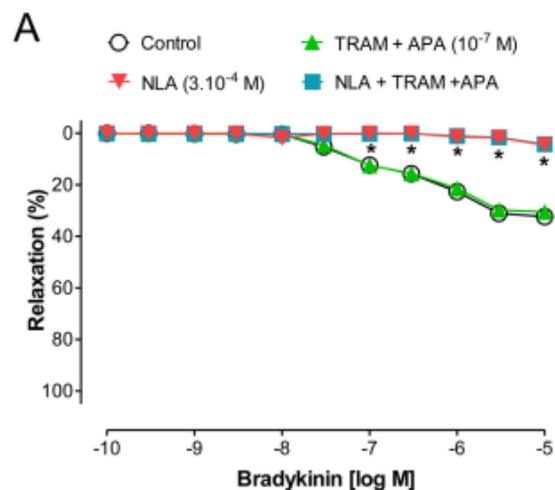


Table 1. Clinical characteristics of coronary artery bypass graft surgery patients

Patient number	Age	Sex	BMI	Diabetes	Hypertension	Dyslipidemia
P1	72	M	31.51	+	-	+
P2	81	M	30.12	-	+	+
P3	79	M	28.08	+	+	+
P4	81	F	34.21	+	+	-
P5	82	M	27.08	+	+	-
P6	71	M	24.98	+	+	+
P7	84	F	32.46	-	+	+
P8	78	M	29.74	+	+	+
P9	65	M	33.46	-	+	+
P10	76	F	24.65	-	+	+
P11	84	M	27.10	+	+	-
P12	66	M	19.91	-	+	-
P13	50	M	27.06	-	+	+
P14	77	M	21.45	+	+	-
P15	71	F	28.12	-	+	+
Total	74.5±2.4	27.3% (4/15)	28.0±1.1	53.3% (8/15)	93.3% (14/15)	66.7% (10/15)

M=Male; F=Female. Diabetes was defined as a previous history of diabetes mellitus. Total values are given as mean±S.E.M. for Age and BMI (Body Mass Index), and as percent (n/15) for female ratio and the presence of Diabetes, Hypertension and Dyslipidemia.