Biochemical and Biophysical Research Communications **269,** 257–264 (2000)
doi:10.1006/bbrc.2000.2279, available online at http://www.idealibrary.com on IDEAL®

Effects of Shear Stress on Eicosanoid Gene Expression and Metabolite Production in Vascular Endothelium as Studied in a Novel Biomechanical Perfusion Model

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Received January 17, 2000

This study investigated the effects of shear stress on gene expression of prostacyclin synthesis-related enzymes cyclooxygenases (COX-1 and COX-2), prostacyclin synthase (PGS), and thromboxane synthase (TXS) and their metabolites prostaglandin (PGI_2) and thromboxane A_2 (TXA_2) in endothelium of intact conduit vessels. Paired human umbilical veins were perfused at high/low shear stress (25/<4 dyn/cm^2) at identical intraluminal pressure (20 mmHg) for 1.5, 3, or 6 hours in a computerized vascular model. High shear perfusion induced a significant, monophasic upregulation of PGS and TXS gene expressions after 6 hours. COX-1 and COX-2 mRNA showed a biphasic response with peaks at 1.5 and 6 hours, with a nadir level at 3 hours. Shear-induced gene expression was associated with a significantly greater accumulation of 6-keto prostaglandin $F_{1\alpha}$ and TXA_2 in the perfusion medium. Thus, shear stress independently of perfusion pressure alters the expression of prostacyclin synthesis-related enzymes and the biosynthesis of their vasoactive metabolites.

Key Words: **prostanoids; shear stress; gene expression; real-time PCR; mRNA.**

Prostaglandins are the major eicosanoids synthesized by vascular smooth muscle and endothelial cells, and the vasculature is an important target organ for their bioactive metabolites (1–3). Biomechanical forces acting on the vessel wall can modulate the production of eicosanoid metabolites (4–6). Fluid shear stress has been shown to dose and time-dependently regulate the secretion of PGI_2 and PGE_2 both in cultured endothelial cells, vascular smooth muscle cells, and osteoblasts (7–10). However, little is known about how shearing and tensile biomechanical forces interact, i.e. what effect shear stress has in the presence of physiological intraluminal pressure on the expression of genes encoding the various enzymes involved in the eicosanoid metabolic pathways. Unfortunately, characterization of such effects has been hampered by the difficulty in available experimental models to accurately measure and, thereby, simulate well-defined levels of fluid shear stress in a whole-vessel preparation. In fact, no physiologically relevant vascular model has been available by which biomechanical stimulation could be studied in intact conduit vessels with preserved cellular communication between vascular endothelial and smooth muscle cells.

We have recently developed a new, physiologically relevant vascular perfusion model, by means of which any combination of hemodynamic forces could be simulated *ex vivo* in intact human or animal vessels (11). This computerized perfusion model makes possible detailed studies of responses of the endothelium to predefined levels of shear stress. The present study was designed to investigate the temporal effects of shear stress on gene expression of the major eicosanoid enzymes (COX-1, COX-2, and prostacyclin and thromboxane synthases) and the resulting changes of secretion patterns of the two major metabolites prostaglandin I_2 and thromboxane A_2 in the present of physiological levels of intraluminal pressure.

MATERIALS AND METHODS

Preparation Procedure

Complete umbilical cords were obtained immediately after delivery from single, full-term vaginal deliveries (Department of Obstetrics, Sahlgrenska University Hospital/Östra, Göteborg). The umbilical vein was divided into two parts, one used for each circuit. Each perfused segment had a length of approximately 20 cm and both were carefully rinsed with PBS solution to remove any remaining blood. All experiments were run in parallel on vessel pairs, and the two vascular segments from the same umbilical cord were exposed to

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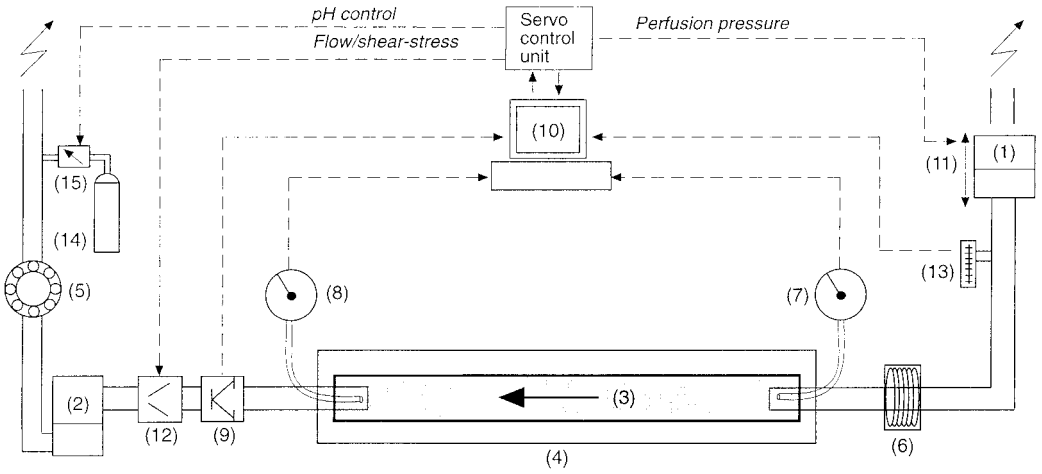

