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PLACENTAL GROWTH FACTOR (PIGF) IS A POTENT VASODILATOR OF RAT AND HUMAN RESISTANCE ARTERIES

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Abstract

The objectives of this study were to determine whether PIGF exerts a vasodilatory effect on rat uterine vessels (arcuate arteries and veins) and to examine regional differences in reactivity by comparing these responses to those of comparably-sized mesenteric vessels. We also sought to examine and compare its effects on human uterine and subcutaneous vessels. All vessels were studied *in vitro*, under pressurized (rat) or isometric wire-mounted (human) conditions, and exposed to a range of PIGF concentrations. Inhibitors of nitric oxide and prostaglandin synthesis were included in an effort to understand the causal mechanism(s). In rat uterine arteries, the effects of receptor inhibition and activation using selective ligands for VEGFR-1 (PIGF) vs. VEGFR-2 (VEGF-E) were determined, and real-time RT-PCR was performed to evaluate the effect of pregnancy on relative abundance of VEGFR-1 and VEGFR-2 message in the vascular wall. PIGF was a potent vasodilator of all vessels studied, with greatest sensitivity observed in rat uterine arteries. Pregnancy significantly augmented dilator sensitivity to PIGF, and this effect was associated with selective upregulation of VEGFR-1 message in the pregnant state. The contribution of nitric oxide was appreciable in rat and human uterine arteries, with lesser effects in rat uterine veins and mesenteric arteries, and with no observable effect in human subcutaneous vessels. Based on these results, we conclude that PIGF is a potent vasodilator of several vessel types in both humans and rats. Its potency and mechanism varies with physiological state and vessel location, and is mediated solely by the VEGFR-1 receptor subtype. Gestational changes in the uterine circulation suggest that this factor may play a role in modulating uterine vascular remodeling and blood flow during the pregnant state.

Keywords

VEGF; nitric oxide; uterine circulation; rat; human resistance arteries; VEGFR-1; Flt-1

INTRODUCTION

Earlier studies from our laboratory have shown that rat uterine resistance arteries are highly sensitive to dilation to vascular endothelial growth factor (VEGF), and that this response is further enhanced during pregnancy through a mechanism linked to estrogen [10,11].

PlGF is a member of the VEGF growth factor family that was discovered in 1991. Unlike some VEGF isoforms, which bind to VEGFR-1 (Flt) and/or VEGFR-2 (KDR or Flk), PlGF binds specifically to the VEGFR-1 receptor. Thus far, four studies have examined the effects of placental growth factor (PlGF) on vascular reactivity, with reports of vasodilatory action in isolated renal [8], placental [12], pulmonary [5] and mammary vessels [15]. The mechanism of dilation has not been determined. Vasodilation to VEGF has been more widely reported and is associated with signaling through the VEGFR-2 receptor via a mechanism that involves activation of phosphoinositide 3-kinase (PI3K) and Akt-dependent phosphorylation of eNOS, resulting in increased NO production [e.g. 13]. In contrast, one report concluded that the vasodilator response may involve both types of receptor [5].

The physiologic role of PlGF is not known, however, studies have shown that its circulating concentrations exceed those of VEGF by >40-fold during normal gestation, although its affinity for the VEGFR-1 receptor is only 1/10th of the affinity of VEGF [1]. During preeclampsia, a disease associated with hypertension and uteroplacental underperfusion, free PlGF concentrations are significantly reduced due to elevations of a soluble form of the VEGFR-1 receptor (sFlt-1) [8,14]. Adenoviral overexpression of sFlt-1 in rats results in a preeclampsia-like syndrome characterized by hypertension, proteinuria, and renal endothelial glomerular endotheliosis [8]. Recently, it has been suggested that a ratio of sFlt-1 to PlGF may be a useful predictive marker for the development of preeclampsia [14].

Based on these observations, we hypothesized that PlGF, like VEGF, may exert a vasodilatory effect on the peripheral vasculature, and thus modulate regional blood flow and resistance. In view of previous results of enhanced uterine artery dilation to VEGF in pregnancy [2], we also measured uterine artery reactivity to PlGF as a function of concentration, as well as the expression of both the VEGF-1 and VEGF-2 receptor message in uterine arteries from pregnant vs. nonpregnant animals to determine whether changes occur during pregnancy.

Pregnancy-induced, species-related, and regional differences in the vasodilatory actions of PlGF were evaluated by extending reactivity studies to mesenteric arteries in rats, and to myometrial and subcutaneous vessels in humans with and without inhibitors of endothelial dilators (nitric oxide, prostaglandins). We also examined its effect on the rat uterine veins that drain the placenta. Due to placental production and secretion of this growth factor, uterine veins are likely to contain the highest concentrations of PlGF prior to systemic dilution.

METHODS

Isolated rat uterine and mesenteric artery reactivity

Adult (12–14 week-old) pregnant and nonpregnant Sprague-Dawley rats were purchased from Charles River Canada, and shipped to the University of Vermont. All procedures were approved by the Institutional Animal Care and Use Committee. Vessels were obtained from late pregnant (Day 20/22 of gestation; n=23) and age-matched nonpregnant animals (n=13) following euthanasia with an injection of methohexital sodium (Brevital, 50 mg/kg i.p) and decapitation in a small animal guillotine. Vessels from pregnant animals were used for reactivity experiments; in addition, those from nonpregnant animals were used to test for the

effects of pregnancy on PIGF reactivity (n=5), and for determination of receptor message (n=8).

The uterus and a section of the gut 5–10 cm distal to the pylorus were removed and placed in separate petri dishes containing cold (4°C) HEPES-buffered PSS. HEPES-PSS was composed of (mM): 141.8 NaCl, 4.7 KCl, 1.7 MgSO₄, 0.5 EDTA, 2.8 CaCl₂, 1.2 KH₂PO₄, 10.0 HEPES, and 5.0 glucose (pH = 7.4).

Segments (1–2mm long) of uterine arcuate arteries, uterine arcuate veins, and third-order mesenteric arteries were dissected free from connective and adipose tissue and transferred to the chamber of a small vessel arteriograph. One end of the vessel was tied onto a glass cannula and flushed of any luminal contents by increasing the pressure prior to securing the distal end onto a second cannula using a servo-null pressure system (Living Systems Instrumentation). All vessels were initially pressurized (arteries: 50 mmHg; veins: 10 mmHg), and equilibrated for 45–60 min at 37°C prior to the beginning of experimentation. Lumen diameter was measured by trans-illuminating each vessel segment and using a video dimension analyzer (Living Systems Instrumentation) in conjunction with data acquisition software (Ionoptix) to continuously record lumen diameter.

