



Inflammation increases MMP levels via PGE₂ in human vascular wall and plasma of obese women

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Abstract

Background and objectives: Matrix metalloproteinases (MMPs) are involved in several inflammatory processes including obesity-related vascular diseases and graft failure of coronary artery (CA) bypass grafts [internal mammary artery (IMA), saphenous vein (SV)]. In these inflammatory conditions, the release of prostaglandin E₂ (PGE₂) is increased via the activity of inducible microsomal PGE synthase-1 (mPGES-1). Our aim was to investigate whether MMPs and their endogenous inhibitor (TIMPs) may be regulated by PGE₂ under inflammatory conditions in human vasculature and perivascular adipose tissue (PVAT), as well as in plasma of obese patients.

Methods: MMP-1,-2 and TIMP-1,-2 densities were measured in human plasma ($n = 68$) as well as in supernatants of human vascular wall (IMA $n = 16$, SV $n = 14$, CA $n = 13$) and their PVAT. The effects of inflammation and mPGES-1 inhibitor (Compound III, 10 μ M) on MMPs regulation were evaluated. The correlations between PGE₂ and several parameters were calculated in plasma from patients with or without obesity.

Results: The vascular wall and PVAT from SV exhibited the greatest MMP-1,-2 release. An increase of MMP-1,-2 and/or a decrease of TIMP-1 quantities have been detected under inflammation only in vascular wall not in PVAT. These changes under inflammation were completely reversed by inhibition of mPGES-1. In obesity, C-reactive protein (CRP), biomarker of inflammation, and PGE₂ levels were increased. PGE₂ contents were positively correlated with some anthropometric parameters and plasmatic CRP in both genders, while the correlation with the plasmatic MMP-1 density was significant only in women.

Conclusions: The greater MMP activity observed in SV may contribute to the increased prevalence of graft failure. Under inflammation, the greater mPGES-1 and PGE₂ levels lead to enhanced MMP activity in human vascular walls. The positive association between PGE₂ and MMP-1 or CRP has been observed in plasma of women. We suggest that mPGES-1 inhibitors could prevent graft failure and obesity-related vascular remodeling mostly in women.

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Introduction

Among prostanoids, prostaglandin (PG) E₂ is involved in the control of vascular tone, remodeling, and inflammation. PGE₂ is synthesized from arachidonic acid through cyclooxygenases (COXs) and three PGE synthases [1]. When human vascular cells are exposed to inflammatory cytokines such as interleukin-1 β (IL-1 β) and/or lipopolysaccharide (LPS), COX-2, and microsomal PGE synthase-1 (mPGES-1) are co-induced and thereby the release of PGE₂ is strongly (tenfold) increased [2–5].

In many vascular diseases, such as atherosclerosis, aneurysm, or varicose veins, PGE₂ regulates vascular remodeling through the regulation of matrix metalloproteinase (MMP) activity [6–8]. MMP activity is under the

control of tissue inhibitors of MMPs (TIMPs). MMP is involved in many physiological and pathological processes through degradation of extracellular matrix proteins. Studies performed on human vascular preparations have demonstrated that smooth muscle cells constitutively express MMP-2, TIMP-1, and TIMP-2, whereas endothelial cells express MMP-1, MMP-2, TIMP-1, and TIMP-2 [9]. Furthermore, some types of MMPs such as MMP-1, -2, -3, and -9 could be induced after inflammatory stimuli in human vascular cells probably via nuclear factor- κ B [9, 10, 11–13].

Several studies indicated that human blood vessels such as internal mammary artery (IMA) and saphenous vein (SV) produced both MMPs and PGE₂ [14–16]. These vessels are used as a graft material to bypass stenosed coronary arteries (CA) in CA bypass surgery. Our recent study demonstrated that mPGES-1 expression and PGE₂ release were greater in SV than in IMA. Furthermore, inflammatory stimuli increased both mPGES-1 and PGE₂ levels in these vessels [17]. On the other hand, several studies showed that MMP-2 levels were greater in SV than in IMA [14, 18]. However, the contribution of PGE₂ on MMP release under inflammatory conditions has not been thoroughly evaluated in these human vessels.

The graft materials, SV and IMA, are surrounded by perivascular adipose tissue (PVAT), which is also able to synthesize PGE₂ and MMPs [15, 19]. In humans, the quantity of PVAT strongly correlated with obesity, anthropometric measures including waist circumference (WC), body weight, and body mass index (BMI) [20]. In addition, when the patients divided according to quartiles based on increasing peri-aortic adipose tissue, a gradual increase in the estimated cardiovascular risks and more severe systemic inflammation in terms of higher C-reactive protein (CRP) level were observed [20]. In fact, several studies demonstrated that inflammation observed in PVAT could contribute to vascular inflammation in obesity, which is defined as a low-grade inflammatory disease [19, 21–23]. One recent study emphasized the impairment of beneficial effects of PVAT in obese patients. Furthermore, loss in weight in these patients contributes to reduction of pro-inflammatory mediators released from PVAT, a decrease in macrophage number and restoration of PVAT beneficial effect on vascular tone [24]. In line with human studies, animal models of obesity revealed the increased PVAT mass and adipocytes size, and also greater release of pro-inflammatory cytokines have been observed [25, 26]. In subjects with cardiovascular diseases or undergoing bypass surgery, increased CRP, PGE₂, and MMP plasma levels have been well established [27–33]. However, in the plasma of obese patients where PVAT produces more pro-inflammatory cytokines [23, 34], the association between these inflammation-induced mediators has not yet been studied.

The aim of the study was to investigate whether MMPs and TIMPs may be regulated by PGE₂ under inflammatory conditions related to obesity. This regulation was determined both in vitro with human vessels and their PVAT and in vivo with plasma from obese patients.

Methods

Human vascular preparations

The study was performed on isolated segments of human IMA ($n = 16$, 12 males and 4 females aged 73 ± 3 years) and SV ($n = 14$, 9 males and 5 females aged 65 ± 5 years), and their isolated PVAT obtained from patients who had undergone CA bypass surgery. Moreover, CA and their isolated PVAT ($n = 13$, 10 males and 3 females aged 46 ± 7 years) were obtained after cardiac transplantation from the patients with non-occlusive cardiomyopathy. Vascular preparations used in this study were macroscopically without atherosclerotic lesions of fatty streaks. The Ethics Committee of INSERM (the French National Institute for Health and Medical Research) approved the study plan (approval number: 11–101). These tissues are considered as surgical waste in accordance with French ethical laws (L.1211-3-L.1211-9). All experiments with human subjects were performed in accordance with the Declaration of Helsinki.