FIG. 1. Schematic of the computerized perfusion model. 1 = up-stream perfusion medium reservoir; 2 = down-stream reservoir; 3 = blood vessel; 4 = perfusion chamber; 5 = peristaltic pump; 6 = heat exchanger; 7 = up-stream pressure transducer; 8 = down-stream pressure transducer; 9 = electromagnetic flow meter detector; 10 = data acquisition computer; 11 = motor-driven height regulator; 12 = proportionating solenoid valve; 13 = pH meter; 14 = gas mixture; 15 = magnetic valve.

either high and low shear stress, respectively. In this way, each vessel served as its own control. All veins were perfused in the anterograde direction from the placental to the fetal end, i.e. in the same direction as *in vivo*.

The Biomechanical Perfusion System

The main components of the perfusion system are described below. A detailed technical description of the perfusion system has recently been given elsewhere (11). As shown in Fig. 1, organ chambers and the lower perfusion reservoirs were placed in a stainless steel waterbath kept at 37 ± 0.5°C by a circulation thermostat (Tempette Junior TE-8J, Labassco, Sweden). A heat exchanger (D720, Dideco Sweden AB, Sweden) was connected to the perfusion line immediately before each vessel to ensure a constant perfusate temperature of 37.0 ± 0.1°C. From the start each circuit contained a total of 500 mL Tyrode's saline solution as perfusion medium, supplemented with penicillin-streptomycin to prevent growth of bacteria in the system. The flow rate in each perfusion system was measured in-line by an electromagnetic flow meter detector (Toshiba Tosmac 334, Model 334, Frontech Processautomation AB, Sweden). Pressure catheters were inserted into the fetal and placental ends of the vessel through the up- and down-stream cannules of each vessel for measurement of intraluminal pressure. The perfusion medium was constantly bubbled with a controlled amount of gas mixture of 90% N_2, 5% O_2, and 5% CO_2 (AGA Gas AB, Sweden) to maintain target pH, pO_2, and pCO_2 throughout the experiment. A pH-electrode (Microprocessor pH-meter RE 357, Skafte & Claesson, Sweden) was immersed in each upper perfusion reservoir and kept within the physiological range of 7.2–7.4 by means of a feedback computer routine controlling the magnetic gas valves (Type 200, Bürkert Contromatic AB, Sweden).

Control and Regulation of the Perfusion System

To establish and maintain the target combinations of perfusion pressures, shear stress, and flow in each circuit, a fast data acquisition and control computer system was developed. In brief, readings from pressure transducers, and flow and pH meters were recorded and digitized through a data acquisition PCI-MIO-16XE-50 board and fed into a Macintosh Power PC 7600/120 MHz. The software was a custom-assembled program developed by our group in LABVIEW 4.0 (11). Digital output signals control perfusion pressure by regulating the height of the upper perfusion reservoir and flow rate by the aperture of the proportionating flow valve to generate and maintain pre-determined targets combination of mean perfusion pressure, flow rate, and/or shear stress. The software continuously monitors real-time perfusion pressure, mean perfusion pressure, pressure drop over the vessel, flow rate, pH values, shear stress, vascular resistance, and Reynolds number (to ensure laminar flow conditions). A hemodynamic validation of the system has recently been presented elsewhere (11).

Shear Loading

In the first series, 11 paired umbilical veins were exposed to high (target 25 dyn/cm^2) or low (target $<$4 dyn/cm^2) shear stress at a constant target mean perfusion pressure of 20 mm Hg for 6 hours. In the second and third series, 5 and 7 paired umbilical veins were exposed to high (target 25 dyn/cm^2) or low (target $<$4 dyn/cm^2) shear stress at the same level of perfusion pressure as above but for 3 and 1.5 hours, respectively. A summary of hemodynamic data is shown in Fig. 2. Samples of perfusion medium were collected after the 10 min washout period (baseline sample) through the ports and thereafter every 30 min during the remaining hours for measurement of prostanoid secretion. Perfusate samples were immediately frozen and stored at −70°C until analysis.

Reverse Transcription PCR

Isolation of total RNA and reverse transcription. Following perfusion, endothelial cells were isolated by incubating each vessel with 0.1% collagenase for 12 min at 37°C. Total RNA was extracted according to Chomczynski (12). Reverse transcription of 1 μg total RNA was carried out in a total volume of 20 μL reaction mixture.

