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The 5-series F_2-isoprostanes possess no vasomotor effects in the rat thoracic aorta, the human internal mammary artery and the human saphenous vein

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1 Among the F_2-isoprostanes, the 15- and the 5-series are currently used as markers of lipid peroxidation in vascular diseases. 15-F_{2t}-IsoP (also named iP$F_{2\alpha}$-III) exerts a vasoconstriction in most vessels, whereas no data is available concerning 5-F_{2t}-IsoP (also named iP$F_{2\alpha}$-VI), which is more abundant in plasma.

2 The aim of this study was to determine whether 5-F_{2t}-IsoP possess any vascular effects on various vessels including the isolated rat thoracic aorta, the human internal mammary artery and the saphenous vein.

3 In organ baths, 5-F_{2t}-IsoP and its 5-epimer did not affect the basal tone of any vessel, unlike 15-F_{2t}-IsoP. These compounds possessed no antagonist effects on 15-F_{2t}-IsoP-induced contractions, No dilator effect was observed in comparison with sodium nitroprusside and acetylcholine on the rat aorta.

4 In conclusion, we show that unlike 15-F_{2t}-IsoP, 5-F_{2t}-IsoP and its 5-epimer possess no vasomotor effects and as such are unlikely to be involved in the pathogenesis of vascular diseases. Further studies are required to test whether these mediators may have effects on systems not being measured in the current study.

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Abbreviations: 5-epi-5-F_{2t}-IsoP, 5-epi-5-F_{2t}-Isoprostane; 5-F_{2t}-IsoP, 5-F_{2t}-Isoprostane; 15-F_{2t}-IsoP, 15-F_{2t}-Isoprostane; Ach, acetylcholine; SNP, sodium nitroprusside; TP-receptor, thromboxane/PGH_2-receptor

Introduction

Isoprostanes are a family of compounds produced *in vivo* by non-enzymatic free radical-induced peroxidation of arachidonic acid (Morrow *et al*., 1990). Depending on which of the labile hydrogen atoms is first abstracted by free radicals, three initial arachidonoyl radicals can be formed, which then form four prostaglandin H_2 isomers. Fully reduced, they can form four F_2-isoprostane regioisomers (Figure 1), each of which is comprised of eight diastereoisomers. F_2-isoprostanes are initially formed esterified on phospholipids and then released in free form by phospholipases (Morrow *et al*., 1992). Among these F_2-isoprostanes, 15-F_{2t}-IsoP (Taber *et al*., 1997) also named isoprostaglandin $F_{2\alpha}$ type III (Rokach *et al*., 1997) or 8-iso $PGF_{2\alpha}$, and 5-F_{2t}-IsoP (isoprostaglandin $F_{2\alpha}$ type VI), respectively from the 15-series and the 5-series, are currently quantifiable in plasma and urine, and are extensively used as clinical markers of lipid peroxidation in vascular diseases (Patrono & Fitzgerald, 1997, Cracowski *et al*., 2001). Elevated levels of 5-F_{2t}-IsoP have been described in atherosclerosis (Pratico *et al*., 1997), myocardial reperfusion following thrombolysis (Reilly *et al*., 1997) and percutaneous transluminal coronary angioplasty (Iuliano *et al*., 2001).

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15-F_{2t}-IsoP induces vasoconstriction in numerous animals and human vessels, *via*. TP-receptor activation (Kromer & Tippins, 1996; 1998; Oliveira *et al*., 2000; Cracowski *et al*., 2001). In contrast, no data is available concerning the vascular properties of 5-F_{2t}-IsoP. Such observations are of the utmost interest since 5-F_{2t}-IsoP and its 5-epimer have been shown to be generated in greater concentrations than 15-F_{2t}-IsoP in humans (Li *et al*., 1999).

Therefore, the aim of this study was to determine the vascular effects of 5-F_{2t}-IsoP and its 5-epimer on the isolated rat thoracic aorta, the human internal mammary artery and the human saphenous vein.