Organ cultures

The IMA, SV, and CA were dissected free from PVAT, cut into rings of 2–4 mm width. “Control condition” indicates that the preparations presented few or no inflammatory markers [35–37], these vessels and their isolated PVAT were placed immediately into 12-well plates containing RPMI supplemented with PSA (penicillin 1000 IU/ml, streptomycin 100 μ g/ml, amphotericin 0.25 μ g/ml). In addition to RPMI and PSA, some vessels and their isolated PVAT were incubated with both IL-1 β (100 ng/ml) and LPS (100 μ g/ml) [3, 17, 38]. Both inflammatory agents were suspended in RPMI supplemented with PSA and this condition corresponds to “Inflammation.” Some preparations were incubated in the presence of mPGES-1 inhibitor (compound III, C3, 10 μ M, [39]) under inflammatory condition and named as “Inflammation + mPGES-1 inhibitor.” The volume of the culture medium was adjusted to 1 ml for 70 mg of tissue. All tissue incubations were performed at 37 °C in a humidified atmosphere of 5% CO₂ in air using a culture incubator [17]. After 24 h incubation, supernatants were kept at –80 °C for MMPs and TIMPs measurements.

Human plasma samples

In the present study, we recruited 68 randomly selected subjects, included 21 non-obese (healthy lean) subjects with BMI between 18.5 and 25 kg/m² and 47 obese subjects with BMI ≥ 30 kg/m². Patients characteristics are presented in Table S1. All participants gave written informed consent and the study was approved by Farhat Hached Hospital Ethics Committee for research on humans (approval number: 11–2011). Information on each participant's life style and health status was obtained through an interview, including questions regarding smoking history, prescription medicines for diseases such as dyslipidemia, arterial hypertension, or cardiovascular diseases. Subjects with a history of cardiovascular disease, liver, renal, or thyroid disease, smoking habit, malignancy, diseases responsible for microvasculopathy, and subjects using medications that might affect lipid and glucose metabolism or alter the endothelial or smooth muscle dependent responses were excluded from the study. Other exclusion criteria were women in the menstrual cycle and pregnant women.

Anthropometric measurements and biochemical analyses in human plasma

Participants were first examined anthropometrically. Height (m) and weight (kg) were taken with participants dressed in light weight clothing without shoes and BMI was calculated (kg/m²). The WC was measured at the midway point between the lower rib margin and the crest of the ileum in a horizontal plane at standing position (cm) and hip circumference was measured by placing a tape measure around the patient's hip at the level of the prominences over the greater trochanters of both femurs (cm). After we calculated waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR). Blood samples were collected from subjects in tubes after 12 h overnight fast, the blood was maintained at 4 °C then centrifuged (4000 × *g* for 10 min). Plasma was distributed in aliquots and stored at – 80 °C until the batched measurements of parameters. Total cholesterol and triglycerides were determined by the cholesterol oxidase and the glycerol oxidase methods (Elitech Diagnostic, France). High-density lipoprotein cholesterol was measured by the immune-inhibition method (Elitech Diagnostic, France) and low-density lipoprotein cholesterol was calculated with the Friedwald formula. Apolipoprotein A1, ApoB and high-sensitivity CRP were also measured. All biochemical parameters were determined on an automated Synchron CX7 Clinical System (Beckman, Fullerton, CA).

Enzyme-linked immunosorbent assays

MMP-1, MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2 levels in plasma or supernatants of human vessels and PVAT were determined by enzyme-linked immunosorbent assays (ELISA) using commercially available kits according to the manufacturer's instructions. The concentration of PGE₂ was measured in plasma using an EIA kit according to the manufacturer's instructions. The PGE₂, MMPs, and TIMPs concentrations were expressed as ng/ml of plasma or ng/mg of wet weight tissue. Technical replicates were used to ensure the reliability of single values.

Statistical analysis

All results obtained from different patients (*n*) were expressed as a mean ± SEM. Depending on data distribution (normal or non-normal), statistical analysis was performed by Mann–Whitney rank sum test, Student's *t*-test (paired data derived from the same patient or unpaired), one-way analysis of variance with Bonferroni's correction for multiple comparisons post hoc tests, and Pearson's or Spearman's correlations analysis were performed. *P*-value < 0.05 indicates that data are significantly different. Statistical analyses were performed using GraphPad Prism (Version 7, La Jolla California, USA).

Drugs and materials

The protease inhibitor cocktail, IL-1β, LPS, antibiotics, and antimycotic were purchased from Sigma-Aldrich (St. Louis, MO, USA). PGE₂ EIA kit was obtained from Cayman Chemical (Ann Arbor, MI, USA). Human MMP-1 (DYS901), human MMP-2 (DY902), human MMP-3 (DY513), human MMP-9 (DY911), human TIMP-1 (DY970), and human TIMP-2 (DY971) were from R&D Systems (DuoSet®ELISA). RPMI was obtained from Gibco Invitrogen (Paisley, UK). C3 [Compound III; [1-(1-isopropyl -5,6 -dimethyl-1H- benzoimidazol-2-yl)-piperidine-4-carboxylic acid cyclopentylamide] was a generous gift from NovaSAID AB and Dr. Per-Johan Jakobsson (Karolinska Institutet, Stockholm, Sweden).

Results

The effect of inflammation on the release of MMP and TIMP in vascular preparations

The MMP-1 and MMP-2 levels were significantly increased under inflammatory conditions in IMA, SV, and CA, whereas TIMP-1 content was lower in IMA and CA

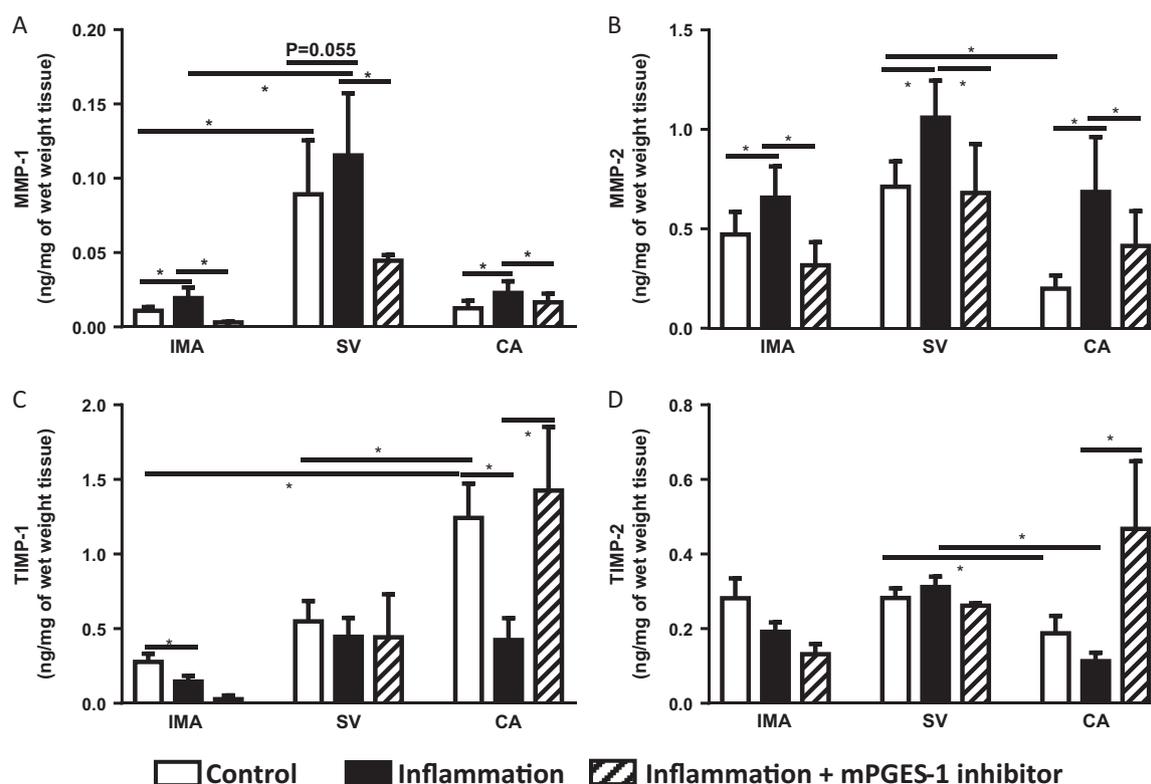


Fig. 1 The effects of inflammation and microsomal prostaglandin E synthase-1 (mPGES-1) inhibitor (C3, 10 μ M) on the release of matrix metalloproteinases (MMP-1, -2) and tissue inhibitors of MMP (TIMP-1, -2) from internal mammary artery (IMA), saphenous vein (SV), and coronary artery (CA). The productions of MMP and TIMP were