Following equilibration, all vessels were precontracted with phenylephrine to produce a 50–70% reduction in baseline diameter. Once constriction was achieved, PIGF-2 (mouse, R&D Systems) was added in increasing concentrations (0.1 pM – 10 nM), and the resulting dilation recorded. Separate vessel segments were preincubated with L-NNA (0.2 mM) to inhibit nitric oxide prior to the administration of PIGF.

To determine the receptor subtype responsible for PIGF-induced dilation in the uterine circulation, vessels were incubated with inhibitory antibodies to either VEGFR-1 (R&D Systems; 10 µg/ml) or VEGFR-2 (R&D Systems; 1 µg/ml) for 15 minutes prior to constriction with phenylephrine. These inhibiting antibodies were utilized at the maximal effective concentration specified by the manufacturer, and confirmed experimentally in preliminary tests in our lab.

Once the concentration-response characteristics were defined, a separate group of vessels was prepared as described, and incubated with VEGF-E, an isoform of VEGF that is selective for VEGFR-2 activation alone (1 nM arteries, 10 nM veins; Research Diagnostics Inc.), or in combination with PIGF (0.1 nM in arteries, 5 nM in veins).

Vascular reactivity of human subcutaneous and myometrial arteries

These studies were carried out in collaboration with the Karolinska Institute, Sweden, by two co-authors (K.K. and L.L.). The investigation was undertaken with the approval of the local research ethics committee. Biopsies of subcutaneous fat and myometrium were obtained from healthy pregnant women with a median age of 26 years (range 24–34) and at a median gestational age of 38 weeks (range 37–39) undergoing elective cesarean section due to breach presentation (n=4) or psychological reasons (n=3). Myometrial arterial segments were obtained from the upper edge of the transverse incision in the lower uterine segment during C-section. Biopsies were immediately placed in cold physiological salt solution (PSS, mM: NaCl 119, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.17, NaHCO₃ 25, KH₂PO₄ 1.18, EDTA 0.026, glucose 5.5) and utilized for isolation of small arteries, which were mounted in the organ baths of a four-channel wire myograph (Multimyograph, model 610, Danish Myo Technology; Aarhus, Denmark), as previously described [7]. Each organ bath contained warmed (37°C) physiological saline solution (PSS) that was continuously bubbled with 5% CO₂ carbon dioxide/95% O₂ oxygen. Following a 30 minute equilibration period, a passive circumference–tension curve was created for each segment in order to set optimum

resting tension. Endothelium-dependent vasodilatation was assessed by the addition of a single concentration of bradykinin (BK, 1 $\mu\text{mol/L}$) to each chamber after pre-constriction with norepinephrine (NE, 1 $\mu\text{mol/L}$). Arteries that did not achieve at least 70% vasorelaxation to BK were excluded from the study.

Both myometrial and subcutaneous arteries were pre-constricted with norepinephrine (3 μM), and concentration-response curves to PIGF (0.01–10 nM) were generated before and after a 30 minute incubation with a solution containing a combination of L-NAME (300 μM) and indomethacin (Indo, 10 μM) to inhibit production of endothelial NO.

Measurement of message for VEGFR-1 and VEGFR-2 by RT-PCR

Main uterine arteries were isolated and flash-frozen in liquid nitrogen for subsequent extraction and purification of mRNA, followed by real-time RT-PCR Taqman assay to evaluate the mRNA expression of VEGFR-1 and VEGFR-2. Proprietary sequences were purchased from ABI. Total RNA was extracted using Trizol (Invitrogen) as described by the manufacturer. RNA concentrations were determined by measuring the absorbance at a wavelength of 260 and 280 nm. RNA was then converted to cDNA using random hexamer primers and the Superscript TM First-strand Synthesis System, and stored at -70°C until ready for use. Real-time quantification of target mRNA transcripts was performed using an ABI Prism 7000 Sequence Detection System. All samples were quantified in a multiplex reaction with 18s ribosomal RNA to control for differences in sample preparation. Control (Ribosomal VIC RNA control; ABI) and targeted cDNA were amplified in a single reaction tube. The efficiency of amplification of each target transcript was evaluated by plotting cycle threshold to log (sample concentration) for each standard curve. Reactions were carried out using Universal Master Mix (ABI). Amplification was performed over 50 cycles of denaturation at 95°C for 15 sec, and annealing/extension at 60°C for 60sec. Cycle threshold values were determined for each reaction using real-time plots of fluorescence versus cycle. Relative quantification was determined using the standard curve method. Target cDNA expression was normalized to 18s ribosomal RNA, and each sample was run in triplicate to obtain an average value.

Drugs and solutions

All chemicals were purchased from Sigma Chemical (St. Louis, MO), including: salts for buffer preparation, L-NNA, indomethacin, phenylephrine, diltiazem, papaverine. PIGF-2 (mouse) and antibodies to VEGF receptors were purchased from R&D Systems; VEGF-E was obtained from Research Diagnostics Inc.

Statistical analysis

Data are expressed as mean values \pm standard error of the mean (SEM), where n is the number of arterial segments studied. Paired or unpaired Student's t -tests were used as appropriate to determine the significance of differences between sets of data, and differences considered significant at $p \leq 0.05$. When more than two treatment groups were evaluated, as in the VEGF-E and antibody studies, differences in responses between groups were determined with ANOVA followed by a multiple comparisons test (Tukey's) to evaluate the significance of differences between treatment means.

In wire-mounted human vessels, relaxation to PIGF was expressed as percent inhibition of the contraction induced by NE. Differences in responses between groups were determined by comparing concentration-response curves using a two-way repeated-measures ANOVA, using PIGF concentration as a within-subject factor and artery type or incubational conditions as a between-subject factors. All data are presented as mean values \pm SEM; n represents the number of vessels (one vessel per woman).

Calculations

Pharmacologic sensitivity to vasodilation by PIGF (IC_{50}) was calculated for each vessel by normalizing the fractional response to a particular concentration of PIGF to the maximal response obtained at the highest concentration of drug tested. Efficacy was defined as the % of maximal dilation, which was determined at the end of each experiment by the addition of diltiazem (10 μ M) and papaverine (100 μ M), a relaxing solution that induces complete vascular smooth muscle relaxation. In wire-mounted vessels efficacy was defined as the % of maximal dilation relative to pre-constrictor level.