Oligonucleotides for TaqMan PCR assay. Oligonucleotide primers and TaqMan probes were designed by using Primer Express version 1.0 (Perkin-Elmer Applied Biosystems Inc.), based on sequences from the GenBank database (Table 1). Constitutively expressed glyceraldehyde phospho-dehydrogenase (GAPDH) was selected as external endogenous control to correct for potential variation in RNA loading, cDNA synthesis, or efficiency of the amplification reaction. To verify that the amplification product was a target gene, the amplified RT-PCR product of one sample was sequenced. To ensure that GAPDH was not effected by shear stress all the samples were also related to a second endogenous control,

TABLE 1

Oligonucleotide Primers and Probes Used for TaqMan Real-Time Quantitative PCR

Gene	Oligonucleotide	Sequence	Position
COX-2 U04636	Sense primer	5′-C 5′-AGC CCT TCC TCC TGT GCC T-3′	2946–2964
	Antisense primer	5′-AAT CAG GAA GCT GCT TTT TAC CCT T-3′	2998–3000
			3426–3446
	Probe	5′-(FAM) ATG ATT GCC CGA CTC CCT TGG GTG T (TAMRA)p-3′	2966–2990
COX-1 M-31812	Sense primer	5′-GGA TGC CTT CTC TCG CCA G-3′	−1409–1426
	Antisense primer	5′-ATG TGG TGG TCC ATG TTC CTG-3′	1472–1492
	Probe	5′-(FAM) TTG CTG GCC GGA TCG GTG G (TAMRA)p-3′	1428–1438
			1460–1467
GAPDH J04038	Sense primer	5′-C 5′-CCA CAT CGC TCA GAC ACC AT-3′	1435–1454
	Antisense primer	5′-CCA GGC GCC CAA TAC G-3′	3123–3138
	Probe	5′-(FAM) AAG GTG AAG GTC GGA GTC AAC GGA TTT G (TAMRA)p-3′	1459–1486
PGS D-83393-83402	Sense primer	5′-TGC TTG ATA GCG TGC TGA GTG	2702–2707
			2728–2742
	Antisense primer	5′-TCC ACC ACA ACC TCG CG-3′	2778–2793
	Probe	5′-(FAM) TCA GGC TTA CAG CTG CCC CCT TCA TC (TAMRA)p-3′	2749–2774
TXA D-36075-36087	Sense primer	5′-CCT TAG GAG GCG GAG AGA CTT C-3′	2791–2796
			2947–2962
	Antisense primer	5′-GTC TTG CAC GCC CAT GG-3′	3051–3067
	Probe	5′-(FAM) AAA TGG TCC TGG ATG CCC GAC ATT C (TAMRA)p-3′	3017–3042

β-actin. No significant difference in the expression response of the target genes was observed when data were related to the alternative control gene (data not shown).

Quantitative real-time RT-PCR assay. Relative quantification of mRNAs were performed on an ABI Prism 7700 Sequence Detector (Perkin-Elmer Applied Biosystems Inc., Foster City, CA, USA) equipped with 96-well thermal cycler in the present of 5 pmol specific probe for each gene. The probes were labeled with a reporter dye (FAM; 6-carboxy-fluorescein; emission maximum 518 mm) at the 5′ end and a quencher dye (TAMRA; 6-carboxy-tetramethyl-rhodamine; emission maximum 582 nm) at the 3′ end via a linker arm nucleotide (LAN). Typically, PCR was carried out in a 50 μl mix containing; 1:8 of the cDNA templates, 1× TaqMan Buffer A, 5 mM $MgCl_2$, 0.2 mM dNTP mix (20 mM dUTP and 10 mM of dATP, dCTP, and dGTP), 1.25 U Taq Gold polymerase, 0.5 U AmpEraseUNG, and 15 pmol of both forward and reverse primers. The samples were sere subjected to 50 cycles of two-step PCR after denaturation at 95°C for 10 min. The AmpErase uracil-N-glycelase was activated before the denaturation step by heating the mix for 2 min at 52°C. Each cycle consisted of a 15 sec denaturation step and a 1 min combined annealing/extension step at 60°C. All samples were amplified simultaneously in triplicate in one assay run. Variation coefficients for C_T values for triplicate reactions were excellent (less than 2% for the gene products). PCR consumables were supplied by Perkin-Elmer Applied Biosystems Inc., Foster City, CA, USA.

The TaqMan chemistry. This method is based on the use of a fluorogenic probe designed to hybridize within the target sequence and to generate a signal that accumulates during PCR cycling in a manner proportional to the concentration of amplification products. Briefly, during PCR cycling, the probe first specifically hybridize to the corresponding template, and then it is cleaved via the 5′ → 3′ exonuclease activity of Taq DNA polymerase. This cleavage results in an increase of fluorescence emission of FAM reporter, without effecting the emmision of quencher dye. This sequence of events occurs in each PCR cycle, without interference in the enzymatic reaction and PCR product accumulation. Since the exonuclease activity of Taq polymerase acts only if the fluorogenic probe is annealed to the target and the enzyme can not hydrolyze with the probe when it is free in solution, the increase of fluorescence is proportional to the amount of the PCR product. By this principle the measurement of the fluorescence in each sample provides a homogenous signal which is specifically associated with the amplified target and quantitatively related to the amount of PCR.