Methods

Isolated preparations

In accordance with French law and the local ethical committee guidelines for animal research, male Wistar rats (370–430 g IFFA CREDO, Lyon, France) were housed in climate controlled conditions and provided with standard rat chow. Animals were anaesthetized with an intraperitoneal injection of 60 mg kg^{-1} sodium pentobarbital (Sanofi, Libourne, France). Heparin (150 IU, Sanofi Winthrop, Gentilly, France)

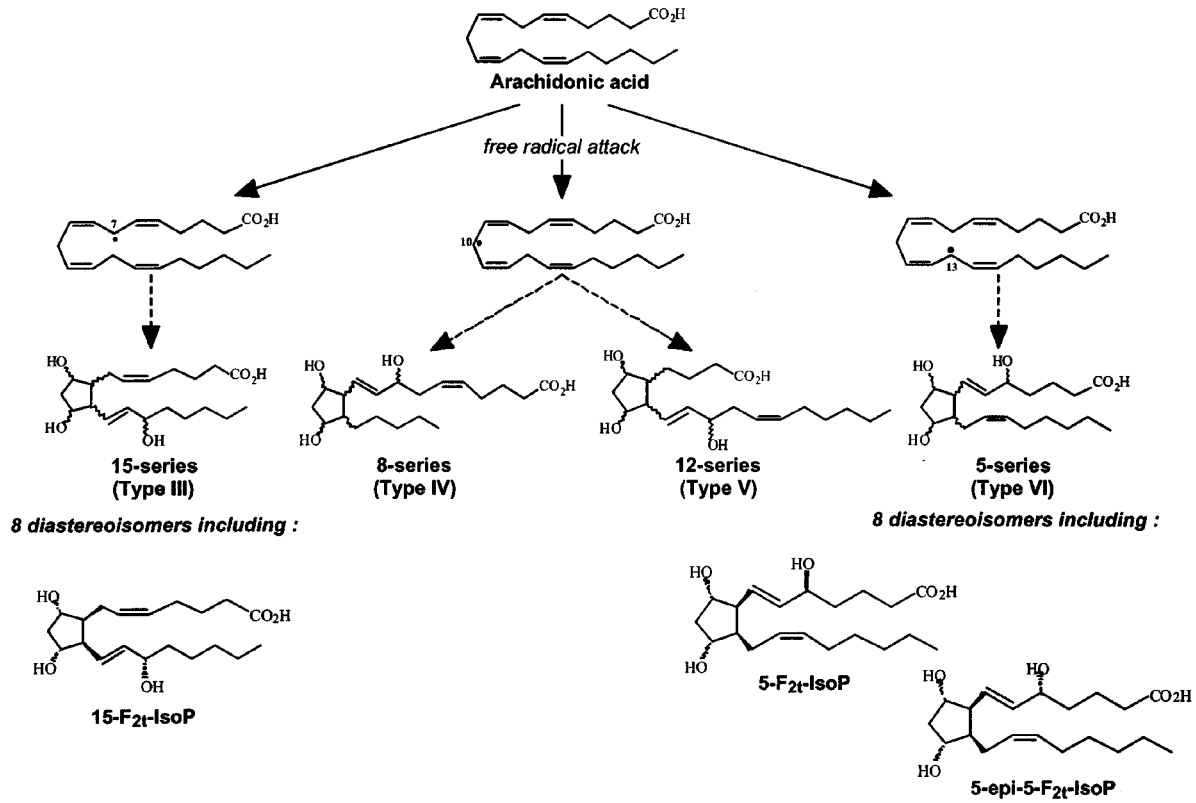

Figure 1 F_2-Isoprostanes formation from arachidonic acid, leading to four F_2-Isoprostane regioisomers. For simplicity, the intermediate compounds are not shown.

was injected intravenously. Then, the thoracic aorta was quickly excised, cleaned of connective tissue and cut into 4-mm lengths. Six rings were taken from each thoracic aorta. The endothelium was removed from some aortic rings by gently rolling the tip of a plastic forceps inside the vessel.

Human internal mammary arteries and saphenous veins were obtained from patients undergoing coronary bypass surgery. The discarded distal ends of the arterial and venous grafts were immediately placed in oxygenated HEPES-buffered Krebs solution maintained at 4°C and transferred to the laboratory within 2 h. The HEPES-buffered Krebs solution had following composition (mM): NaCl (130), KCl (3.8), $CaCl_2$ (2.1), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $NaHCO_3$ (14.8), glucose (10.4) and HEPES (10). Blood vessels were dissected free from connective tissue and cut into 4-mm lengths.