RESULTS

PIGF induced significant dilation in all arterial types studied, although there were significant differences in sensitivity, efficacy, and relative contribution of NO to the observed effects.

Vasodilatory effects of PIGF on rat uterine vessels; effects of gestation; expression of message for VEGFR-1 vs. VEGFR-2

Arcuate arteries and veins obtained from the uterine circulation dilated readily to PIGF. As previously observed with VEGF [10], arteries obtained from pregnant animals were significantly (1000x) more sensitive than those from age-matched nonpregnant controls (IC_{50} values: NP = 2.7 ± 1.4 nM; LP = 0.003 ± 0.0015 nM; $p < 0.01$), although the extent of maximal dilation (efficacy) was comparable (NP = $86 \pm 5.8\%$; LP = 78 ± 11 ; $p > 0.05$).

Moreover, the sensitivity of LP uterine arteries to the vasodilator effects of PIGF was significantly (200x) greater than that of veins (IC_{50} value: 0.6 ± 0.17 nM in veins; Figure 1A) and dilated to a greater extent than veins (efficacy averaged 78% and 39% of maximal dilation, respectively, at maximal concentrations; Figure 1B).

In rat uterine arcuate arteries, the vasodilatory actions of PIGF were completely eliminated by preincubation with L-NNA (0.2 mM), an inhibitor of nitric oxide synthase. Conversely, in veins, approximately 30% of the dilation remained (Figure 2).

This vasodilatory effect was due to activation of the VEGFR-1 receptor, as preincubation of vessels with an antibody to the R1 receptor eliminated >90% of the observed dilation, while pre-exposure to an anti-R2 antibody was completely without effect (Figure 3). The addition of a selective agonist for the VEGFR-2 receptor (VEGF-E) was also without effect, and there was no amplification of the response to PIGF + VEGF-E vs. PIGF alone (Figure 4).

Real-time RT-PCR measurement of mRNA showed that message for both receptor subtypes was present in the uterine artery wall. VEGFR-1 but not VEGFR-2 message was upregulated during gestation (Figures 5A and B).

Effects of PIGF on reactivity of rat mesenteric arteries

Third-order mesenteric arteries dilated to PIGF ($\approx 50\%$ of maximal dilation at the highest concentration, 10 nM) to a lesser extent than comparably-sized uterine arteries (compare Figure 6A vs. 1A); dilation was also considerably slower than that observed in uterine vessels, taking 15–45 instead of 5–15 minutes to stabilize. Preincubation in L-NNA (0.2 mM) did not affect efficacy, but decreased sensitivity (IC_{50} values: Control: 0.22 ± 0.04 nM; L-NNA: 1.20 ± 0.47 nM; $p < 0.05$; Figures 6A and 6B).

Effects of PIGF on human myometrial and subcutaneous arteries

Both types of human vessels relaxed to PIGF in a concentration-dependent manner (Figure 7). The extent of dilatation to the maximal concentration of PIGF (10 nM) was almost twice

as large in myometrial vs. subcutaneous arteries ($79 \pm 7\%$ vs. $45 \pm 8\%$; $p < 0.05$), Figure 7), which were also more sensitive to PIGF-induced vasodilation (IC_{50} : 1.1 ± 0.38 nM vs. 3.0 ± 0.45 nM; $p < 0.05$, Figure 7 and 8).

Preincubation with L-NAME (300 μ M) and indomethacin (Indo, 10 μ M) eliminated approximately 50% of the relaxation response to PIGF in myometrial arteries (e.g., at 10 nM: $79 \pm 7\%$ PSS vs. $44 \pm 14\%$ L-NAME + Indo, $n=5$, $p < 0.05$, ANOVA), however, it had no effect on efficacy in subcutaneous arteries ($51 \pm 7\%$ PSS vs. $48 \pm 9\%$ in L-NAME + Indo, $n=6$).

The data shown in Figure 7 indicate the extent of dilation under each condition relative to pre-constriction level (efficacy). Calculation of pharmacologic sensitivity requires the normalization of this parameter to the maximal effect observed in response to the compound in question (PIGF) for each vessel. Hence, the data in Figure 7 were normalized in this manner to assess sensitivity, and are re-plotted in Figure 8. As indicated above, there was a difference in sensitivity to PIGF between subcutaneous and myometrial arteries ($p < 0.05$, ANOVA), however, after inhibition of NOS and COX products, the sensitivity of myometrial arteries was comparable to that obtained in subcutaneous arteries before and after L-NAME + Indo, ($p > 0.05$, ANOVA). After preincubation with L-NAME + Indo, IC_{50} values were: 2.2 ± 0.65 nM (myometrial) to 2.4 ± 0.5 nM (subcutaneous), and were no longer statistically different ($p > 0.05$).

DISCUSSION

The main findings of this study are that: (1) PIGF is a potent vasodilator of resistance arteries and veins from the rat uterine circulation, with arteries displaying significantly greater sensitivity and efficacy than veins; (2) in rat uterine arteries, pregnancy significantly enhanced sensitivity to PIGF without altering its efficacy; (3) PIGF dilation is principally mediated by the release of NO in rat uterine arteries, while other, still-unidentified factors contribute to the vasodilation of uterine veins. (4) In rat tissues, message for both VEGF receptor subtypes is present in the uterine artery wall, and VEGFR-1 (but not VEGFR-2) message is significantly increased in pregnancy. (5) Human uterine (myometrial) arteries also dilate in response to PIGF, although the contribution of nitric oxide to the vasorelaxation, while significant, is proportionately less in human vs. rat uterine arteries. Finally, (6) both rat mesenteric and human subcutaneous arteries dilate to PIGF in an NO-independent manner. Together, these observations demonstrate the broad and mechanistically-diverse actions of PIGF on the vascular wall of resistance arteries and veins. They also support the importance of PIGF/VEGFR-1 signaling in uterine blood flow regulation during pregnancy, and raise the potential of an influence on blood pressure and peripheral resistance, as well as venous capacitance.

In this study, we were interested in probing the role of the tissue (non-soluble) VEGFR-1 receptor in uterine circulatory function during pregnancy. An *in vitro* approach using isolated vessels allowed us to quantitate the vasodilatory effects of PIGF, a selective agonist for VEGFR-1. As the results indicate, PIGF is an extremely potent vasodilator, with significant effects documented at sub-nanomolar concentrations, particularly in uterine arteries. The observation of a venodilatory effect is novel, and the fact that PIGF levels would be highest in the veins that drain the placenta suggests that it may play a role in regulating venous tone. Further, uterine venorelaxation is associated with an increased permeability of the vascular wall [2] and, in view of the well-documented proximity of arteries to veins in the uterine circulation of several animal species, as well as in the human, its permeability-enhancing action may facilitate signal transfer from vein to artery, and

thereby potentially alter uterine resistance artery tone, placental blood flow, and vascular structure [3].