A linear TaqMan amplification plot has the characteristic sigmoidal shape, Fig. 3. After a certain number of amplification cycles, probe cleavage elevates the reporter emission intensity above baseline. The subsequent exponential phase in the curve represents the logarithmic amplification of PCR product.

Collection and analysis of fluorescence emission data. The increase in fluorescence signal in each of the 96 wells are monitored in real time during PCR amplification by 7700 Sequence Detector (Perkin-Elmer Applied Biosystems Inc.) equipped with a CCD (Charge-Coupled device) camera for measuring the fluorescent emission spectra from 500–650 nm. Frosted MicroAmp Optical Tubes (part no. N801–O933, Perkin-Elmer) were used as reaction tubes and tube caps were specially designed to prevent light scattering. Reaction conditions were programmed on a Power Macintosh 7100 (Apple Computer) linked directly to the Model 7700 Sequence Detector.

Methodological validation. The quantity of total cellular RNA extracted from each vessel segment was similar in vessels exposed to high or low shear. Transcript levels of the endogenous control GAPDH were independent of shear stimulation. In addition, no effect of high shear stimulation on the control gene was observed when GAPDH mRNA levels were expressed relative to β-actin.

For quantification of gene expression, standard curves were prepared for both the targets and the endogenous reference in a series of two-fold dilutions from 1:1 to 1:32. For each experimental sample, the amount of target and endogenous reference was determined from the corresponding standard curve. Then, the target amount was divided by the endogenous reference amount to obtain a normalized target value. Log input amount was calculated according to the formula: $10^{\wedge}$((cell containing C_T value) − b)/m; where b = y-intercept of standard curve line, and m = slope of standard curve line, Fig. 3.

Biochemical assays. Prostanoid concentrations in the perfusion medium were determined by specific enzyme-immunoassays (EIA). The amount of prostacyclin was measured as the accumulation of the stable metabolite 6-keto-prostaglandin $F_{1\alpha}$ (RPN 221). TXA_2 was measured as the metabolite tromboxane B_2 (RPN 220). Both assays were purchased from Amersham Life Science, England. Samples were analyzed in duplicates and all samples from one paired experiment were analyzed within one assay-run.

To exclude any contamination of endotoxins in the system, samples were taken at five positions in the system (port before and after the vessel, perfusion fluid reservoir, perfusion pressure chamber, and waterbath) and analyzed for endotoxins at the Department of Microbiology, Göteborg University, Göteborg. No detectable endotoxin levels were found.

Statistical Analysis

Data are expressed as mean and standard error of the mean (SEM) unless otherwise stated. Parametric methods, ANOVA were used for evaluation of changes in response to the different perfusion conditions. For comparison of gene expression during the different stimulation conditions Student's t-test was used after logarithmic transformation of data. Significance tests were considered significant at $p < 0.05$ (two-tailed test).

RESULTS

Hemodynamics

Average intraluminal pressure, shear stress, and flow rate in the three perfusion series are shown in Fig. 2. Average shear stress levels in the 6-hour series were 25.7 ± 0.5 versus 2.3 ± 0.2 dyn/cm^2 in high and low shear circuits, respectively ($p < 0.0001$) (Fig. 3). The corresponding mean levels in the 3- and 1.5-hour series were 24.7 ± 0.1 versus 2.8 ± 0.1 dyn/cm^2 ($p < 0.0001$), and 24.5 ± 0.4 versus 3.6 ± 0.3 dyn/cm^2 ($p < 0.0001$), respectively. Mean perfusion pressure was maintained at 20 mmHg in both circuits in all perfusion series (high versus low shear circuits, ns throughout).

Expression of COX-1, COX-2, PGS, and TXS

COX-1, COX-2, PGS, and TXS are the key enzymes involved in the production of the prostanoids PGI_2 and TXA_2. As shown by real-time RT-PCR in Fig. 4, the COX-1 mRNA was significantly up-regulated already after 1.5 hours (146% $p < 0.05$) of high shear stimulation. The expression decreased to a level significantly below baseline (76% $p < 0.05$) after 3 hours. After 6 hours of perfusion, the mRNA level again increased significantly (120% $p < 0.04$).

A similar biphasic response pattern was observed for COX-2 mRNA. An upregulation was detected after 1.5 hours, which fell short of statistical significance (128% $p < 0.74$, ns). As with COX-1, a nadir was observed after three hours of high shear perfusion (71% $p < 0.04$). After 6 hours the expression of COX-2 increased significantly (130% $p < 0.03$), Fig. 4.

As shown in Fig. 4, PGS and TXS were expressed at similar levels during high and low shear perfusion both after 1.5 and 3 hours (ns throughout). Thereafter, the expression of both PGS and TXS was induced after 6 hours of high shear perfusion (150%, $p = 0.03$ and 200%, $p = 0.05$, respectively).

