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Differential Regulation of Pregnancy Associated Plasma Protein-A in Human Coronary Artery Endothelial Cells and Smooth Muscle Cells

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Abstract

Background—Pregnancy-associated plasma protein-A (PAPP-A), a metalloproteinase that serves to modulate local insulin-like growth factor (IGF) action, is upregulated in atherosclerotic plaque. However, little is known about the cellular mechanisms underlying this elevated PAPP-A.

Objective—To continue study of PAPP-A expression and its regulation in human vascular cells, with a focus on endothelial cells.

Design—Primary cultures of human coronary artery endothelial cells (ECs) were treated without and with cytokines, growth factors, or low density lipoprotein (LDL). PAPP-A mRNA, protein, and protease activity were assessed using real-time PCR, ultra-sensitive PAPP-A ELISA and cell-free proteolysis of IGF binding protein (IGFBP-4), respectively. In addition, vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), monocyte chemotactic protein (MCP-1), IGF-I, IGF-I receptor, and IGFBP-4 and -5 mRNA expression levels were determined.

Results—ECs in culture show little basal PAPP-A expression. The pro-inflammatory cytokines, tumor necrosis factor (TNF)- α and interleukin (IL)- β , stimulated PAPP-A expression (TNF- α >> IL-1 β), whereas there was no effect of IL-6, transforming growth factor- β , IGF-I, insulin, fibroblast growth factor or epidermal growth factor in these cells. Stimulation of PAPP-A expression by TNF- α was associated with significantly increased VCAM, ICAM, and MCP-1 expression but without major changes in other IGF system components. TNF- α -induced VCAM, ICAM, and MCP-1 expression (≤ 4 h) preceded PAPP-A expression (24 h). The anti-oxidant, N-acetyl cysteine, inhibited TNF- α -induced PAPP-A expression without altering the induction in VCAM, ICAM, and MCP-1. Treatment with native or oxidized LDL had no effect on PAPP-A expression in ECs. Comparative results in human coronary smooth muscle cells indicated qualitative and quantitative differences in PAPP-A expression and regulation between the two vascular cell types.

Conclusions—Human coronary artery ECs express PAPP-A mRNA and functional protein when activated by the pro-inflammatory cytokine, TNF- α . This study complements work on PAPP-A expression in human coronary artery SMCs and human monocyte-derived macrophages and suggests an interactive model of PAPP-A regulation and action in human atherosclerotic plaque.

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List of special characters used: α , β , $^{\circ}$, μ , $\sim$

Keywords

Pregnancy-associated plasma protein-A; Insulin-like growth factor; Tumor necrosis factor- α ; Endothelial cells; Interleukin-1 β ; Low density lipoprotein; Vascular cell adhesion molecule

INTRODUCTION

Pregnancy Associated Plasma Protein-A (PAPP-A) is a metalloproteinase that serves to modulate local insulin-like growth factor (IGF) action through degradation of IGF binding proteins *in vitro* and *in vivo*.^{1,2} PAPP-A expression, as assessed by immunohistochemistry, is upregulated in ruptured and eroded human atherosclerotic plaque, co-localizing with activated smooth muscle cells, macrophages, and endothelial cells.^{3,4} Since plaque-derived PAPP-A is being considered as a new biomarker^{2,3} and may be a potential therapeutic target in atherosclerosis, a better understanding of its cell source and regulation is important. To determine the mechanisms underlying elevated PAPP-A in atherosclerotic plaque, we initiated studies in primary cultures of human vascular cells involved in plaque development. We previously reported that human coronary artery smooth muscle cells (SMCs) constitutively express and secrete PAPP-A.⁵ Peptide growth factors had no effect; however, the proinflammatory cytokines, interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α , were potent stimulators of PAPP-A expression in these cells, with relative responsiveness of SMCs to cytokines being IL-1 β > IL-6, TNF- α .^{2,6} Interestingly, in spite of intense immunostaining for PAPP-A co-localizing with activated macrophages in atherosclerotic plaque,^{3,4} we recently showed that macrophages did not express PAPP-A under basal conditions or in response to any activators, cytokines or growth factors tested. Rather, PAPP-A associated with and was internalized by activated macrophages.⁷ The question remained 'Is PAPP-A expressed by vascular endothelial cells?' and, if so, 'How is it regulated?'.