Earlier [10], we reported a significant effect on uterine artery sensitivity to VEGF and, in a subsequent study, determined this to be secondary to elevated circulating levels of estrogen by studying oophorectomized animals in combination with selective hormone replacement []. These findings did not distinguish the receptor subtype involved in VEGF vasodilation since VEGF activates both VEGFR-1 and VEGFR-2 receptors. In this study, we demonstrate a similar change in reactivity to PIGF, a selective agonist for the VEGFR-1 receptor subtype, and implicate a transcriptional mechanism as the underlying cause since: (1) VEGFR-1 but not VEGFR-2 message was significantly upregulated in pregnancy and, as shown by the experiments that utilized receptor blocking antibodies, (2) only the VEGFR-1 receptor appears to be involved in the vasodilatory process, at least in the vessels studied herein. This finding does not preclude altered post-receptor signaling as an additional, or complementary mechanism for enhanced vasodilator sensitivity.

The important question from a physiological standpoint is whether PIGF signaling contributes to the profound increase in uterine blood flow that characteristically occurs in the pregnant state. In this regard, it was recently noted [6] that fetal and placental weights were significantly reduced in a rat model in which PIGF/VEGF signaling was attenuated by adenoviral overexpression of sFlt). Although uterine blood flow was not measured in that study, there is a long-standing association between reduced uteroplacental perfusion and reduced fetal and placental weights in studies that utilize a surgical approach to impose a state of reduced uteroplacental perfusion in rats (the RUPP model [e.g. 16,17]. More directly pertinent to this study, it was recently shown that, in addition to the hypertension that is associated with RUPP, there is also a significant (~800%) increase in plasma sFlt-1a concentration. As might be predicted, this excess of soluble receptor was associated with significant reductions in the circulating free concentrations of both PIGF and VEGF [18].

The actions of PIGF extend to the human, as it was a potent vasodilator of human myometrial resistance arteries, with IC_{50} values in the sub-nanomolar range. As in rat uterine arteries, in which NO accounted for >90% of the dilation to PIGF, NO contributed significantly to human uterine artery vasodilation, since its inhibition eliminated >40% of the vasodilation. In contrast, eNOS inhibition was of little consequence in PIGF-induced dilation of rat mesenteric and human subcutaneous arteries, demonstrating regional differences in its mechanism of action.

The experiments with anti-VEGFR-1 and anti-VEGFR-2 antibodies, and the complete absence of a vasodilatory effect of VEGF-E - at least in rat uterine vessels - confirm that dilation is mediated entirely through VEGFR-1 receptor. Although this is not surprising in view of the specificity of PIGF for VEGFR-1 signaling, it is noteworthy in that other reports have associated vasodilation and the production of nitric oxide most closely with VEGFR-2 signaling through mechanisms involving phospholipase C and Akt-1 [6,13].

It is also interesting to note that only VEGFR-1 and not VEGFR-2 message was upregulated in gestation, pointing to an unrecognized role for this receptor, and of signal transduction pathways subsequent to its activation in the adaptation of the maternal uterine circulation to pregnancy. Combined with two earlier reports that showed an increased sensitivity to VEGF dilation in rat uterine arteries from pregnant rats [10], and of its linkage to estrogen [11], the current findings support the hypothesis that enhanced vasodilation may result from an estrogen-induced increase in VEGFR-1 receptors in the vascular wall. Changes in other signaling pathways downstream to the receptor (e.g. production of NO or other vasodilators) cannot be ruled out, however, and will be evaluated in subsequent studies.

Acknowledgments

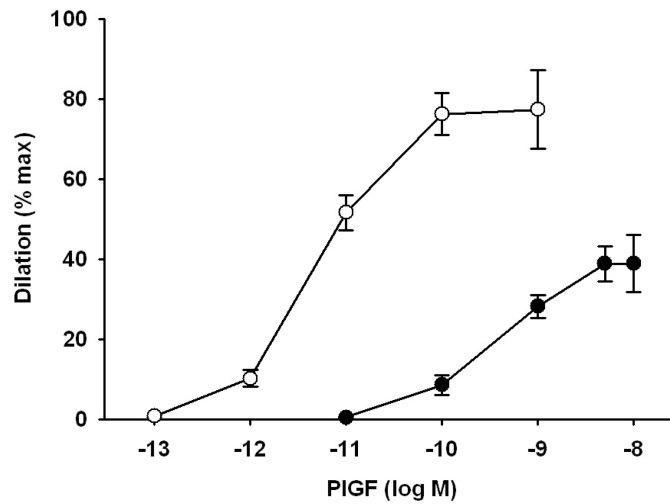
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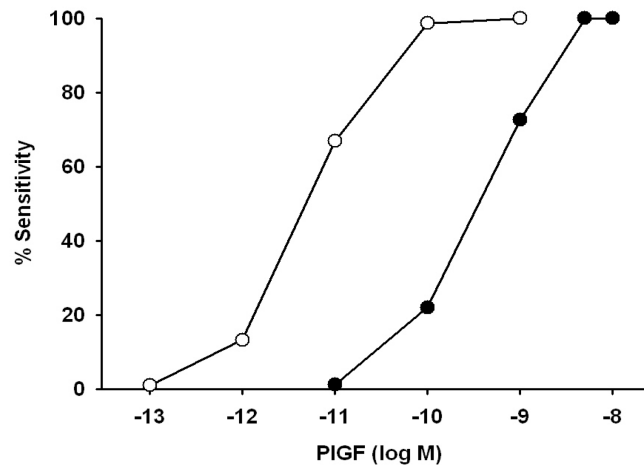
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A.



B.

**Figure 1.**

A. Concentration-response curve showing dilation of pressurized rat uterine arcuate arteries (50 mmHg; n=5; open circles) and veins (10 mmHg; n=3; filled circles) to PIGF. Efficacy (maximal dilation) of arteries was twice that of veins (78% vs. 39%; $p < 0.01$). B. Same data shown as sensitivity (normalized to maximal dilation obtained in each vessel to PIGF). Note approximately 200x greater sensitivity in arteries vs. veins (IC_{50} values in text).