Prostaglandin Secretion during High and Low Shear-Stress Perfusions

Cumulative perfusate concentrations of eicosanoid metabolites during various shear conditions are shown in Fig. 5. The two metabolites accumulated significantly during the 6-hour perfusion period (ANOVA time effect, $p < 0.0001$ throughout).

Perfusion under high shear-stress conditions induced a significantly greater PGI_2 secretion as measured by the accumulation of the metabolite 6-keto prostaglandin $F_{1\alpha}$ than perfusion under low shear conditions (ANOVA condition × time interaction, $p = 0.001$). However, the production rate during the high shear condition was significantly higher only during the first 60 min of perfusion (contrast analysis, p =

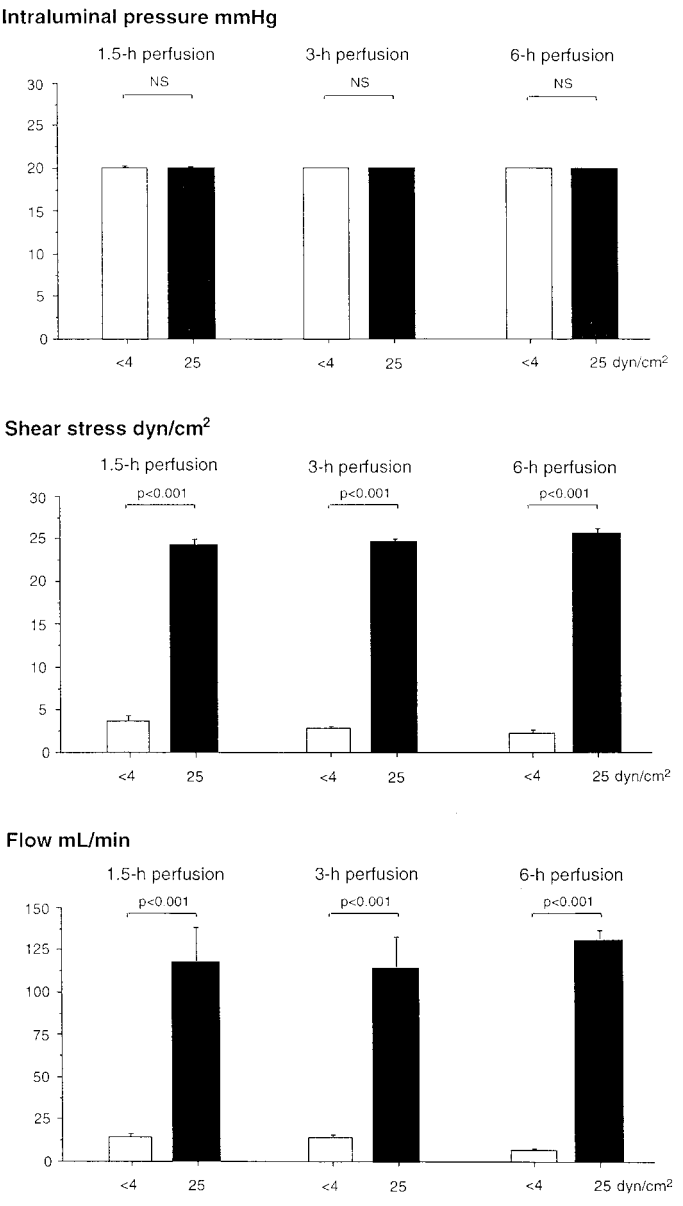

FIG. 2. Summary of hemodynamics during 1.5 (n = 5 paired vessels), 3 (n = 5), and 6 (n = 11) hours at high (target 25 dyn/cm^2) and low (<5 dyn/cm^2) shear stress. Mean intraluminal pressure was maintained at identical target levels (20 mmHg) in all experiments. Significance levels by t-test.