Experimental design

The methods used for the measurement of isometric tension were as previously reported (Cracowski *et al*., 2000; Stanke-Labesque *et al*., 2001). Briefly, rings were suspended in organ chambers filled with 6 ml of Krebs solution maintained at 37°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide. Segments were mounted between two stainless steel wires. The upper wire was fixed to a force transducer through which changes in isometric forces were continuously displayed on a recorder. The rings were initially stretched and were allowed to equilibrate for 60 min. The rings were then challenged twice with KCl (90 mM) at a 10-min interval. The endothelial function was assessed by testing the relaxant effect of acetycholine (1 μM) on aortic rings precontracted with methoxamine (3 μM). Following a further 60-min period, concentration–contraction curves were made. Only one cumulative concentration–contraction curve was established in each ring. Four rings were run in parallel.

The vasomotor effects of 5-F_{2t}-IsoP and 5-epi-5-F_{2t}-IsoP were tested on rat thoracic aortic rings: (1) The contractile responses were compared to 15-F_{2t}-IsoP. The role of the endothelium was assessed by comparing the response to 5-F_{2t}-IsoP and 5-epi-5-F_{2t}-IsoP in rings with an intact or denuded endothelium. (2) In order to test an antagonist activity of 5-F_{2t}-IsoP or 5-epi-5-F_{2t}-IsoP on the contractile response to 15-F_{2t}-IsoP, concentration–responses curves to 15-F_{2t}-IsoP were obtained 30 min after pretreatment with 5-F_{2t}-IsoP and 5-epi-5-F_{2t}-IsoP (10^{-5} M). (3) To determine the potential dilator effects, the rings were contracted by phenylephrine (10^{-7} M). When a stable plateau was reached, 5-F_{2t}-IsoP and 5-epi-5-F_{2t}-IsoP were added in a cumulative fashion (10^{-10}–10^{-5} M), and compared to sodium nitroprusside and acetylcholine-induced relaxation (10^{-9}–10^{-4} M).

Contractile experiments were performed on rings of internal mammary arteries and saphenous veins in order to study 5-F_{2t}-IsoP and 5-epi-5-F_{2t}-IsoP effects on both arterial and venous human vessels in comparison with 15-F_{2t}-IsoP.

Drugs

15-F_{2t}-IsoP (8-iso-prostaglandin $F_{2\alpha}$) was purchased from Cayman (Ann Arbor, U.S.A.), sodium nitroprusside (SNP), acetylcholine (Ach) and phenylephrine from Sigma (Saint

Quentin Fallavier, France). 5-F_{2t}-IsoP and 5-epi-5-F_{2t}-IsoP were synthesized according to our procedure (Durand *et al*., 2001). All isoprostanes were dissolved in methanol at 10^{-2} M. Stocks solutions were then diluted in distilled water before being added to the organ baths. The highest concentration of methanol was 0.1%, which had no direct effect on the vascular tone in preliminary experiments.

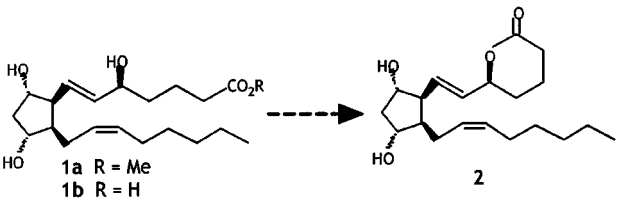

Figure 2 Potential lactonization of 5-series F_2-isoprostanes.

The 5-series of isoprostanes (5-F_{2t}-isoP) is the only group of isoprostanes that have an OH function on the C-5 relative to the COOH and are expected to form a six-membered-ring lactone 2 (Figure 2). The lactone 2 being much less polar than the unlactonized hydroxy acid 1b, it was easily to check the stability of such isoprostanes by thin layer chromatography. We have also, checked the stability of the methyl ester of 5-F_{2t}-isoP 1a and analogues by ^{1}H and ^{13}C NMR spectroscopy. Using these two different techniques, we are able to conclude that the 5-F_{2t}-isoP 1b and all epimers are stable compounds, when they are kept at -20°C in a solution of methyl alcohol under nitrogen, up to 1 year.