In addition, low density lipoproteins (LDL), in particular oxidized LDL (oxLDL), have been implicated in vessel injury and initiation of early lesion formation through multiple effects on vascular cells, including activation of ECs and stimulation of SMC proliferation.^{8,9} Previous studies indicated effects of oxLDL on the IGF system, specifically IGF-I and IGF-I receptor expression in SMCs.^{10–12} The effect of oxLDL on PAPP-A expression in SMCs or in ECs has not been determined.

In this study we investigated the effect of cytokines, growth factors, and LDL on PAPP-A expression in human coronary artery endothelial cells (ECs) and compared responses in human coronary artery SMCs.

MATERIALS AND METHODS

Materials

TNF- α and IL-1 β were purchased from Research Diagnostics Inc. (Flanders, NJ). IL-6, soluble IL-6 receptor and transforming growth factor (TGF)- β were from R & D Systems, Inc. (Minneapolis, MN). Bone morphogenic protein (BMP)-2 was from Genetics Institute (Cambridge, MA), and N-acetyl cysteine (NAC) was from Sigma Chemical Corp. (St. Louis, MO). Reagents for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from BioRad Laboratories (Richmond, CA). Human LDL ($d = 1.019–1.063$ g/ml) was isolated by density ultracentrifugation from EDTA-treated plasma. The LDL supernatant was dialyzed against isotonic saline to remove EDTA. Oxidation of LDL was obtained by incubating LDL with 5 μ M CuSO₄ at 37°C for 3 h, and then dialyzed overnight against saline prior to addition to cells. The extent of oxidation of the LDL preparations was determined by measuring thiobarbituric acid reactive substance (TBARS) (PeroXOquant,

Pierce, Rockford, IL). LDL processed in parallel but without CuSO₄ was 1.7 TBARS/μg protein and oxLDL was 15.6 TBARS/μg protein.

Cell culture

Human coronary artery endothelial cells (ECs) and smooth muscle cells (SMCs) were obtained from Clonetics (Walkersville, MD), and cultured in the recommended Endothelial Cell and Smooth Muscle Growth Medium, respectively, containing 5% fetal bovine serum (FBS). Cells of the fourth to sixth passage were used for experiments. Cells were washed and changed to 0.5% FBS with the indicated experimental additions. At the end of the incubation, conditioned medium was collected, centrifuged to remove debris and stored at -70°C. Cell numbers were determined using a Coulter Counter (Coulter Electronics, Hialeah, FL). Alternatively, total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Valencia, CA) and treated with deoxyribonuclease (DNA-free, Ambion, Inc., Austin, TX). Four hundred nanograms of RNA were reverse transcribed using TaqMan RT reagents (PE Applied Biosystems, Foster City, CA) according to manufacturer's instructions.

Real-time PCR

Real-time quantitative PCR analyses for mRNA expression were performed using the primer sequences in Table 1 and the iCycler iQ Detection System. Amplification plots were analyzed with iCycler iQ Detection System analysis software v3.0.6070 (Bio-Rad, Hercules, CA).

PAPP-A ELISA

PAPP-A levels in cell-conditioned medium were measured using an Ultra-sensitive PAPP-A ELISA kit kindly provided by Diagnostic Systems Laboratories, Inc. (Webster, TX). Minimum sensitivity is 0.24 mIU/L, with intra- and inter-assay coefficients of variation of 4.7% and 4.2%, respectively.