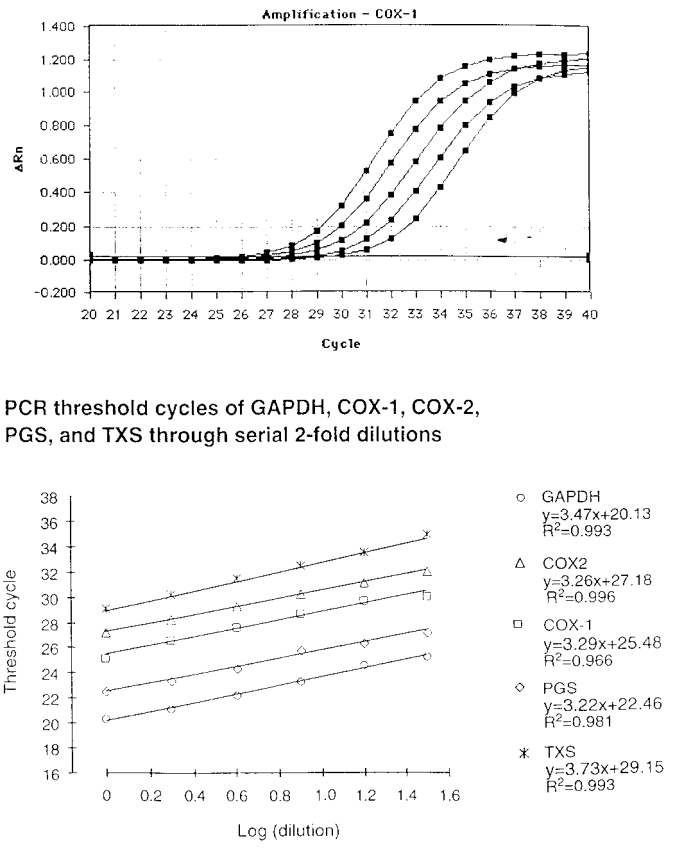

FIG. 3. Upper panel: Amplification plots show twofold serially diluted COX-1 cDNA. Total RNA from unstimulated endothelial cells was reverse-transcribed, and amplified using the TaqMan chemistry. Plot demonstrates the predicted gradual rightward shift of C_t values with each dilution step. Lower panel: Validation of amplification efficiency for COX-1, COX-2, PGS, TXS, and GAPDH. As indicated, their amplification efficiencies were similar for the target genes and the endogenous control throughout a series of twofold dilutions from 1:1 to 1:32.

0.0003), but thereafter PGI_2 production rates declined and leveled off to similar plateau levels in both systems.

Secretion patterns of TXA_2 were markedly different from that of PGI_2. High shear stress increased the production of TXA_2 as compared to the low shear condition (ANOVA condition $\times$ time interaction, $p = 0.0009$). The stimulatory effect of shear on the production rate of TXA_2 was evident both during the first (contrast analysis, $p = 0.001$) and the last three hours ($p = 0.0009$) of perfusion, whereas the production rate during the intermediate 2 hours was similar in both systems. The average production rate during high and low shear conditions was for PGI_2 (1.14 ± 0.18 vs 0.69 ± 0.12 pg/cm $\times$ min) and for TXA_2 (0.18 ± 0.04 and 0.13 ± 0.04 pg/cm $\times$ min). The increased production rate of the two prostanoids metabolites during high shear conditions was statistically significant ($p < 0.05$).

Prostaglandin Secretion in Deendothelialized Vessels

Accumulation of prostanoid metabolites was very low in denuded vessels perfused under high shear or high pressure conditions (data not shown).

DISCUSSION

The results of the present study show that shear stress independently of intraluminal pressure affects the gene expression of prostacyclin synthesis-related enzymes and modulate vascular eicosanoid formation. Taken together our data show that shear stress has significant and time-dependent stimulatory effects on the prostacyclin-related enzymes. To date, such combined effects of biomechanical forces have been difficult to explore on the level of a whole blood vessel, since available perfusion models have not permitted a distinct discrimination of shearing and tensile forces.

The pattern of shear-stimulated prostacyclin production was similar to what has previously been reported in human and experimental animal conduit vessels exposed to fluid flow (13–15). Our findings are also in accordance with findings in cell-culture systems (7). Regarding production rates, estimations of cell density by scanning electron microscopy revealed an average amount of 2×10^6 endothelial cells per cm umbilical vein. Based on this calculation, the average production rate of 6-keto-$PGF_{1\alpha}$ was approximated to 6.1 pg/min/10^6 cells during the first hour and 1.4 pg/min/10^6 cells during the subsequent two hours of high-shear perfusion. Production rates leveled off at approximately 0.5 pg/min/10^6 cells during the last three hours of perfusion. These levels are comparable to previously reported by Frangos *et al.*, who observed an initial production rate of about 3.7 pg/min/10^6 cells studied in human umbilical vein endothelial cells exposed to 10 dyn/cm^2 (9).

TXA_2 and PGI_2 are derived from the same precursor PGH_2. In line with the hypothesis, our results indicated that shear stress also stimulated the production of TXA_2 during the 6-hour perfusion period. Surprisingly, Okahara and his group recently reported that a shear stress-induced increase in PGI_2 production was not accompanied by the expected concomitant increase in TXA_2 in a cone-plate system (16). Therefore, they suggested that shear stress stimulates PGI_2 production without effecting TXA_2 formation. The disagreement between studies could be explained by the absence of pressure in *in vitro* systems. In the present experiments, TXA_2 showed a delayed shear-stimulated secretion response after 3 hours of perfusion in contrast to the rapid increase in PGI_2 production. A similar slow TXA_2 response has previously been observed in osteoblasts exposed to shear, which may be due to an increased *de novo* synthesis of enzyme(s) required for arachidonic acid metabolism (10).