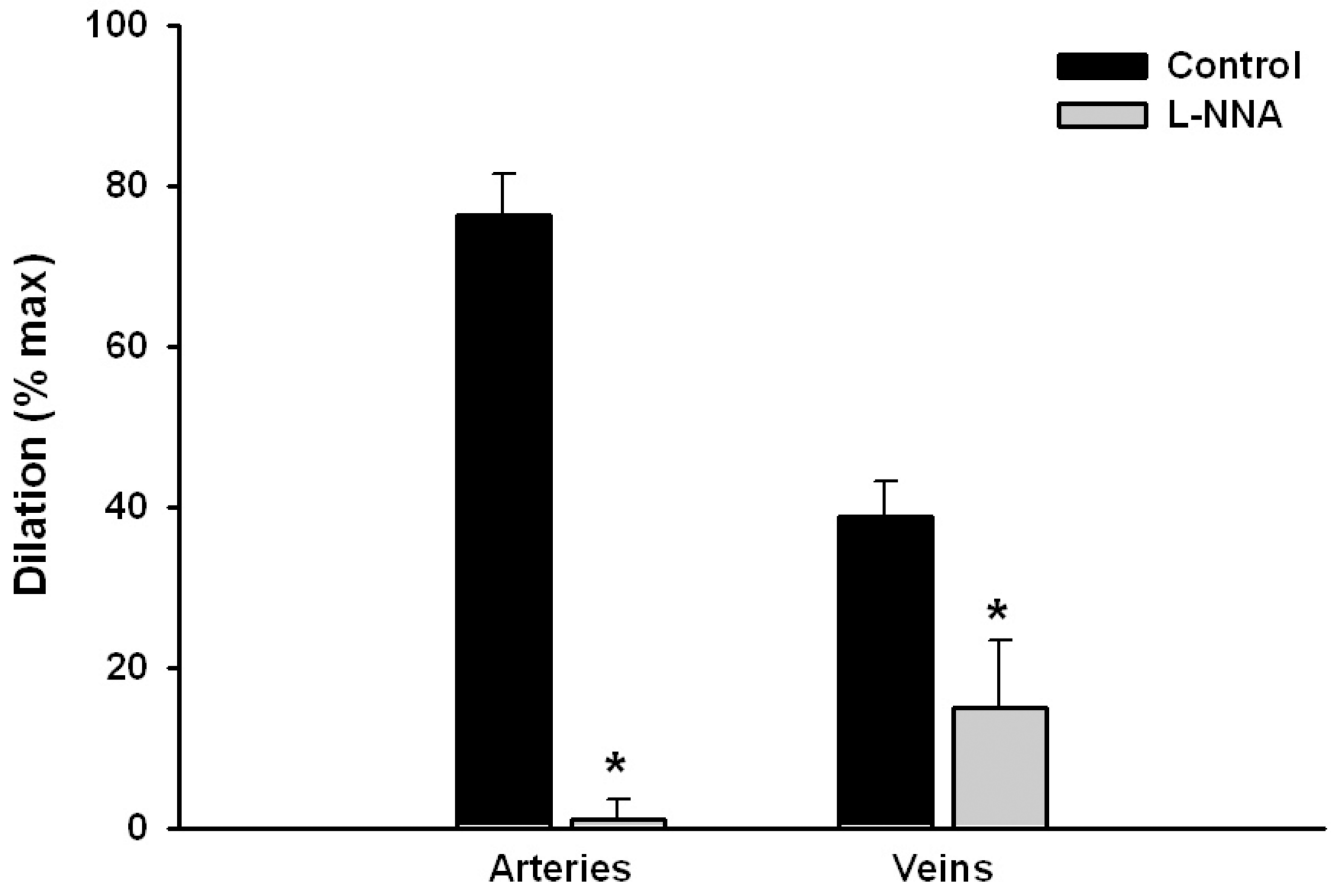


Figure 2. Nitric oxide synthase inhibition (L-NNA, 0.2 mM) eliminated and significantly (>60%) diminished vasodilation to PIGF in rat uterine arteries (n=5) vs. veins (n=3), respectively.