IGFBP-4 protease assay

Cell-free IGFBP-4 proteolysis was assayed as previously described.^{1,5,6,13} Conditioned medium (5X concentrated) was incubated at 37°C for 24 h with ¹²⁵I-IGFBP-4 and 5 nM IGF-II. IGF-II binds IGFBP-4 and increases its susceptibility to cleavage by PAPP-A.¹⁴ Reaction products were separated by SDS-PAGE and visualized by autoradiography for loss of intact and generation of 18 kD radiolabeled fragments.

Statistical analyses

Data are presented as mean ± SEM. Statistical analyses were performed using ANOVA, followed by multiple comparisons. Results are considered statistically significant at P < 0.05.

RESULTS

Cytokines and Growth Factors

The time course of PAPP-A mRNA expression in ECs under basal and cytokine-stimulated conditions is presented in Figure 1. There was little PAPP-A expression in ECs under basal conditions over the 24 h treatment period (<100 copies mRNA/ng total RNA). Both TNF-α and IL-1β treatment increased PAPP-A mRNA expression, but significant increases were not seen until 24 hours. TNF-α appeared to be more effective than IL-1β, with 30-fold versus 6-fold increases in PAPP-A mRNA at 24 h. IL-6 (50 ng/ml ± 100 ng/ml soluble receptor) and transforming growth factor (TGF)-β (10 ng/ml), cytokines that were potent stimulators of PAPP-A expression in human coronary artery SMCs and human osteoblasts, respectively,^{6,15} had no effect on PAPP-A expression in ECs. Furthermore, none of the growth factors tested

[IGF-I (10 nM), insulin (1 μ M), fibroblast growth factor (100 ng/ml), epidermal growth factor (10 ng/ml)] affected PAPP-A expression in EC (data not shown).

Induction of PAPP-A expression was associated with EC activation, as assessed by vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), and monocyte chemotactic protein (MCP-1) expression.^{8,16} Treatment with TNF- α or IL-1 β significantly increased VCAM mRNA expression at all time points tested: 1, 4, 8, 24 h (Fig. 2A). TNF- α had maximal effects (~100-fold) at 4–8 h, whereas IL-1 β had peak effects (~50-fold) at 4 h. ICAM and MCP-1 expression was also rapidly upregulated with cytokine treatment of ECs (Figs 2B, 2C). VCAM, ICAM, or MCP-1 mRNA levels did not change with IL-6, TGF- β , IGF-I or insulin treatment (data not shown).

Dose-response experiments confirmed TNF- α >> IL-1 β in stimulating PAPP-A protein expression and secretion in 72 h EC-conditioned medium (Fig. 3). Maximal stimulation (20-fold) was seen at 10 nM TNF- α with a half-maximal effect at ~1 nM. IL-1 β treatment did not elicit dose-dependent increases in PAPP-A. TNF- α -stimulated ECs secreted functional PAPP-A as demonstrated in Figure 4. There was no detectable proteolysis of radiolabeled IGFBP-4 in basal EC-conditioned medium. Incubation of ¹²⁵I-IGFBP-4 in medium from ECs treated with TNF- α exhibited IGF-dependent loss of intact IGFBP-4 and generation of radiolabeled fragments at 18 kD, characteristic of PAPP-A proteolytic activity.^{1,5,6,14,15}

The 24-h lag between TNF- α (or IL-1 β) treatment and a significant increase in PAPP-A mRNA expression suggested an intermediate factor mediating the cytokine effect. Two candidates for this putative intermediate factor were explored: bone morphogenic protein-2 (BMP-2) and reactive oxygen species (ROS). TNF- α has been reported to increase EC expression of BMP-2, which is a member of the TGF- β superfamily.¹⁷ BMP-2 has been shown to activate ECs and is upregulated in atherosclerosis.¹⁸ Oxidative stress and ROS, which is also upregulated in atherosclerotic plaque, mediate TNF- α effects in other systems.^{19,20} In our experiments, direct addition of BMP-2 to ECs failed to stimulate PAPP-A expression, indicating that BMP-2 is unlikely to be the mediator of TNF- α 's effect in this regard. On the other hand, a potent anti-oxidant, NAC, completely prevented the ability of TNF- α to stimulate PAPP-A expression in ECs (Fig. 5A). NAC did not affect the TNF- α -stimulated increase in VCAM, ICAM, or MCP-1 expression (Figs. 5B, 5C, 5D).