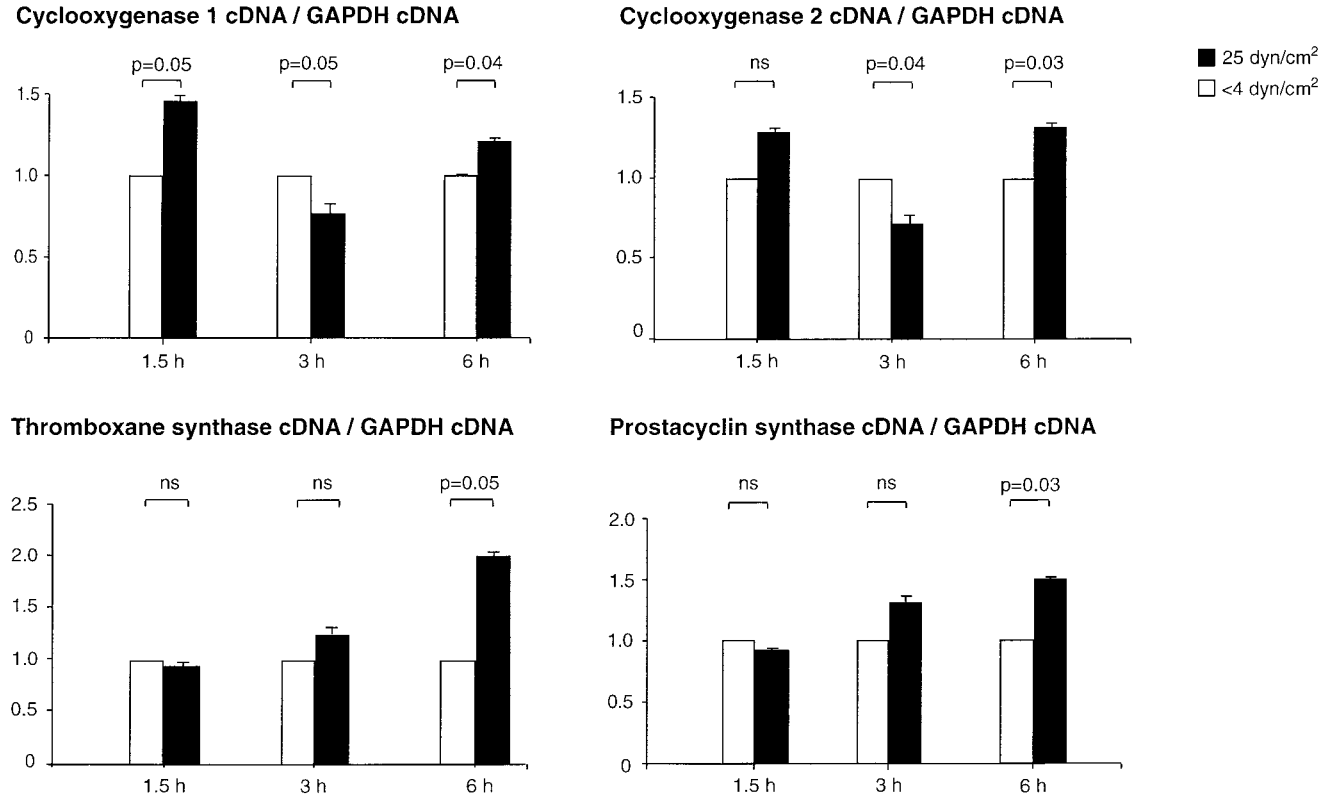

FIG. 4. Upper panel: Bar graphs show expression of COX-1 and COX-2 cDNA normalized to GAPDH for vessels exposed to high/low shear stress at identical levels of intraluminal pressure during 1.5 (n = 7 paired vessels), 3 (n = 5), and 6 (n = 11) hours of perfusion. Lower panel: Bar graphs show expression of PGS and TXS cDNA normalized to GAPDH in the three experimental series.

Several enzymes are involved in the formation of the prostanoid metabolites. COX-1 is considered to be constitutively expressed in most cell types, including endothelial cells. However, Wang and co-worker have recently shown that COX-1 contains the shear stress responsive element (SSRE), GGTCTC, in its promoter region (17). It has recently been shown that when cultured endothelial cells were exposed to high shear at the level of 24 dyn/cm^2 in a modified cone-plate viscometer the expression of COX-1 was upregulated after one hour and this increase lasted for 12 hours (16). The present results confirm the early effect of high shear on transcription of this enzyme, but we also detected a bi-phasic effect; after an initial up-regulation its expression decreased at 3 hours for a later induction at 6 hours.

COX-2 gene expression in the vascular endothelium is induced by several factors, including hemodynamic forces acting on the vessel wall. Topper *et al.* used an *in vitro* model of monolayers of HUVECs exposed to 10 dyn/cm^2 of laminal shear stress (18, 19). The response of COX-2 gene expression was biphasic, with the first and second peaks occurring after one and six hours of stimulation, respectively. Okahara and co-workers reported that shear stress increased COX-2 mRNA after one hour with a peak response at six hours. In a previous study we have shown that shear increases the expression of COX-2 both at the transcriptional and translation levels (20), which is in accordance with the present observations. Also, they are in line with findings by Okahara with the exception that similar to COX-1, the transcription of COX-2 was suppressed after 3 hours of shear stimulation.