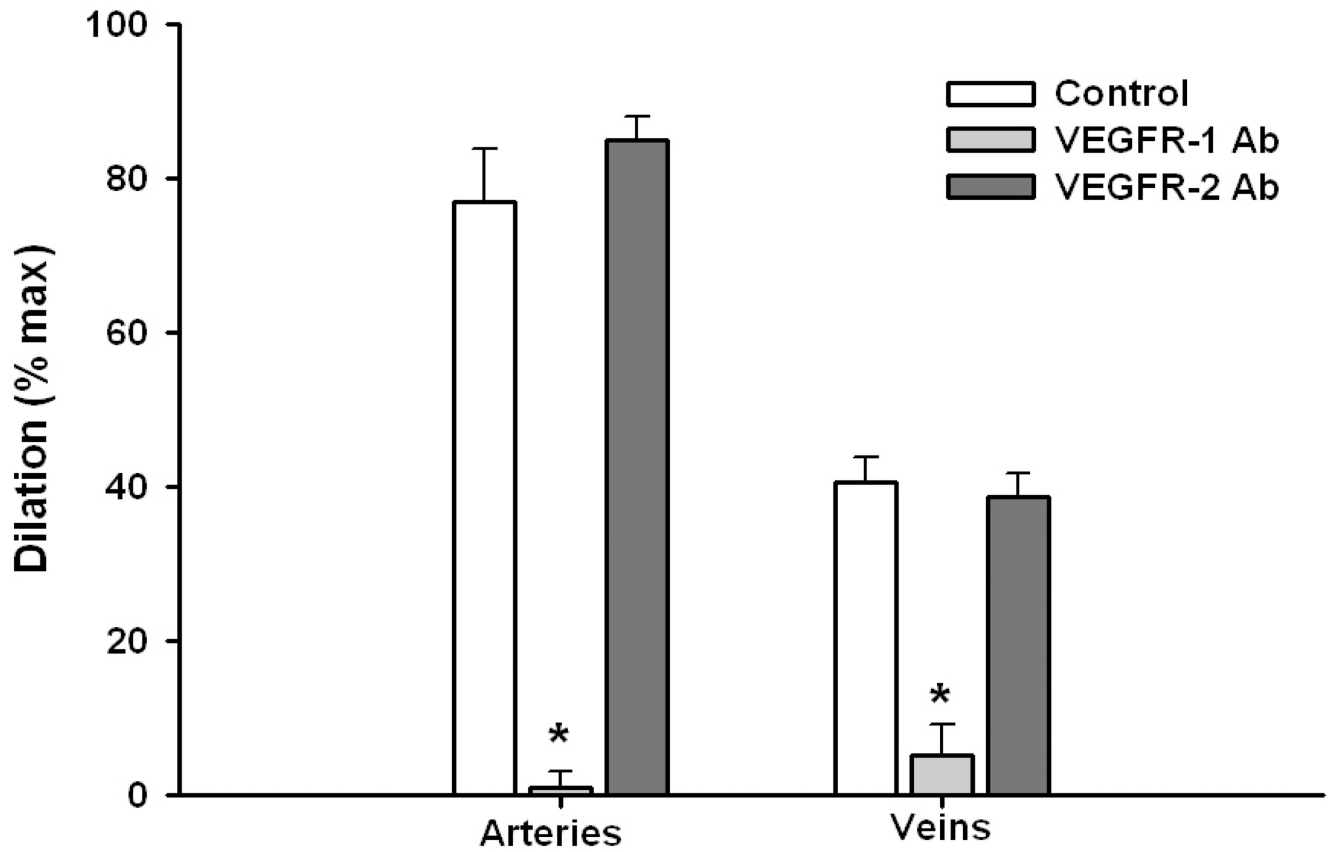


Figure 3. Effects of preincubation in a solution containing antibodies to VEGFR-1 (10 mg/ml) and VEGFR-2 (1 mg/ml) on vascular dilation to PIGF. Nearly complete (>90%; $p < 0.001$) inhibition of dilation was observed in response to the VEGFR-1 antibody; while there was no observable effect of VEGFR-2 inhibition ($p > 0.05$; $n = 4$ for each set of data).

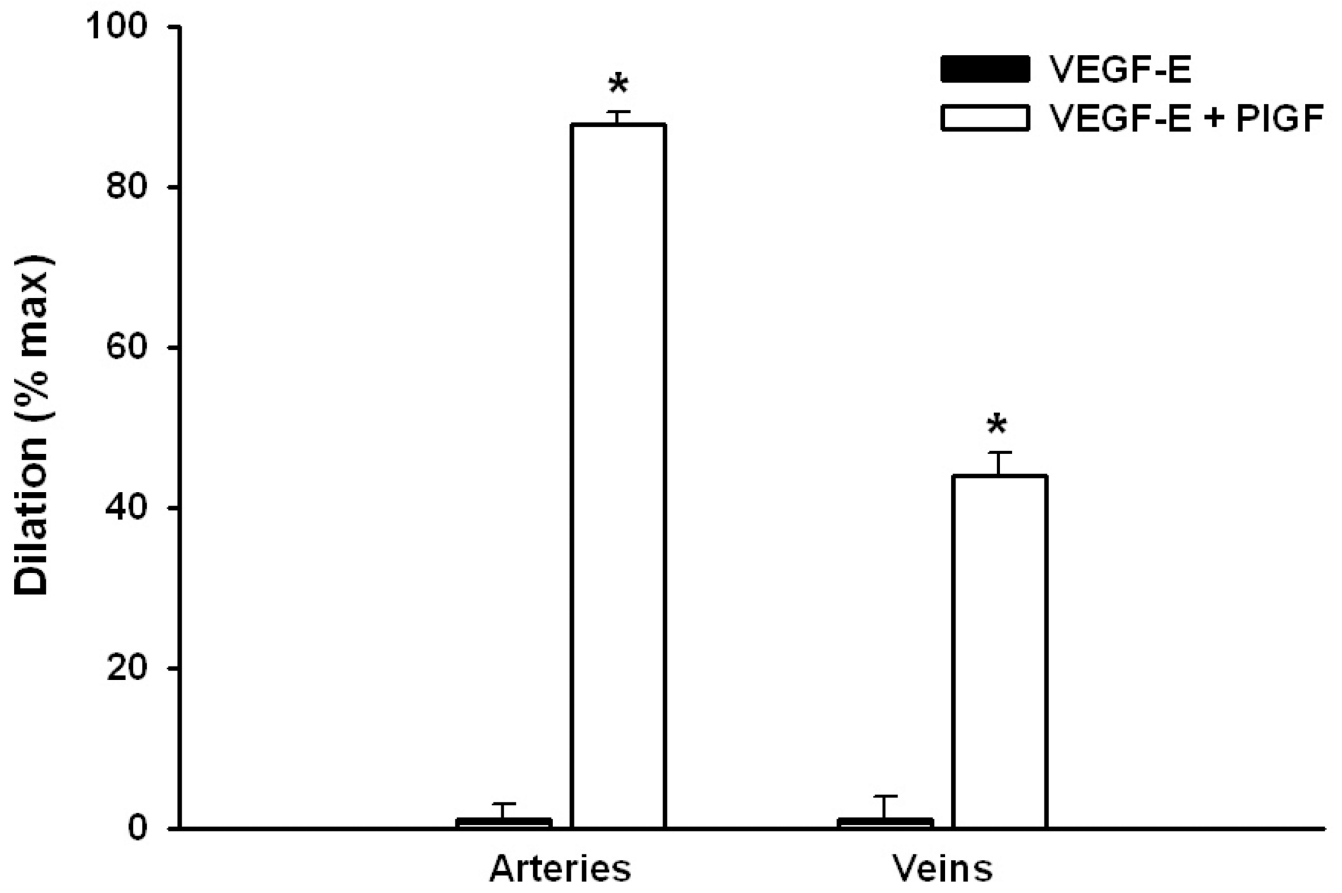


Figure 4.

Uterine arcuate arteries (n=4) and veins (n=3) failed to dilate to VEGF-E (10 nM), an agonist that is selective for VEGFR-2; subsequent addition of PIGF (a selective VEGFR-1 agonist; 1 nM - arteries; 10 nM - veins) produced significant dilation of both types of vessels.