Low Density Lipoproteins

To determine possible contributions of LDL on PAPP-A expression, ECs and SMCs were treated with 60 μ g/ml native or oxLDL. RNA was harvested at 8 and 24 h. In addition, some cells were pretreated with LDL and oxLDL for 48 hr and then stimulated with TNF- α , since a recent report indicated oxLDL by itself did not regulate VCAM expression in human aortic ECs, but it could enhance TNF- α stimulation.⁸ ECs showed the predicted increase in PAPP-A mRNA expression with TNF- α , however there was no significant effect of LDL or oxLDL on PAPP-A mRNA either alone or with TNF- α (data not shown). In contrast to ECs, SMCs have high basal expression of PAPP-A mRNA (>1000 copies/ng total RNA). SMCs showed no significant increases in PAPP-A mRNA expression with native and oxLDL alone. However, pretreatment of SMCs with LDL, but not oxDL, enhanced TNF- α stimulation of PAPP-A expression (Fig. 6).

Expression of other components of the IGF system in ECs and SMCs in response to treatment with TNF- α and oxLDL is presented in Table 2. Both early (8 h) and late (24 h) treatment effects are presented for both cell types. ECs had very low levels of IGF-I and IGFBP-5 mRNA, and, although there was a statistically significant increase in IGF-I mRNA and decrease in IGFBP-5 mRNA after 8 h of TNF- α treatment, the physiological significance of these changes is questionable. IGF-I receptor (IGF-IR) and IGFBP-4 mRNA levels were relatively high in

control ECs. IGF-IR and IGFBP-4 mRNA levels were significantly lower in TNF- α -treated than control levels at 8 h but not at 24 h, which appears to be due to a decrease in control levels at 24 h. Oxidized LDL significantly increased IGFBP-4 mRNA levels in ECs at 24 h. SMCs had very low levels of IGF-I, moderate levels of IGF-IR and IGFBP-5, and high levels of IGFBP-4 mRNA under basal conditions. TNF- α treatment had no significant effect on IGF-I or IGF-IR mRNA expression but decreased IGFBP-4 and IGFBP-5 mRNA levels. Oxidized LDL had no significant effect on IGF-I, IGF-IR, IGFBP-4 or IGFBP-5 mRNA expression in SMCs. Although the response to oxLDL is presented in Table 2, similar effects were seen with LDL, including the significant increase in IGFBP-4 mRNA expression in ECs after 24 h of treatment.

DISCUSSION

This study demonstrates that cultured human coronary artery ECs express PAPP-A mRNA and functional protein when activated by the pro-inflammatory cytokine, TNF- α . There was some stimulation of PAPP-A expression with IL-1 β , however IL-1 β was far less potent than TNF- α . This is opposite of what we see with human coronary artery SMCs, where IL-1 β is more potent than TNF- α in stimulating PAPP-A expression.⁶ The time course of stimulation by these cytokines also differs between ECs and SMCs. In ECs, there was no significant increase in PAPP-A expression until 24 h post-treatment, whereas, in SMCs, PAPP-A expression was evident at 4 hr, peaked (15–25-fold) at 8 h, with elevated expression (3- to 5-fold) retained at 24 h after stimulation.⁶ Furthermore, IL-6 is a potent stimulator of PAPP-A in SMCs,² but there was no effect of this cytokine in ECs. This was true even though IL-6 soluble receptor was also present. Along the same line, TGF- β , the most potent stimulator of PAPP-A in human bone cells,¹⁵ had no effect on PAPP-A expression in ECs or SMCs. These data indicate that regulation of PAPP-A expression by cytokines is cell-specific.