Prostacyclin synthase catalyzes the conversion of prostaglandin H_2 to prostaglandin I_2. PGS is also reported to contain a shear stress-responsive element in its promoter region (21). The present study shows that prostaglandin synthesis was induced after six-hour perfusion under high-shear conditions, while there was only a non-significant induction after 3 hours, and no effect at all after 15 hours. Prostaglandin H_2 is also a substrate for thromboxane synthase, which produces thromboxane A_2. In this study we have shown that thromboxane synthase gene expression is upregulated by shear stress in the same pattern as was observed for prostacyclin synthase.

In the model we describe a novel theoretical approach to the calculation of shear stress made possible by continuous on-line measurement of wall shear (11). Compared with previous systems (22–26), the major advantage is the possibility to regulate shear stress independently of pressure or fluid flow. Furthermore,

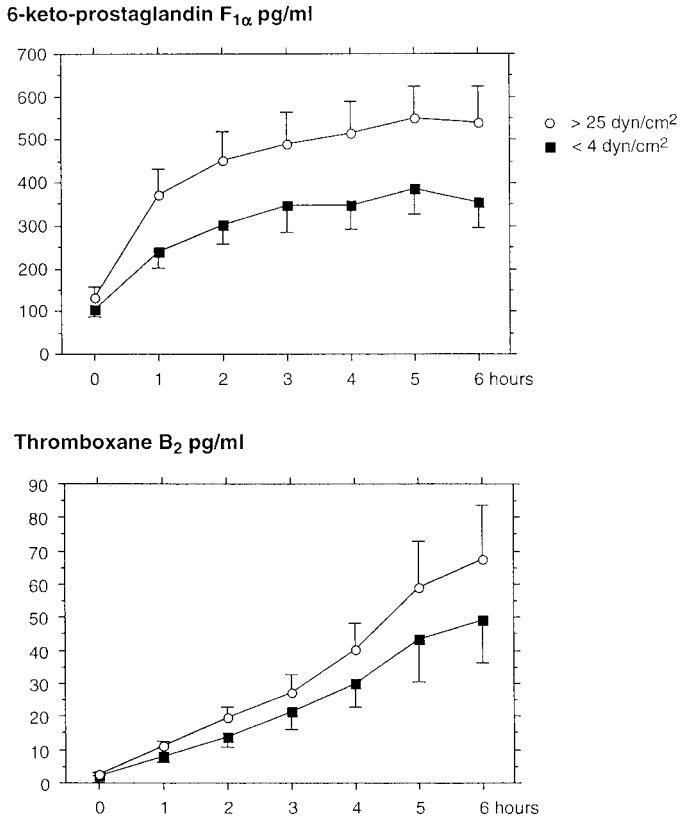

FIG. 5. Cumulative perfusate concentrations of eicosanoid metabolites during high (target 25 dyn/cm^2; open circles) and low (< 5 dyn/cm^2; closed squares) shear conditions. The two metabolites accumulated significantly during the 6-hour perfusion period (ANOVA time effect, $p < 0.0001$). High shear stress conditions induced a significantly greater PGI_2 production than perfusion under low shear conditions (ANOVA condition × time interaction $p = 0.001$). High shear stress also increased the production TXA_2 ($p = 0.0009$).

in comparison with cell culture devices, studies are performed with a preserved relation between endothelial and vascular smooth muscle cells. Use of intact vessels is physiologically relevant, since its mechanoelastic properties determine the deformation forces taken up by the cells in response to mechanical stimulation. Our study was performed in human umbilical vessels to facilitate comparison with previous studies using this type of vessel (23, 26) or cultured human umbilical vein endothelial cells (HUVEC) (27–29). However, the model is generally applicable to a variety of human or animal conduit vessels, provided that there is a large enough pressure drop across the segment to permit calculation of shear stress. With a modified approach to shear calculation, the model can be adapted for microvascular studies.

In conclusion, the present study is the first to investigate the effects of defined shear in combination with a physiological intraluminal pressure in an intact conduit vessel on gene expression of eicosanoid enzymes and production of their major metabolites. Shear stress independently of pressure modulated the gene expression of four important prostacyclin synthesis-related enzymes and formation of the two important vascular eicosanoid metabolites PGI_2 and TXA_2. The release pattern of PGI_2 differed substantially from that of thromboxane A_2, which suggests that these pathways are, at least partly, differentially regulated. The new, computerized biomechanical perfusion system we have developed may be a useful tool to elucidate specific effects of various biomechanical forces on intact mammalian conduit vessels.

ACKNOWLEDGMENTS

The authors thank laboratory technicians Hannele Korhonen and Camilla Ejdestig for excellent technical assistance throughout the study. The cooperation of the midwives and nurses at the Department of Obstetrics, Sahlgrenska University Hospital/Östra, Göteborg for collecting the umbilical cords is gratefully acknowledged.

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