measured in the supernatants of organ culture after 24 h incubation. The release of MMP and TIMP was expressed as ng/mg of wet weight tissue. * $P < 0.05$ indicates significantly different. Values are means \pm SEM derived from [n values: 3–16 (IMA), 3–14 (SV), 3–13 (CA)] different patients

(Fig. 1a–c). On the other hand, TIMP-2 levels remain unchanged under inflammation (Fig. 1d). The release of MMP-1 and MMP-2 from SV was greater vs. IMA and CA, respectively (Fig. 1a, b). The levels of TIMP-1 were significantly higher in CA vs. other vessels, whereas TIMP-2 levels were lower in CA as compared with SV (Fig. 1c, d).

The effect of mPGES-1 inhibitor on the release of MMP and TIMP in vascular preparations

The increased levels of MMP-1 and MMP-2 observed under inflammatory conditions were reversed by an mPGES-1 inhibitor (C3, 10 μ M) (Fig. 1a, b). On the other hand, the decreased levels of TIMP-1 under inflammation, as well as TIMP-2 levels were restored in the presence of mPGES-1 inhibitor in CA (Fig. 1c, d).

The effect of inflammation on the release of MMP and TIMP in PVAT

Under inflammatory conditions, the levels of MMPs and TIMPs released from PVAT derived from IMA, SV, or CA were not altered (Fig. 2a–d). The MMP-1, -2, and TIMP-1

levels were significantly higher in PVAT from SV vs. PVAT from IMA and CA either in control or inflammatory conditions (Fig. 2a–c).

The role of obesity on CRP, PGE₂, MMP-1, and MMP-9 levels in human plasma

CRP and PGE₂ levels were significantly increased in human plasma from obese patients (BMI ≥ 30 kg/m²) vs. non-obese group (BMI between 18.5 and 25 kg/m²) in both women and men (Fig. 3a, b). MMP-1 and MMP-9 contents were increased only in human plasma from obese women patients vs. non-obese women, whereas it was not modified in obese men patients (Fig. 3c, d).

The correlations between PGE₂ levels and MMP or CRP levels in human plasma

PGE₂ levels were significantly positively correlated with CRP levels in both men and women (Fig. 4a, b). Furthermore, there was also a positive correlation between MMP-1 levels and PGE₂ or CRP levels only in women but not in men (Fig. 4c–f). Moreover, PGE₂ contents were also

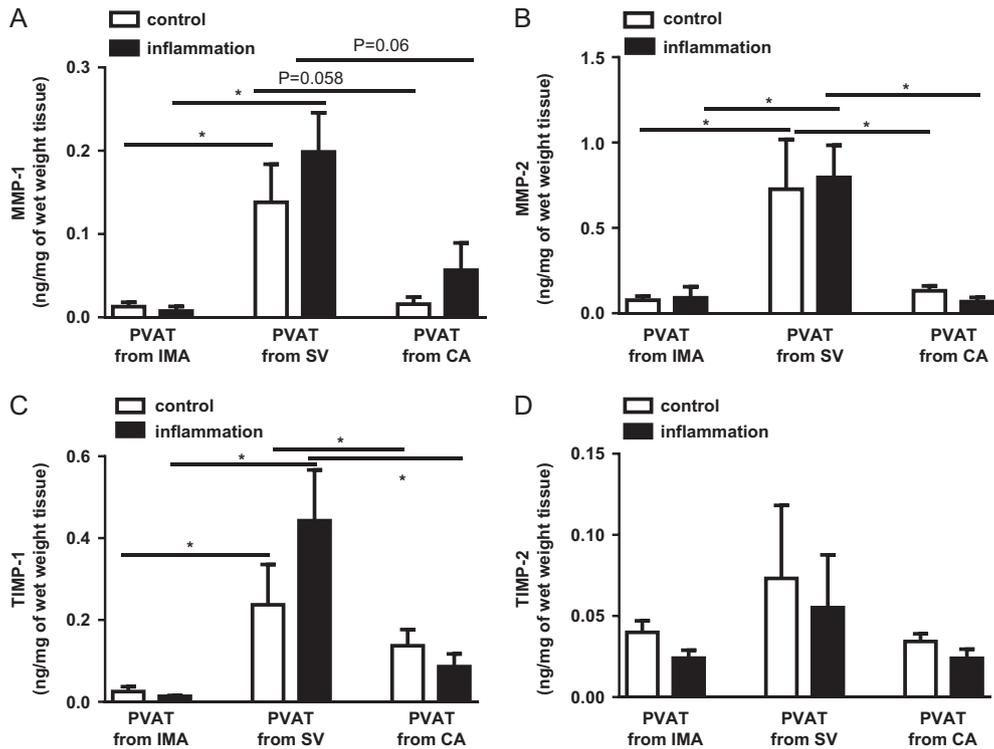


Fig. 2 The effects of inflammation on the release of matrix metalloproteinases (MMP-1, -2) and tissue inhibitors of MMP (TIMP-1, -2) from perivascular adipose tissue (PVAT) of internal mammary artery (IMA), saphenous vein (SV), and coronary artery (CA). The productions of MMP and TIMP were measured in the supernatants of organ

culture after 24 h incubation. The release of MMP and TIMP was expressed as ng/mg of wet weight tissue. **P* < 0.05 indicates significantly different. Values are means ± SEM derived from [n values: 3–10 (IMA), 3–4 (SV), 3–11 (CA)] different patients

correlated with TIMP-1 levels in women (Table 1). On the other hand, there was no significant correlation between PGE₂ and MMP-2, MMP-3, MMP-9, or TIMP-2 in both genders (Table 1).

The correlations between PGE₂ levels and anthropometric parameters in human plasma

A positive correlation was found between PGE₂ levels and WHR values in human plasma independently of gender status. Moreover, in men, PGE₂ levels were positively correlated with all anthropometric parameters (BMI, WHtR, WHR, and WC) (Table 2).