The stability of 5-F_{2t}-IsoP in Krebs' solution was checked by gas chromatography-electronic impact mode mass spectrometry, using 15 F_{2t}-IsoP-d_4 as the internal standard with a methodology derived from 15-F_{2t}-IsoP quantification (Bessard *et al*., 2001). We added 100 μl of 5-F_{2t}-IsoP (10^{-3} M) in 900 μl Krebs' solution, and maintained at 37°C during 0, 30

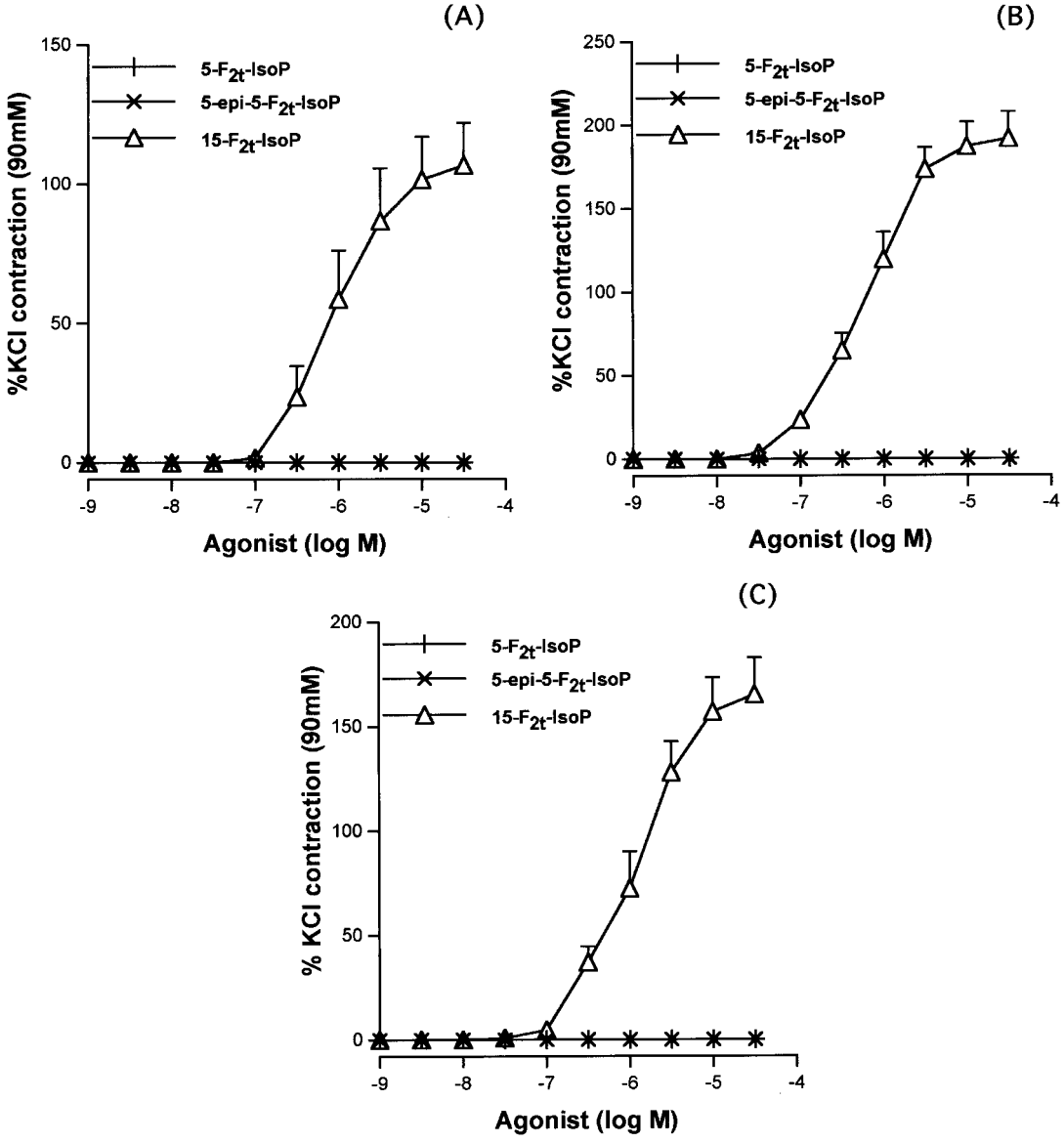

Figure 3 Concentration–contraction curves to 5-F_{2t}-IsoP and its epimer (5-epi-5-F_{2t}-IsoP) in comparison with 15-F_{2t}-IsoP in the rat aorta (A), the human internal mammary artery (B) and the saphenous vein (C). ($n=6$ in all groups).

and 60 min. The respective concentrations were 10^{-4} M, 9.2 10^{-5} M and 9.4 10^{-5} M (ANOVA: *NS*).