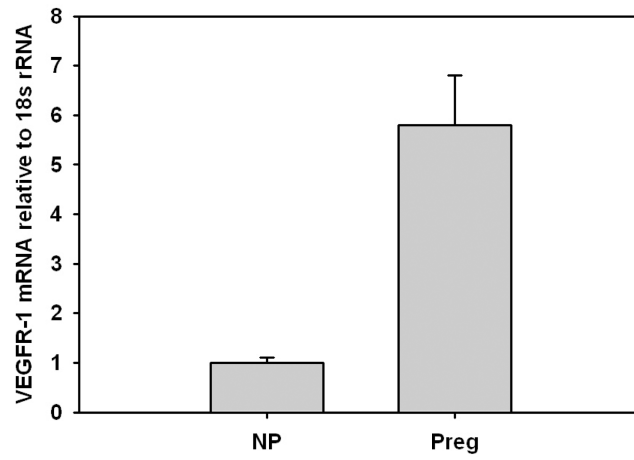
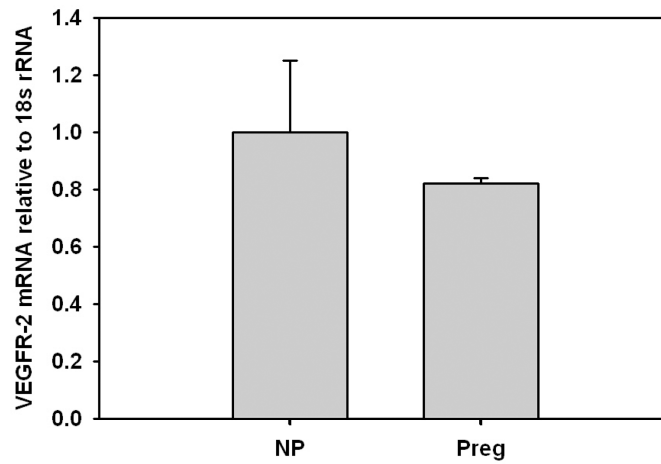
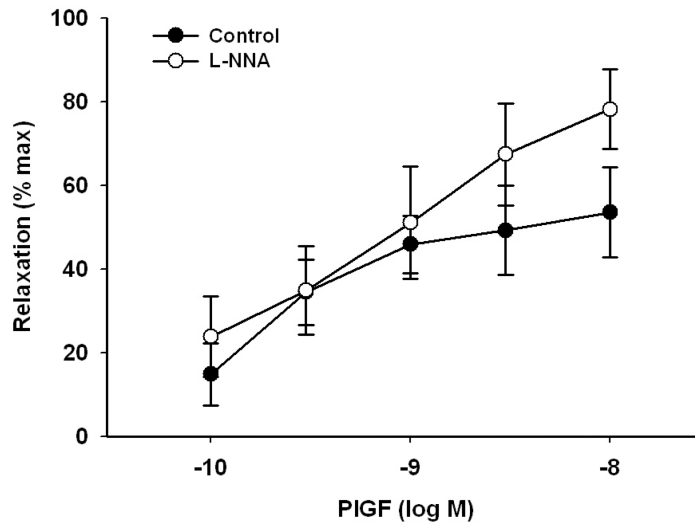
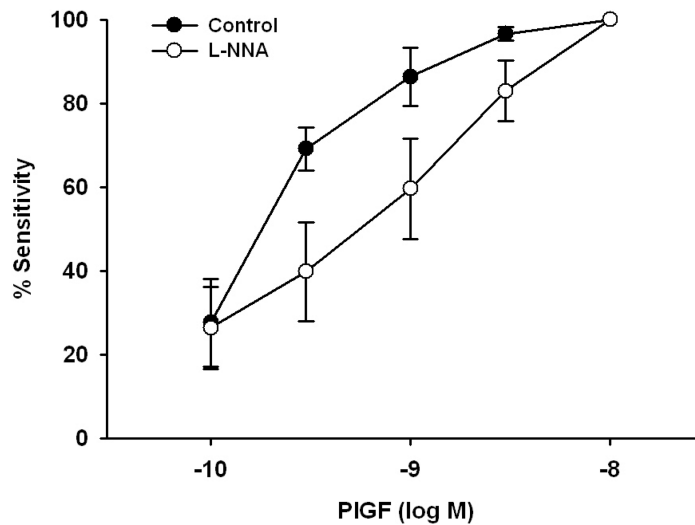
A.**B.**

Figure 5. Message for VEGFR-1 (A) and VEGFR-2 (B) as determined by real-time RT-PCR Taqman assay in uterine arteries from nonpregnant (NP, n=8) and pregnant (Preg) rats. Gestation resulted in a significant ($p<0.05$) upregulation of VEGFR-1 message (A); in contrast, no changes were observed in VEGFR-2 message expression (B).

A.



B.

**Figure 6.**

Graphs showing concentration-dependent dilation of isolated third order rat mesenteric arteries in response to PIGF in the absence (control; n=5) and presence (n=4) of nitric oxide synthase inhibition with L-NNA (0.2 mM). A. There was no observable effect of L-NNA on efficacy, with slightly greater dilation observed in its presence ($p>0.05$) at the highest concentration of PIGF (10 nM). B. Based on IC_{50} values ($p<0.05$; see text), sensitivity to dilation was decreased approximately five-fold in the presence of L-NNA.