Discussion

Increased MMP activity is involved in vascular remodeling observed in several inflammatory processes [9, 10, 12] such as CA bypass graft failure or obesity-related vascular diseases. In the present work, we studied in vitro vascular inflammation with graft materials (IMA, SV) and CA, as well as in vivo inflammation with plasma of obese patients. Our results showed that PGE₂ increases MMP levels via

mPGES-1 enzyme under inflammation. Our study suggests that mPGES-1 inhibitor could be a novel approach to decrease inflammation-induced MMP activity for prevention of obesity-related diseases and graft failure.

In control conditions, we have demonstrated that higher MMP-1 and MMP-2 contents were released from SV vs. IMA and CA, respectively (Figs. 1a, b). In accordance with our results, several studies showed greater MMP levels in SV vs. IMA [14, 18]. Regulation of MMP activity is one of the key events in the development of intimal hyperplasia, which could be the reason of graft failure after bypass surgery [16]. The greater MMP levels observed in SV vs. IMA (Fig. 1a, b) could be responsible for the increased prevalence of SV graft failure through intimal hyperplasia [40, 41]. In addition, intra- and postoperatively, the bypass grafts are exposed to inflammatory conditions, graft failure could become more obvious because of increased MMP levels in this condition (Fig. 1a, b)

In our previous studies [15, 17], we have measured and compared PGE₂ levels in IMA, SV, and CA in both control and inflammatory conditions. Inflammation significantly increased PGE₂ levels via inducible mPGES-1 enzyme in these vessels. SV produced approximately fivefold more PGE₂ amount when compared with IMA either from their

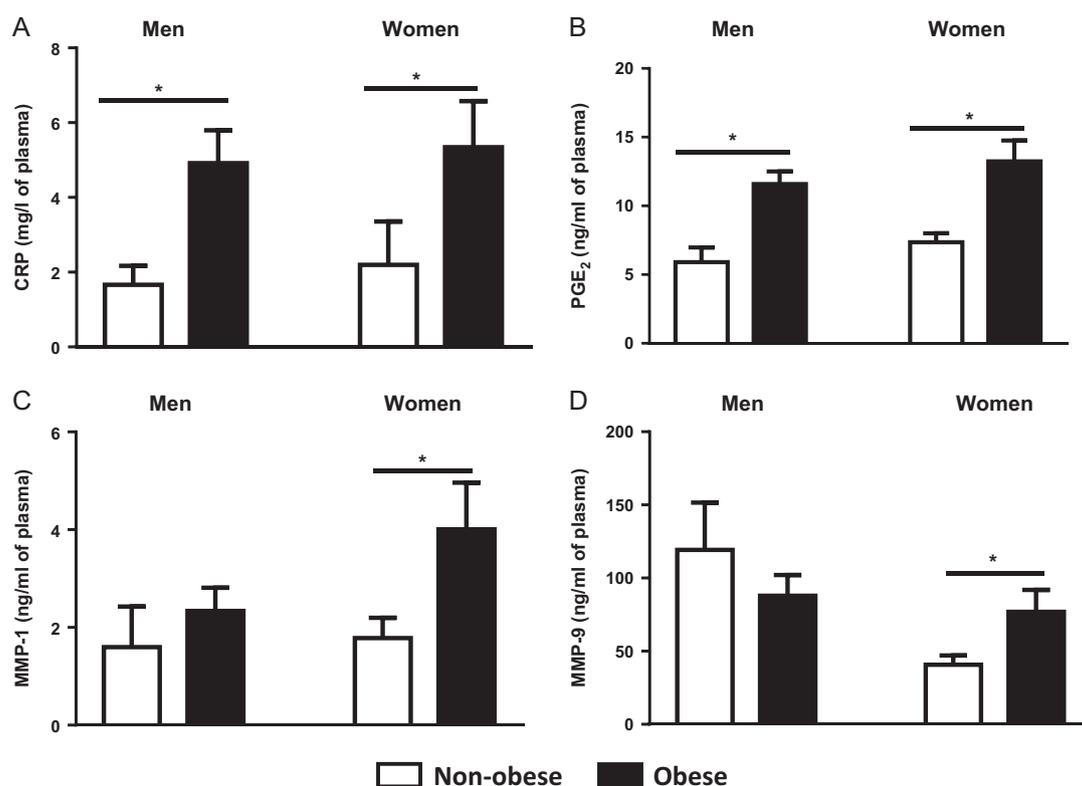


Fig. 3 The CRP (C-reactive protein), PGE₂ (prostaglandin E₂), MMP-1 (matrix metalloproteinase-1), and MMP-9 levels in human plasma of non-obese (BMI between 18.5 and 25 kg/m²) or obese patients (BMI ≥

30 kg/m²). **P* < 0.05 indicates significantly different. Values are means ± SEM derived from different patients (*n* = 39 for men, *n* = 24 for women)

vascular wall or their respective PVAT [15, 17]. This difference was even more obvious when these vascular preparations were submitted to inflammatory conditions [2, 17]. As in the cases of MMP levels, IMA released similar amount of PGE₂ as CA and when CA derived from atherosclerotic patients they had a greater capacity to synthesize PGE₂ in comparison with patients without atherosclerosis [35].

Under inflammatory conditions, both PGE₂ and MMP-1,-2 releases were positively associated in human vessels [17] (Fig. 1a, b). This association has been reported in vascular preparations derived from patients (atherosclerosis, aneurysm), in vascular models of inflammation [7, 8, 42]. In non-pathological conditions, this association has also been detected. In SV without inflammation, the MMP-1/(TIMP-1 or TIMP-2) ratio was significantly decreased in the presence of a selective PGE₂ receptor antagonist (GW62768X) [6, 7]. Our results presented in Fig. 1 demonstrate that the upregulation of MMP-1/2 by endogenous PGE₂ is also detectable in IMA and CA. Unlike other studies, here we demonstrated that the regulatory role of endogenous PGE₂ on MMP synthesis is supported by the use of a selective inhibitor of mPGES-1 (C3, 10 μM). C3 reversed the inflammation-increased MMP levels in these three different human vessels (Fig. 1).

Measuring only MMPs levels is not considered sufficient to interpret MMP activity, as MMP activity is mostly determined as a ratio between MMPs and their endogenous inhibitors, TIMPs [43]. Therefore, we also quantified TIMPs levels in this study. Our results indicated that TIMP-1 was downregulated by inflammation in both IMA and CA (Fig. 1c). When we calculated our results as an MMP-1/TIMP-1 or MMP-2/TIMP-2 ratio (data not shown), we observed that in vitro inflammation induced more pronounced enhancement in IMA and CA vs. SV. That could be explained by the lower increase in PGE₂ production under inflammation by SV (1.3-fold) as compared with IMA (2-fold) and CA [17].