Data analysis

Concentration–contraction curves were expressed as a percentage of KCl 90 mM-induced contraction. Relaxation curves were expressed as a percentage of the initial phenylephrine (10^{-7} M) contraction. Maximum contraction (Emax) and potency (pEC_{50}) were calculated to determine the arterial segment reactivity. Emax was expressed as a percentage of KCl 90 mM-induced maximal contraction. The effective concentration of agent that caused 50% of maximum contraction (EC_{50}) was calculated from each curve by a logistic, curve-fitting equation. EC_{50} values were expressed as pEC_{50} ($-\log EC_{50}$). Data were expressed as mean ± s.e.m. Unpaired *t*-tests were used to test the statistical significance between two means. More than two means were compared with the use of analysis of variance. Values of $P<0.05$ were considered significant.

Results

Vasomotor effects on rat aorta

5-F_{2t}-IsoP and 5-epi-5-F_2-IsoP induced no variation of the baseline on the rat aorta (Figure 3A), unlike 15-F_{2t}-IsoP ($pEC_{50}=5.98\pm0.17$; $E_{max}=106\pm15\%$). Similarly no contraction was observed in endothelium denuded rings. Pretreatment with 5-F_{2t}-IsoP (10^{-5} M) and 5-epi-5-F_{2t}-IsoP (10^{-5} M) had no effect on 15-F_{2t}-IsoP concentration–response curves ($pEC_{50}=5.98\pm0.17$; 5.91 ± 0.26 and 5.72 ± 0.34; $E_{max}=106\pm15\%$; $94\pm22\%$ and $105\pm7\%$ in the presence of vehicle, 5-F_{2t}-IsoP (10^{-5} M) and 5-epi-5-F_{2t}-IsoP (10^{-5} M) respectively, NS). SNP and Ach (10^{-9}–10^{-4} M) induced a significant aortic relaxation (SNP: pEC_{50} 8.14 ± 0.26, E_{max} $106\pm5\%$ and Ach: pEC_{50} 6.7 ± 0.87, E_{max} $90\pm3\%$), whereas both 5-F_{2t}-IsoP and 5-epi-5-F_{2t}-IsoP, had no effect on rat aortic rings precontracted with phenylephrine (10^{-7} M) (Figure 4).

Contractile responses on human internal mammary arteries and saphenous veins

5-F_{2t}-IsoP and 5-epi-5-F_{2t}-IsoP had no vasoconstrictor effect on human internal mammary arteries and saphenous veins (Figure 3B,C), unlike 15-F_{2t}-IsoP that induced a concentration–dependent vasoconstriction ($pEC_{50}=6.21\pm0.1$ and 5.85 ± 0.1; $E_{max}=191\pm16\%$ and $165\pm18\%$ in internal mammary arteries and saphenous veins, respectively).

Discussion

5-F_{2t}-IsoP and its 5-epimer did not affect the basal tone of the rat thoracic aorta as well as the human internal mammary artery and the saphenous vein. In addition, these compounds had neither antagonist effects on 15-F_{2t}-IsoP-induced contractions, nor dilator effects on the rat thoracic aorta. Therefore, 5-F_{2t}-IsoP, unlike 15-F_{2t}-IsoP, had no vasomotor effect on arterial and venous blood vessels, which remained consistent between species.