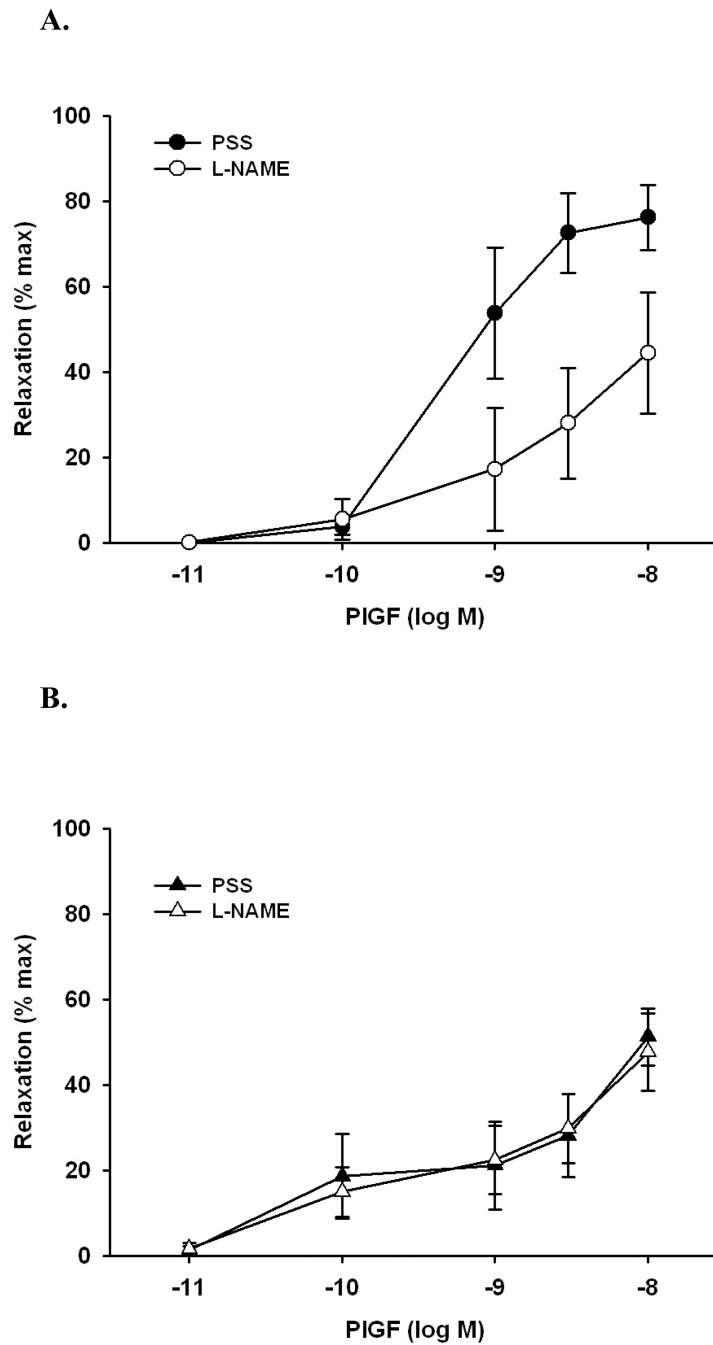


Figure 7.

Concentration-response curves showing the efficacy of PIGF in isometric (wire-mounted) myometrial (A, n=5) and subcutaneous (B, n=6) resistance arteries from pregnant women before and after incubation with a solution containing N^w-nitro-L-arginine methyl ester (L-NAME; 300 μ M) plus indomethacin (Indo; 10 μ M). Note proportionately greater relaxation of myometrial vs. subcutaneous vessels. At the highest concentration (10 nM), preincubation with L-NAME and indomethacin eliminated approximately 50% of the relaxation response to PIGF in myometrial arteries, however, it had no effect on efficacy in subcutaneous arteries.

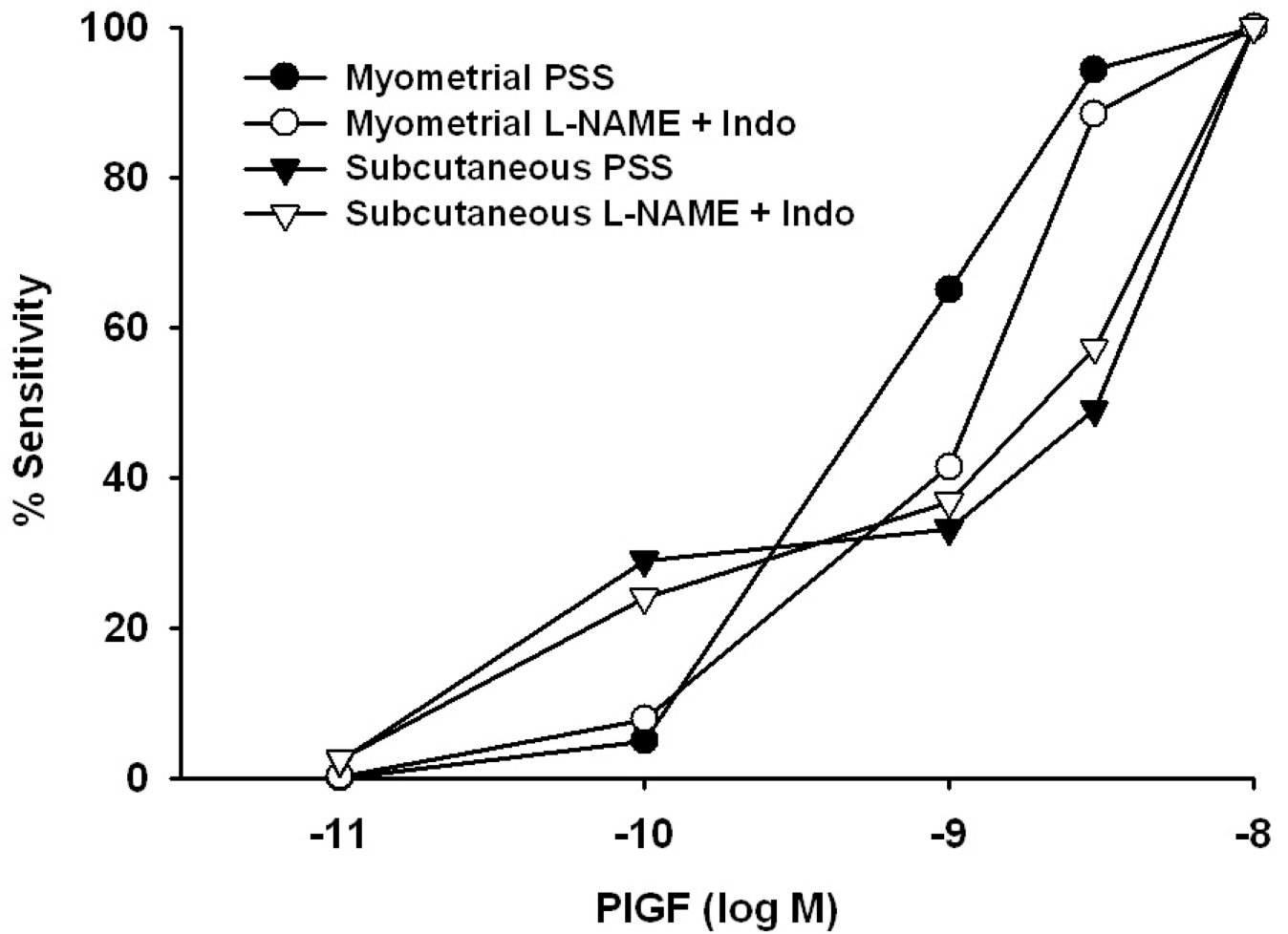


Figure 8.

Graph summarizing relative sensitivity of human uterine myometrial and subcutaneous vessels with and without pre-exposure to nitric oxide synthase and cyclooxygenase inhibition. There was a difference in sensitivity to PIGF between subcutaneous and myometrial arteries ($p < 0.05$, ANOVA), however after pre-exposure to these inhibitors the sensitivity of myometrial and subcutaneous arteries was comparable ($p > 0.05$, ANOVA).