Several lines of evidence suggest that PVAT may contribute to improve graft patency whereas this beneficial effect may be lost in obese patients where PVAT released more pro-inflammatory cytokines [24, 25, 44–46]. For this reason, we have also measured and compared MMP activity between vascular wall and PVAT for each vessel both in control and inflammatory conditions. When we calculated our results as an MMP-1/TIMP-1 or MMP-2/TIMP-2 ratio, we found that these ratios were greater in PVAT than in vascular wall of the corresponding vessel (4–12-fold for MMP-1/TIMP-1 ratio; 2–4-fold for MMP-2/TIMP-2 ratio). These results suggested that not only vascular wall but also

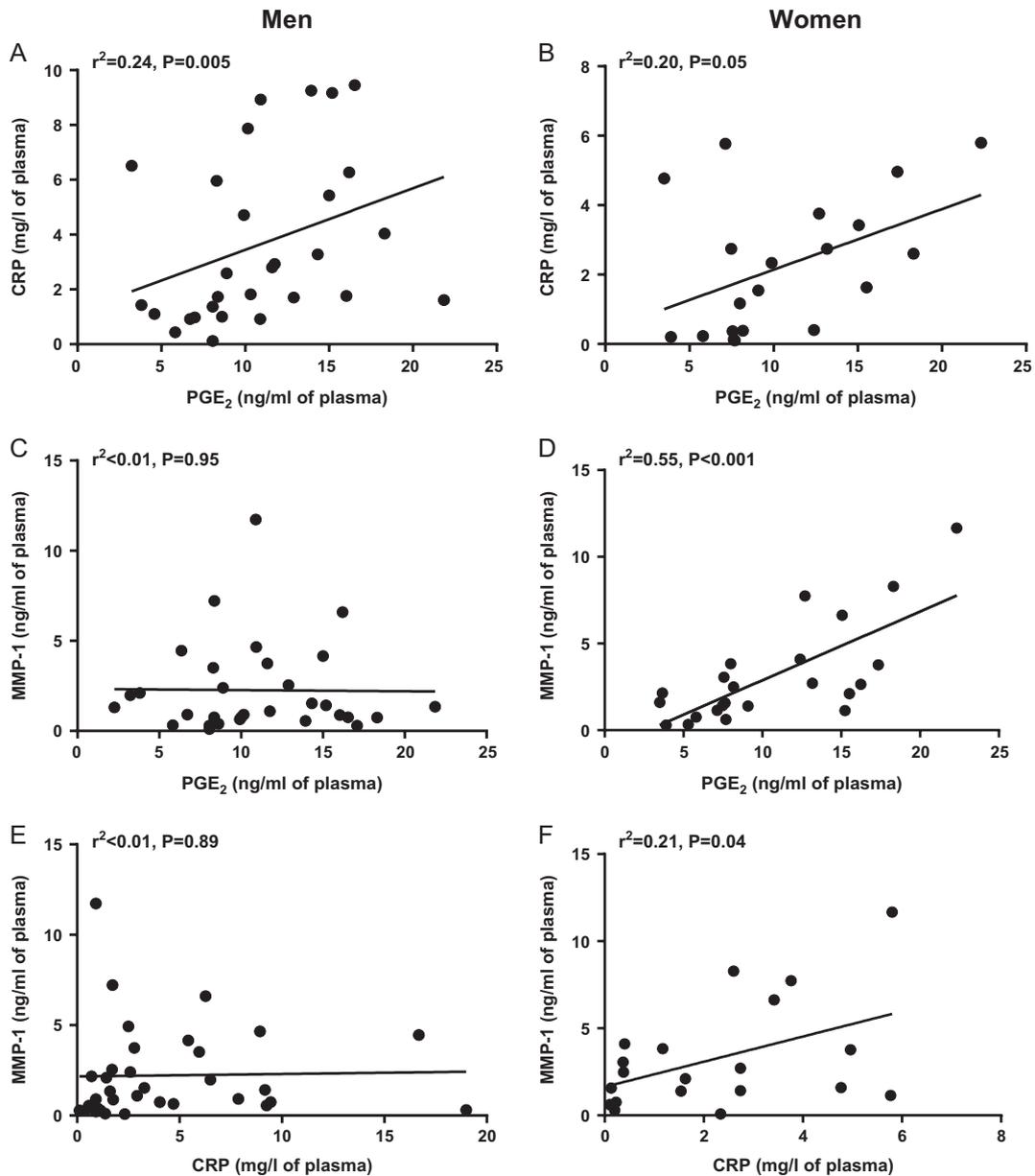


Fig. 4 The correlations between prostaglandin E₂ (PGE₂) and C-reactive protein (CRP) or matrix metalloproteinase-1 (MMP-1) levels in human plasma. $P<0.05$ indicates significant correlations

(Spearman's or Pearson's analysis). Data are derived from different patients ($n = 34$ for men, $n = 24$ for women)

PVAT could provide MMPs and contribute to the vascular remodeling. Furthermore, greater MMP contents shown in vascular wall of SV vs. other vessels have been also detected in PVAT from SV vs. PVAT from other vessels (Figs. 1a, b, 2a, b).

Our in vitro results demonstrate that, in contrast to the MMP levels observed in the vascular wall (Fig. 1a, b), those in PVAT (Fig. 2a–d) were not enhanced under inflammatory conditions. In line with our results, in human adipocytes incubation with LPS did not modify MMP-2 levels [47]. However, in some rodent PVAT, inflammation was

responsible for increased MMP-2/-9 levels [48, 49]. It is possible that these results could be due to a different regulation of MMPs between vascular wall and PVAT, as well as different species used in these studies.

Obesity is defined as a low-grade inflammatory disease [23], which is supported by increased CRP measurement, marker of inflammation [50], in plasma of obese patients (Fig. 3a). In these samples, we have investigated the interactions among inflammatory mediators (CRP, PGE₂, and MMP). There was an upregulation of PGE₂ levels in plasma of obese patients (Fig. 3b) as well as in omental adipose

Table 1 The correlations between prostaglandin E₂ (PGE₂) and matrix metalloproteinases (MMP) or tissue inhibitors of matrix metalloproteinase (TIMP) in human plasma

	Men		Women	
	r ²	P	r ²	P
MMP-1	< 10 ⁻²	0.95	0.55	< 10 ^{-3*}
MMP-2	0.02	0.37	0.11	0.12
MMP-3	0.06	0.17	0.04	0.35
MMP-9	0.09	0.08	0.01	0.69
TIMP-1	0.02	0.42	0.36	0.002*
TIMP-2	0.01	0.66	0.04	0.36

*P < 0.05, significantly different; Spearman's or Pearson's analysis. Data are derived from n = 31–34 for men, n = 23–24 for women.

Table 2 The correlations between prostaglandin E₂ (PGE₂) and anthropometric parameters in human plasma

	Men		Women	
	r ²	P	r ²	P
BMI	0.35	< 10 ^{-4*}	0.10	0.13
WHtR	0.30	0.001*	0.12	0.12
WHR	0.27	0.003*	0.19	0.05*
WC	0.23	0.006*	0.14	0.09

BMI body mass index, WC waist circumference, WHR waist-to-hip ratio, WHtR waist-to-height ratio *P < 0.05, significantly different; Spearman's or Pearson's analysis. Data are derived from n = 30–34 for men, n = 21–24 for women.

tissue [51] derived from obese vs. healthy patients. Moreover, in rodent obese models, the release of PGE₂ in plasma or adipose tissue was usually greater than in the control group [52–55].