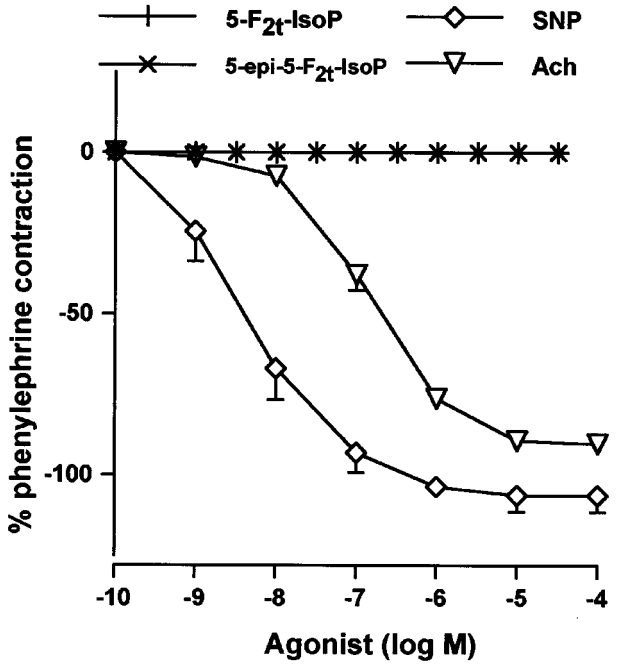

Figure 4 Concentrations–relaxation curves to 5-F_{2t}-IsoP and its epimer (5-epi-5-F_{2t}-IsoP) in comparison to sodium nitroprusside (SNP) and acetylcholine (Ach) in rat aortic rings precontracted with phenylephrine (10^{-7} M) ($n=6$ in all groups).

15-F_{2t}-IsoP induces a vasoconstriction, mediated by TP-receptor stimulation, which may be modulated by the endothelium (Cracowski *et al*., 2001). Although the 5- and the 15-series F_2-Isoprostanes are both produced through free radical peroxidation of arachidonic acid, they differ from their initial arachidonoyl radical that leads to major differences in the lateral chain structure (see Figure 1). The 15-F_{2t}-IsoP shares similar side chains with the classical prostanoids thromboxane A_2 and prostaglandin $F_{2\alpha}$ in contrast to the lack of 15S-hydroxyl in the 5-series isoprostanes, which is essential to high agonist potency on prostanoid receptors. In line with this hypothesis, the 5-series F_2-Isoprostanes had no vasomotor effect in the vessels examined.

Substantial evidence has been accumulated to support the use of urinary isoprostanes analysis as a non invasive index of lipid peroxidation *in vivo* (Roberts & Morrow, 2000). Attention was initially focused on 15-F_{2t}-IsoP quantification. Recently, other isomers, 5-F_{2t}-IsoP, 5-F_{2c}-IsoP and 5-epi- 5-F_{2t}-IsoP, were detected and quantified in plasma, coronary sinus or urine by mass spectrometry (Pratico *et al*., 1998; Li *et al*., 1999; Iuliano *et al*., 2001). To date, the major differences between the 5- and the 15-series F_2-Isoprostanes were their respective concentrations: 5-F_{2t}-IsoP levels were found to be approximately 3–4 times higher than 15-F_{2t}-IsoP (Li *et al*., 1999; Iuliano *et al*., 2001), enabling an easier quantification. The second difference is that their formation in human diseases may be dissociated: a proportional increase was found in cigarette smoking (Pratico *et al*., 1998), hypercholesterolemia (Reilly *et al*., 1998; Li *et al*., 1999) and percutaneous coronary angioplasty (Iuliano *et al*., 2001), whereas the urinary levels of the 15-series F_2-Isoprostanes, but not of the 5-series were elevated in cardiac failure (Li *et al*., 1999). The present study shows a third

difference between 15-F_{2t}-IsoP and 5-F_{2t}-IsoP that is of the utmost importance: 15-F_{2t}-IsoP is a vasoconstrictor, which has been hypothesized to be involved in the pathogenesis of coronary vasospasm (Iuliano *et al*., 2001), whereas 5-F_{2t}-IsoP has no vasomotor effects, and as such is not likely to be involved in the pathogenesis of vascular diseases. Our study does not rule out the possibility that the 5-series may share other biological activity of 15-F_{2t}-IsoP such as platelet aggregation inhibition (Cranshaw *et al*., 2001), neutrophil adhesion (Fontana *et al*., 2001), cardiomyocyte hypertrophy (Kunapuli *et al*., 1998). Further studies are required to test whether these mediators may have effects on systems not being measured in the current study.

In conclusion, most attention on isoeicosanoid analysis has focused recently on 5-F_{2t}-IsoP, which is more abundant in biologicals fluids than 15-F_{2t}-IsoP. We show that unlike 15-F_{2t}-IsoP, 5-F_{2t}-IsoP and its epimer possess no vasomotor effects and as such are unlikely to contribute to the pathogenesis of vascular diseases.

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