TNF- α -induced endothelial activation plays a critical role in atherogenesis. Activated ECs are characterized by increases in VCAM, ICAM, and MCP-1, which are important for recruitment of monocytes.^{8,16} In apolipoprotein E-knockout mice, VCAM and ICAM expression was localized to ECs in lesion-prone sites and preceded lesion formation.²¹ PAPP-A expression in ECs was associated with and followed VCAM, ICAM, and MCP-1 expression induced by TNF- α . As discussed below, activation appears to be necessary, but not sufficient, for PAPP-A expression in ECs.

The different time courses for cytokine-stimulation of PAPP-A expression in ECs and SMCs suggest different pathways of regulation in these two vascular cell types. In human fibroblasts, PAPP-A-regulated expression by TNF- α and IL-1 β was mediated by NF κ B activation and the JNK pathway.²² Although NF κ B activation may be involved in TNF- α -induced PAPP-A expression in ECs, the 24 h lag between stimulus and response suggests some intermediate factor/pathway. TNF- α has been shown to induce NF κ B-dependent inflammatory markers, including BMP-2 and VCAM, in human coronary artery ECs.¹⁸ In this study, direct addition of BMP-2 to ECs had no effect on PAPP-A expression. On the other hand, pretreatment with NAC, a potent anti-oxidant, inhibited TNF- α -induced PAPP-A expression. This suggests, but does not prove, a role for ROS in the regulation of PAPP-A expression. Interestingly, NAC did not block TNF- α -induced VCAM, ICAM, or MCP-1 expression, thus dissociating any direct effect of these molecules on PAPP-A expression. Interestingly, Marui et al.¹⁶ reported that activation of VCAM gene expression by TNF- α in human umbilical vein ECs was sensitive to inhibition by anti-oxidants but ICAM was not. These apparently discrepant results may be due to the different functional and regulatory properties that characterize ECs derived from different vascular beds. Further investigation will be necessary to determine signaling pathways mediating the stimulation of PAPP-A expression in ECs. LDL cholesterol is central to the development of atherosclerosis. Previous studies suggested that oxidized LDL increased

VCAM and MCP-1 expression in ECs, although this effect was strain-dependent in mice.²³ Khan et al.⁸ found that oxLDL alone had no effect, but that pretreatment with oxLDL increased VCAM expression in human aortic ECs. In our study, treatment of ECs with oxLDL had no effect on VCAM, ICAM, MCP-1, or PAPP-A expression without or with TNF- α . Blood vessel origin of the EC may play a role in response to oxLDL on adhesion molecule expression. In SMCs, LDL or oxLDL alone had no significant effect; however, LDL, but not oxLDL, potentiated TNF- α -stimulated PAPP-A expression. This could be due to the state of the LDL. Our “native” LDL underwent a small amount of oxidation during preparation, and it is known that minimally oxidized, moderately oxidized and highly oxidized LDL can have different and sometimes opposite effects. Furthermore, there is differential response in SMCs to oxLDL in different mouse strains.²⁴

We also looked at IGF-I, IGF-IR, IGFBP-4 and IGFBP-5 mRNA expression in ECs and SMCs in response to TNF- α and LDL treatments. We were especially interested in LDL. LDL and oxLDL significantly increased IGFBP-4 mRNA expression in ECs, evidence that the preparations used had biological effects. There was no significant effect of LDL or oxLDL on IGF-I, IGF-IR, IGFBP-4 or IGFBP-5 mRNA expression in SMCs. This was somewhat surprising since Scheidegger et al.¹⁰ reported that LDL increased IGF-I mRNA, oxLDL decreased IGF-I and IGF-IR mRNA, and both LDL and oxLDL increased IGFBP-4 in rat aortic SMCs. The differential responses could be due to differences in extent of oxidation, concentration of LDL, species, and/or vessel of origin. It is of note that our ECs and SMCs were derived from human coronary arteries to minimize these differences when comparing responses of ECs to SMCs.