In our study, plasma PGE₂ was positively correlated with some markers, such as CRP levels and anthropometric parameters such as BMI, WHtR, WHR, and WC (Fig. 4a, b, Table 2). In addition to PGE₂, MMP-1 and MMP-9 levels were also increased in plasma of obese women (Fig. 3c, d) and there was a positive correlation between PGE₂ and MMP-1 or TIMP-1 levels in women but not in men (Fig. 4c–f, Table 1). Taken together, these results (Fig. 4, Tables 1 and 2) showed that more pronounced correlations were found in women between MMP-1 and PGE₂ or CRP levels. Even though MMP-9 has an important role in cardiovascular disease and obesity [56], there was no correlation between PGE₂ and MMP-9 levels (Table 1). This result suggests that MMP-9 levels were not controlled by PGE₂ in obese and non-obese patients, whereas it was the case in acute coronary syndrome [57].

In literature, several studies showed a gender difference in the regulation of MMPs [58, 59]. In accordance with our results, increased MMP-1 and MMP-9 levels have

been shown in obese vs. normal-weight women, whereas in a mixed-gender study no difference of pro-MMP-1 contents was observed in obesity [56, 60–62]. Sex hormones could participate in MMP regulation in obesity, several studies emphasized a strong involvement of estrogen in adipose tissue metabolism and accumulation [63, 64]. In premenopausal women, estrogen levels and estrogen receptor-alpha quantities were significantly reduced in obesity [65, 66]. In vitro studies exhibited that estrogens significantly decrease MMP-1 levels in many human cells [67, 68]. These data with our results (Fig. 3c, Fig. 4d, f, and Table 1) suggest that reduced levels of estrogens could account for the elevated levels of MMP-1 measured in our premenopausal obese women. Therefore, treatment with mPGES-1 inhibitor either for obesity-related vascular remodeling or prevention of graft failure could mediate more effective results in women. Furthermore, therapeutic approaches might be more urgent in women than in men since several studies exhibited a greater postoperative mortality and morbidity in women compared with men undergoing CA bypass surgery [69–72]. In addition to MMP, variants of *mPGES-1* gene and their association with disease severity demonstrated sexual difference [73].

In conclusion, our previous and present study show that the induction of mPGES-1 enzyme and consequently elevated PGE₂ release under inflammation lead to increased MMP activity in human bypass grafts (IMA, SV) and CA. This positive association between PGE₂ and MMP-1 is confirmed by their correlation determined in plasma of obese women probably through the regulatory role of estrogen. In addition, we have compared MMP levels between bypass grafts and CA. The greater MMP activity observed in SV may contribute to the increased prevalence of graft failure in SV vs. IMA following bypass surgery. Finally, our results suggest that mPGES-1 inhibitor could be a promising drug for prevention of graft failure and obesity-related vascular remodeling by decreasing inflammation-induced MMPs releases mostly in women.

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Compliance with ethical standards

Conflict of interest PJJ is a member of the board of Gensynta Pharma AB. All other authors declare that they have no conflicts of interest.

References

- Gomez I, Foudi N, Longrois D, Norel X. The role of prostaglandin E₂ in human vascular inflammation. *Prostaglandins Leukot Essent Fatty Acids*. 2013;89:55–63.
- Bishop-Bailey D, Pepper JR, Haddad EB, Newton R, Larkin SW, Mitchell JA. Induction of cyclooxygenase-2 in human saphenous vein and internal mammary artery. *Arterioscler Thromb Vasc Biol*. 1997;17:1644–8.
- Bishop-Bailey D, Pepper JR, Larkin SW, Mitchell JA. Differential induction of cyclooxygenase-2 in human arterial and venous smooth muscle: role of endogenous prostanoids. *Arterioscler Thromb Vasc Biol*. 1998;18:1655–61.
- Camacho M, Gerboles E, Escudero JR, Anton R, Garcia-Moll X, Vila L. Microsomal prostaglandin E synthase-1, which is not coupled to a particular cyclooxygenase isoenzyme, is essential for prostaglandin E(2) biosynthesis in vascular smooth muscle cells. *J Thromb Haemost*. 2007;5:1411–9.
- Camacho M, Rodriguez C, Guadall A, Alcolea S, Orriols M, Escudero JR, et al. Hypoxia upregulates PGI-synthase and increases PGI(2) release in human vascular cells exposed to inflammatory stimuli. *J Lipid Res*. 2011;52:720–31.
- Gomez I, Benyahia C, Louedec L, Leseche G, Jacob MP, Longrois D, et al. Decreased PGE(2) content reduces MMP-1 activity and consequently increases collagen density in human varicose vein. *PLoS ONE*. 2014;9:e88021.
- Gomez I, Ozen G, Deschildre C, Amgoud Y, Boubaya L, Gorenne I, et al. Reverse regulatory pathway (H2S / PGE2 / MMP) in human aortic aneurysm and saphenous vein varicosity. *PLoS ONE*. 2016;11:e0158421.
- Yokoyama U, Ishiwata R, Jin MH, Kato Y, Suzuki O, Jin H, et al. Inhibition of EP4 signaling attenuates aortic aneurysm formation. *PLoS ONE*. 2012;7:e36724.
- Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Unemori EN, Lark MW, et al. Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion. *Circ Res*. 1994;75:181–9.
- Montero I, Orbe J, Varo N, Beloqui O, Monreal JJ, Rodriguez JA, et al. C-reactive protein induces matrix metalloproteinase-1 and -10 in human endothelial cells: implications for clinical and sub-clinical atherosclerosis. *J Am Coll Cardiol*. 2006;47:1369–78.
- Qin W, Lu W, Li H, Yuan X, Li B, Zhang Q, et al. Melatonin inhibits IL1beta-induced MMP9 expression and activity in human umbilical vein endothelial cells by suppressing NF-kappaB activation. *J Endocrinol*. 2012;214:145–53.
- Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Libby P. Enhanced expression of vascular matrix metalloproteinases induced in vitro by cytokines and in regions of human atherosclerotic lesions. *Ann N Y Acad Sci*. 1995;748:501–7.
- Reuben PM, Cheung HS. Regulation of matrix metalloproteinase (MMP) gene expression by protein kinases. *Front Biosci*. 2006;11:1199–215.
- Anstadt MP, Franga DL, Portik-Dobos V, Pennathur A, Bannan M, Mawulawde K, et al. Native matrix metalloproteinase characteristics may influence early stenosis of venous versus arterial coronary artery bypass grafting conduits. *Chest*. 2004;125:1853–8.
- Ozen G, Topal G, Gomez I, Ghorreshi A, Boukais K, Benyahia C, et al. Control of human vascular tone by prostanoids derived from perivascular adipose tissue. *Prostaglandins Other Lipid Mediat*. 2013;107:13–7.
- Turner NA, Ho S, Warburton P, O'Regan DJ, Porter KE. Smooth muscle cells cultured from human saphenous vein exhibit increased proliferation, invasion, and mitogen-activated protein kinase activation in vitro compared with paired internal mammary artery cells. *J Vasc Surg*. 2007;45:1022–8.
- Ozen G, Gomez I, Daci A, Deschildre C, Boubaya L, Teskin O, et al. Inhibition of microsomal PGE synthase-1 reduces human vascular tone by increasing PGI₂: a safer alternative to COX-2 inhibition. *Br J Pharmacol*. 2017;174:4087–98.
- Sun Y, Kang L, Li J, Liu H, Wang Y, Wang C, et al. Advanced glycation end products impair the functions of saphenous vein but not thoracic artery smooth muscle cells through RAGE/MAPK signalling pathway in diabetes. *J Cell Mol Med*. 2016;20:1945–55.
- Ozen G, Daci A, Norel X, Topal G. Human perivascular adipose tissue dysfunction as a cause of vascular disease: focus on vascular tone and wall remodeling. *Eur J Pharmacol*. 2015;766:16–24.
- Yun CH, Lin TY, Wu YJ, Liu CC, Kuo JY, Yeh HI, et al. Pericardial and thoracic peri-aortic adipose tissues contribute to systemic inflammation and calcified coronary atherosclerosis independent of body fat composition, anthropometric measures and traditional cardiovascular risks. *Eur J Radiol*. 2012;81:749–56.
- Taylor LE, Sullivan JC. Sex differences in obesity-induced hypertension and vascular dysfunction: a protective role for estrogen in adipose tissue inflammation? *Am J Physiol Regul Integr Comp Physiol*. 2016;311:R714–20.
- Xia N, Li H. The role of perivascular adipose tissue in obesity-induced vascular dysfunction. *Br J Pharmacol*. 2017;174:3425–42.
- Rocha VZ, Libby P. Obesity, inflammation, and atherosclerosis. *Nat Rev Cardiol*. 2009;6:399–409.
- Aghamohammadzadeh R, Greenstein AS, Yadav R, Jeziorska M, Hama S, Soltani F, et al. Effects of bariatric surgery on human small artery function: evidence for reduction in perivascular adipocyte inflammation, and the restoration of normal anticontractile activity despite persistent obesity. *J Am Coll Cardiol*. 2013;62:128–35.
- Ketonen J, Shi J, Martonen E, Mervaala E. Periadventitial adipose tissue promotes endothelial dysfunction via oxidative stress in diet-induced obese C57Bl/6 mice. *Circ J*. 2010;74:1479–87.
- Marchesi C, Ebrahimian T, Angulo O, Paradis P, Schiffrin EL. Endothelial nitric oxide synthase uncoupling and perivascular adipose oxidative stress and inflammation contribute to vascular dysfunction in a rodent model of metabolic syndrome. *Hypertension*. 2009;54:1384–92.
- Bruins P, te Velthuis H, Yazdanbakhsh AP, Jansen PG, van Hardevelt FW, de Beaumont EM, et al. Activation of the complement system during and after cardiopulmonary bypass surgery: postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia. *Circulation*. 1997;96:3542–8.
- Faymonville ME, Deby-Dupont G, Larbuisson R, Deby C, Bodson L, Limet R, et al. Prostaglandin E₂, prostacyclin, and thromboxane changes during nonpulsatile cardiopulmonary bypass in humans. *J Thorac Cardiovasc Surg*. 1986;91:858–66.
- Joffs C, Gunasinghe HR, Multani MM, Dorman BH, Kratz JM, Crumbley AJ 3rd, et al. Cardiopulmonary bypass induces the synthesis and release of matrix metalloproteinases. *Ann Thorac Surg*. 2001;71:1518–23.
- Sesso HD, Wang L, Buring JE, Ridker PM, Gaziano JM. Comparison of interleukin-6 and C-reactive protein for the risk of developing hypertension in women. *Hypertension*. 2007;49:304–10.
- Ogihara T, Gotoh S, Tabuchi Y, Kumahara Y. Involvement of endogenous prostaglandins in salt-induced hypertension. *Acta Endocrinol (Copenh)*. 1985;108:114–8.
- Yasmin, McEniery CM, Wallace S, Dakham Z, Pulsalkar P, Maki-Petaja K, et al. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol*. 2005;25:372.
- Derosa G, Maffioli P, D'Angelo A, Salvadeo SA, Ferrari I, Fogari E, et al. Evaluation of metalloproteinase 2 and 9 levels and their

- inhibitors in combined dyslipidemia. *Clin Invest Med.* 2009;32: E124–32.
34. Virdis A, Duranti E, Rossi C, Dell'Agnello U, Santini E, Anselmino M, et al. Tumour necrosis factor- α participates on the endothelin-1/nitric oxide imbalance in small arteries from obese patients: role of perivascular adipose tissue. *Eur Heart J.* 2015;36:784–94.
 35. British Pharmacological Society. Contractions of human coronary vessels induced by prostaglandin E2 are mediated via EP3 receptor and modulated by perivascular adipose tissue. *Pharmacology.* 2015. <http://www.pa2online.org/abstract/abstract.jsp?abid=32972&author=Ozen&cat=-1&period=-1>.
 36. Gomez I, Benyahia C, Le Dall J, Payre C, Louedec L, Leseche G, et al. Absence of inflammatory conditions in human varicose saphenous veins. *Inflamm Res.* 2013;62:299–308.
 37. Jabs WJ, Theissing E, Nitschke M, Bechtel JF, Duchrow M, Mohamed S, et al. Local generation of C-reactive protein in diseased coronary artery venous bypass grafts and normal vascular tissue. *Circulation.* 2003;108:1428–31.
 38. Foudi N, Louedec L, Cachina T, Brink C, Norel X. Selective cyclooxygenase-2 inhibition directly increases human vascular reactivity to norepinephrine during acute inflammation. *Cardiovasc Res.* 2009;81:269–77.
 39. Leclerc P, Idborg H, Spahiu L, Larsson C, Nekhotiaeva N, Wannberg J, et al. Characterization of a human and murine mPGES-1 inhibitor and comparison to mPGES-1 genetic deletion in mouse models of inflammation. *Prostaglandins Other Lipid Mediat.* 2013;107:26–34.
 40. Kim FY, Marhefka G, Ruggiero NJ, Adams S, Whellan DJ. Saphenous vein graft disease: review of pathophysiology, prevention, and treatment. *Cardiol Rev.* 2013;21:101–9.
 41. Sur S, Sugimoto JT, Agrawal DK. Coronary artery bypass graft: why is the saphenous vein prone to intimal hyperplasia? *Can J Physiol Pharmacol.* 2014;92:531–45.
 42. Sukhova GK, Schonbeck U, Rabkin E, Schoen FJ, Poole AR, Billingham RC, et al. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atherosclerotic plaques. *Circulation.* 1999;99:2503–9.
 43. Moore CS, Crocker SJ. An alternate perspective on the roles of TIMPs and MMPs in pathology. *Am J Pathol.* 2012;180:12–6.
 44. Dashwood MR, Tsui JC. 'No-touch' saphenous vein harvesting improves graft performance in patients undergoing coronary artery bypass surgery: a journey from bedside to bench. *Vasc Pharmacol.* 2013;58:240–50.
 45. Costa RM, Neves KB, Tostes RC, Lobato NS. Perivascular adipose tissue as a relevant fat depot for cardiovascular risk in obesity. *Front Physiol.* 2018;9:253.
 46. Paz MA, Lupon J, Bosch X, Pomar JL, Sanz G. Predictors of early saphenous vein aortocoronary bypass graft occlusion. The GESIC Study Group. *Ann Thorac Surg.* 1993;56:1101–6.
 47. Samuvel DJ, Jin J, Sundararaj KP, Li Y, Zhang X, Lopes-Virella MF, et al. TLR4 activation and IL-6-mediated cross talk between adipocytes and mononuclear cells synergistically stimulate MMP-1 expression. *Endocrinology.* 2011;152:4662–71.
 48. DeVallance E, Branyan KW, Lemaster K, Olfert IM, Smith DM, Pistilli EE et al. Aortic dysfunction in metabolic syndrome mediated by perivascular adipose tissue TNF α and NOX2 dependent pathway. *Exp Physiol.* 2018;103:590–603.
 49. Moe KT, Naylynn TM, Yin NO, Khairunnisa K, Allen JC, Wong MC, et al. Tumor necrosis factor- α induces aortic intima-media thickening via perivascular adipose tissue inflammation. *J Vasc Res.* 2013;50:228–37.
 50. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation.* 2003;107:499–511.
 51. Garcia-Alonso V, Titos E, Alcaraz-Quiles J, Rius B, Lopategi A, Lopez-Vicario C, et al. Prostaglandin E2 exerts multiple regulatory actions on human obese adipose tissue remodeling, inflammation, adaptive thermogenesis and lipolysis. *PLoS ONE.* 2016;11:e0153751.
 52. Cunha NV, de Abreu SB, Panis C, Grassioli S, Guarnier FA, Cecchini R, et al. Cox-2 inhibition attenuates cardiovascular and inflammatory aspects in monosodium glutamate-induced obese rats. *Life Sci.* 2010;87:375–81.
 53. Pham Huu C, Palhares de Miranda AL, Navarro-Delmasure C, Pham Huu Chanh A, Moutier R. Comparative study of the biosynthesis of PGE2, PGF2 α and TXA2 by different organs of genetically hypertensive (SHR) and obese-hypertensive (SHR-fa/fa) rats. *Prostaglandins Leukot Med.* 1987;26:21–32.
 54. Rocha-Rodrigues S, Rodriguez A, Goncalves IO, Moreira A, Maciel E, Santos S, et al. Impact of physical exercise on visceral adipose tissue fatty acid profile and inflammation in response to a high-fat diet regimen. *Int J Biochem Cell Biol.* 2017;87:114–24.
 55. Wu D, Ren Z, Pae M, Han SN, Meydani SN. Diet-induced obesity has a differential effect on adipose tissue and macrophage inflammatory responses of young and old mice. *Biofactors.* 2013;39:326–33.
 56. Luizon MR, Belo VA, Fernandes KS, Andrade VL, Tanus-Santos JE, Sandrim VC. Plasma matrix metalloproteinase-9 levels, MMP-9 gene haplotypes, and cardiovascular risk in obese subjects. *Mol Biol Rep.* 2016;43:463–71.
 57. Gomez-Hernandez A, Sanchez-Galan E, Ortego M, Martin-Ventura JL, Blanco-Colio LM, Tarin-Vicente N, et al. Effect of intensive atorvastatin therapy on prostaglandin E2 levels and metalloproteinase-9 activity in the plasma of patients with non-ST-elevation acute coronary syndrome. *Am J Cardiol.* 2008;102:12–8.
 58. Cho BS, Roelofs KJ, Ford JW, Henke PK, Upchurch GR Jr. Decreased collagen and increased matrix metalloproteinase-13 in experimental abdominal aortic aneurysms in males compared with females. *Surgery.* 2010;147:258–67.
 59. Sokolis DP, Iliopoulos DC. Impaired mechanics and matrix metalloproteinases/inhibitors expression in female ascending thoracic aortic aneurysms. *J Mech Behav Biomed Mater.* 2014;34:154–64.
 60. Melekoglu R, Ciftci O, Eraslan S, Basak N, Celik E. The effects of body mass index on second-trimester amniotic fluid cytokine and matrix metalloproteinase levels. *Gynecol Obstet Invest.* 2018;83:70–5.
 61. Papazoglou D, Papatheodorou K, Papanas N, Papadopoulos T, Gioka T, Kabouromiti G, et al. Matrix metalloproteinase-1 and tissue inhibitor of metalloproteinases-1 levels in severely obese patients: what is the effect of weight loss? *Exp Clin Endocrinol Diabetes.* 2010;118:730–4.
 62. Andrade VL, Petruceli E, Belo VA, Andrade-Fernandes CM, Caetano Russi CV, Bosco AA, et al. Evaluation of plasmatic MMP-8, MMP-9, TIMP-1 and MPO levels in obese and lean women. *Clin Biochem.* 2012;45:412–5.
 63. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor- α knockout mice. *Proc Natl Acad Sci USA.* 2000;97:12729–34.
 64. Pedersen SB, Kristensen K, Hermann PA, Katzenellenbogen JA, Richelsen B. Estrogen controls lipolysis by up-regulating α 2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor α . Implications for the female fat distribution. *J Clin Endocrinol Metab.* 2004;89:1869–78.
 65. Gallicchio L, Visvanathan K, Miller SR, Babus J, Lewis LM, Zacur H, et al. Body mass, estrogen levels, and hot flashes in midlife women. *Am J Obstet Gynecol.* 2005;193:1353–60.