So what might be the role of PAPP-A in ECs? These cells express abundant type I IGF receptors and IGFBP-4. Indeed, IGFBP-4 is the most highly expressed IGFBP in EC microenvironment.²⁵ PAPP-A increases IGF-I bioavailability for receptor activation through proteolysis of IGFBP-4.^{1,2,15} Therefore, ECs are positioned to respond to enhanced local IGF bioavailability. IGF-I does not appear to be a mitogen for ECs,^{26,27} but IGF-I has been shown to regulate migration and angiogenesis and enhance the inflammatory response in ECs.^{28–30}

Why would increased IGF-I bioavailability via PAPP-A promote the development of atherosclerotic plaque? Anwar et al.³¹ reported that TNF- α decreased IGF-I expression in vascular SMCs. Because IGF-I is important for growth and survival of SMCs, it was suggested that the TNF- α -decreased IGF-I played a role in acute coronary syndromes by decreasing SMC viability and promoting plaque instability. Our data indicate very low expression of IGF-I in SMCs and no effect of TNF- α . This could be due to different cell types and different times of analysis. Nonetheless, our work and that of Che et al.²⁶ would alternatively suggest that IGF-I augments proinflammatory events that are induced by TNF- α in ECs and SMCs and that promote atherogenesis. Further study will be necessary to test this model in vivo.

In summary, vascular cells relevant to human atherosclerotic plaque show differential regulation of PAPP-A expression. We propose a model in which activated macrophages in developing plaque secrete pro-inflammatory cytokines that differentially stimulate ECs and SMCs to express and secrete PAPP-A, which can act in an autocrine and paracrine manner to amplify atherosclerotic disease progression through its ability to enhance local IGF action.

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Figure 1. Time course of PAPP-A mRNA expression in ECs

ECs were treated without (Control) and with 10 nM IL-1 β (Grey hatched bars) or TNF- α (solid black bars) for the indicated times. Results are presented as PAPP-A mRNA expression relative to Control (set equal to 1).

*P < 0.05 vs Control.

Figure 2. Time course of adhesion molecule expression in ECs

ECs were treated without (Control) and with 10 nM IL-1 β (Grey hatched bars) or TNF- α (solid black bars) for the indicated times. Results are presented as VCAM (A), ICAM (B), and MCP-1 (C) mRNA expression relative to Control (set equal to 1).

*P < 0.05 vs Control.

Figure 3. Dose response of PAPP-A protein expression in ECs

ECs were treated with the indicated concentrations of IL-1 β (Grey hatched bars) or TNF- α (solid black bars) for 72 h. Results are presented as mIU/L PAPP-A. PAPP-A is significantly elevated compared to no cytokine treatment (0 nM) at all concentrations.

Figure 4. IGFBP-4 proteolysis

^{125}I -IGFBP-4 was incubated in conditioned medium from Control (C) and TNF- α -treated EC without (-) and with (+) IGF-II as described in Materials and Methods. Arrows indicate intact and fragmented IGFBP-4.

Figure 5. PAPP-A (A), VCAM (B), ICAM (C), and MCP-1 (D) mRNA expression in ECs

ECs were treated without (Control) and with BMP-2 (200 ng/ml), NAC (10 nM), TNF- α (10 nM) or pretreated for 60 min with NAC before TNF- α . Results are presented as copies mRNA per ng total RNA.

*P < 0.05 vs Control.

Figure 6. PAPP-A mRNA expression in SMCs: effect of LDL and oxLDL

ECs were treated without (Control) and with 60 μ g/ml LDL, 60 μ g/ml oxLDL or 1 nM TNF- α for 24 h. In addition, some cells were pretreated with LDL or oxLDL for 48 h and then treated with TNF- α . Results are presented as PAPP-A mRNA expression relative to Control (set equal to 1).