66. Nilsson M, Dahlman I, Ryden M, Nordstrom EA, Gustafsson JA, Arner P, et al. Oestrogen receptor alpha gene expression levels are reduced in obese compared to normal weight females. *Int J Obes (Lond)*. 2007;31:900–7.
67. Claassen H, Steffen R, Hassenpflug J, Varoga D, Wruck CJ, Brandenburg LO, et al. 17beta-estradiol reduces expression of MMP-1, -3, and -13 in human primary articular chondrocytes from female patients cultured in a three dimensional alginate system. *Cell Tissue Res*. 2010;342:283–93.
68. Singer CF, Marbaix E, Kokorine I, Lemoine P, Donnez J, Eeckhout Y, et al. Paracrine stimulation of interstitial collagenase (MMP-1) in the human endometrium by interleukin 1alpha and its dual block by ovarian steroids. *Proc Natl Acad Sci USA*. 1997;94:10341–5.
69. Humphries KH, Gao M, Pu A, Lichtenstein S, Thompson CR. Significant improvement in short-term mortality in women undergoing coronary artery bypass surgery (1991 to 2004). *J Am Coll Cardiol*. 2007;49:1552–8.
70. Hassan A, Chiasson M, Buth K, Hirsch G. Women have worse long-term outcomes after coronary artery bypass grafting than men. *Can J Cardiol*. 2005;21:757–62.
71. Woods SE, Noble G, Smith JM, Hasselfeld K. The influence of gender in patients undergoing coronary artery bypass graft surgery: an eight-year prospective hospitalized cohort study. *J Am Coll Surg*. 2003;196:428–34.
72. Vaccarino V, Abramson JL, Veledar E, Weintraub WS. Sex differences in hospital mortality after coronary artery bypass surgery: evidence for a higher mortality in younger women. *Circulation*. 2002;105:1176–81.
73. Korotkova M, Daha NA, Seddighzadeh M, Ding B, Catrina AI, Lindblad S, et al. Variants of gene for microsomal prostaglandin E2 synthase show association with disease and severe inflammation in rheumatoid arthritis. *Eur J Hum Genet*. 2011;19: 908–14.