*P < 0.05 vs Control.

#P < 0.05 pretreatment effect.

Table 1

Human Primer sets	Product (bp)
PAPP-A F-CTCTCTCCCATGTCTAGCCTC R-CCCTTCTCCGCAATTCC	212
IGF-1 F-CTGCCAAGTCAGCTCGCTCTG R-CACTCCCTCTACTTGC GTTCTT	96
IGF-1R F-GAGGCTGAATACCGCAAAGT R-CGGGTCGGTGATGTTGTAGGTG	162
IGFBP-4 F-CTGCAGCCCTCTGACAAGGACG R-ACTGGTGCTCCGGTCTCGAAT	120
IGFBP-5 F-TGTGACCGCAAAGGATTCTACA R-GCAGCTTCATCCCGTACTTGT	100
VCAM-1 F-AGGCAGAGTACGCAAACA R-CACAGGATTTTCGGAGCA	143
ICAM-1 F-ACTAGGCCACGCATCT R-CAGCATACCCAATAGGCA	184
MCP-1 F-GACTAACCCAGAAACATCCAAT R-GAATGAAGGTGGCTGCTATGA	110
RPL19 F-AGCCAATGCCCGAATG R-CCATGAGAATCCGCTTGTT	167

Forward (F) and reverse (R) primer pairs.

Table 2

Expression of IGF System Components in ECs and SMCs.

	Control	Copies mRNA/ng total RNA TNF- α	oxLDL
<u>EC</u>			
<u>8 h</u>			
IGF-I	23 $\pm$ 2	56 $\pm$ 4 [*]	20 $\pm$ 4
IGF-IR	37,806 $\pm$ 333	28,549 $\pm$ 669 [*]	35,736 $\pm$ 4,089
IGFBP-4	9,237 $\pm$ 160	3,208 $\pm$ 124 [*]	8,978 $\pm$ 149
IGFBP-5	80 $\pm$ 6	16 $\pm$ 1 [*]	95 $\pm$ 26
<u>24 h</u>			
IGF-I	18 $\pm$ 5	23 $\pm$ 4	20 $\pm$ 3
IGF-IR	18,120 $\pm$ 1,239	27,054 $\pm$ 332	17,496 $\pm$ 589
IGFBP-4	5,956 $\pm$ 68	4,266 $\pm$ 529	7,165 $\pm$ 138 [*]
IGFBP-5	65 $\pm$ 8	106 $\pm$ 86	115 $\pm$ 15
<u>SMC</u>			
<u>8 h</u>			
IGF-I	123 $\pm$ 27	229 $\pm$ 171	79 $\pm$ 32
IGF-IR	658 $\pm$ 86	342 $\pm$ 64	430 $\pm$ 103
IGFBP-4	29,233 $\pm$ 1,329	18,195 $\pm$ 583 [*]	21,679 $\pm$ 4,579
IGFBP-5	1,187 $\pm$ 165	601 $\pm$ 134 [*]	887 $\pm$ 133
<u>24 h</u>			
IGF-I	63 $\pm$ 1	81 $\pm$ 24	82 $\pm$ 13
IGF-IR	503 $\pm$ 18	567 $\pm$ 16	679 $\pm$ 90
IGFBP-4	26,244 $\pm$ 5,304	18,731 $\pm$ 1,558	25,988 $\pm$ 1,201
IGFBP-5	1,766 $\pm$ 17	698 $\pm$ 62 [*]	1,691 $\pm$ 150

Human coronary artery ECs and SMCs were untreated (Control) or treated with TNF- α (10 nM) or oxLDL (60 μ g/ml) for 8 and 24 h. Results are expressed as copies of mRNA per ng total RNA.

* P < 0.05 vs Control.