Tissue factor-dependent pathway is not involved in exercise-induced formation of thrombin and fibrin

CLAUS WEISS,1 ANGELIKA BIERHAUS,2 RALF KINSCHERF,3 VOLKER HACK,4 THOMAS LUTHER,5 PETER PAUL NAWROTH,2 AND PETER BÄRTSCH1
1Department of Internal Medicine VII/Sportsmedicine, University Hospital, 69115 Heidelberg; 2Section of Vascular Medicine, Department of Medicine IV, University Hospital, 72076 Tübingen, Germany; 3Department of Anatomy and Cell Biology III, University of Heidelberg, 69120 Heidelberg; 4Division of Immunoochemistry, German Cancer Research Institute, 69120 Heidelberg; and 5Department of Pathology, Technical University of Dresden, 01307 Dresden, Germany

Received 24 April 2001; accepted in final form 10 September 2001

Weiss, Claus, Angelika Bierhaus, Ralf Kinscherf, Volker Hack, Thomas Luther, Peter Paul Nawroth, and Peter Bärtsch. Tissue factor-dependent pathway is not involved in exercise-induced formation of thrombin and fibrin. J Appl Physiol 92: 211–218, 2002.—In healthy individuals, prolonged intensive physical exercise leads to an activation of blood coagulation that results in the formation of thrombin and fibrin. This study investigated whether oxidative stress during intensive physical exercise induces tissue factor (TF) via activation of the redox-responsive transcription factor nuclear factor-κB (NF-κB). Twelve young men performed a standardized 1-h maximal run on a treadmill that gave rise to significant increases of markers of thrombin and fibrin formation. The ratio of intracellular reduced to oxidized glutathione as measured by HPLC decreased from 23.3 ± 10.7 to 14.2 ± 6.5 (P < 0.05), indicating the generation of free radicals during exercise. Electrophoretic mobility shift assays from nuclear extracts of peripheral blood mononuclear cells revealed that exercise testing increased NF-κB (p50/p65) binding activity to a NF-κB consensus sequence by 105 ± 68% (P < 0.01) but did not affect NF-κB (p65/c-Rel) binding to a nonconsensus-κB-like site present in the TF promoter. Consistently, there was no exercise-induced increase in TF expression as demonstrated by TF-specific immunofluorescence staining and ELISA. Thus selective activation of NF-κB (p50/p65) during intensive physical exercise does not result in the expression of TF, suggesting that the TF-dependent pathway in peripheral blood mononuclear cells does not account for exercise-induced formation of thrombin and fibrin.

nuclear factor-κB; glutathione; factor VII; hemostasis

PHYSICAL EXERCISE GIVES RISE to an activation of the coagulation system that results in the formation of thrombin and fibrin when exercise is prolonged and exhaustive (10, 22, 38). The mechanisms by which exercise activates hemostasis are unknown. The release of catecholamines and the considerable increase in shear stress might be relevant in particular for the activation of platelets with exercise (9, 34). With respect to exercise-induced thrombin (and fibrin) formation, the increase of factor VIII concentrations (4) and shortening of activated partial thromboplastin time (13, 18) after exercise suggest the intrinsic pathway of coagulation to be involved. This study addressed the question of whether the tissue factor (TF)-dependent (extrinsic) pathway plays a role in the activation of coagulation with exercise. We hypothesized that oxidative stress during intensive physical exercise might lead to activation of the redox-sensitive transcription factor nuclear factor-κB (NF-κB), which has been demonstrated to control inducible TF transcription in mononuclear and endothelial cells (8, 27).

Therefore, we subjected 12 healthy young men to a 1-h maximal run on a treadmill at a standardized intensity corresponding to the anaerobic threshold (33). As shown earlier, this exercise protocol, reflecting very heavy exercise, gives rise to significant formation of thrombin and fibrin that does not result from prolonged exercise at only moderate intensity (38). The expression of TF was investigated on the mRNA and the protein level before and in intervals after exercise. Binding activity of NF-κB was assessed by electrophoretic mobility shift assays (EMSA) and the TF mRNA transcription by reverse transcription-polymerase chain reaction (RT-PCR). Synthesis of TF was analyzed by flow cytometry as well as by measurements of cellular and plasmatic concentrations by using specific ELISAs. As the major cellular antioxidant system, we assessed the intracellular glutathione status, measuring concentrations of reduced and oxidized glutathione by means of high-pressure liquid chromatography (HPLC) in monocytes.

METHODS

Subjects

Twelve healthy, endurance-trained young men (age 24 ± 5 yr) were studied. Physical examination, electrocardiogram, and routine laboratory parameters revealed no pathological

http://www.jap.org

8750-7587/02 $5.00 Copyright © 2002 the American Physiological Society

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
findings, and there was no evidence of thromboembolic disease in their personal and family history. All subjects were nonsmokers and were not allowed to take any medication 2 wk before exercise testing.

The study protocol was approved by the ethical committee for human studies of the Medical Faculty at the University of Heidelberg. All participants gave their written, informed consent before entering the study.

**Exercise Testing**

Incremental graded exercise testing. To assess the individual performance status, participants were subjected to an incremental graded exercise test on a treadmill (slope 1.5%) starting at an initial velocity of 8 km/h with increments of 2 km/h every 3 min until subjective exhaustion. Maximal oxygen consumption ($V_{O_2}\text{max}$) was measured by means of a metabolic cart (Oxycon gamma, Mijnhardt, Bunnik, The Netherlands) applying an open-circuit method. The individual anaerobic threshold was determined as previously described (33) and corresponded to 75–80% of $V_{O_2}\text{max}$.

One-hour exercise tests. Within 2 wk after determination of $V_{O_2}\text{max}$, subjects performed a 1-h run on a treadmill at a standardized velocity corresponding to the anaerobic threshold. Heart rate was recorded continuously by a sport tester (Polar Electro, Kempele, Finland), and plasma lactate was measured every 15 min during exercise to document that exercise intensity during the 1-h run was maintained at the anaerobic threshold. According to the recommendations of the American College of Sports Medicine (3), classification of exercise intensity on the basis of percentage of maximal heart rate (94% in this study) indicates a “very heavy” exercise intensity to which our subjects were exposed. All exercise tests started between 9:00 and 10:00 AM. Anthropometric data and characteristics of exercise testing are summarized in Table 1.

**Blood Sampling**

Venous blood samples were collected before and immediately after exercise as well as after 3, 6, and 24 h of recovery.

![Table 1. Anthropometric data and characteristics of exercise testing in 12 healthy young men subjected to a 1-h maximal run on a treadmill at a standardized velocity corresponding to the anaerobic threshold (33)](http://jap.physiology.org/)

<table>
<thead>
<tr>
<th>n</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.2 ± 1.5</td>
</tr>
</tbody>
</table>

Incremental graded exercise testing

- $HR_{\text{max}}$, 1/min | 190 ± 12
- $HR_{\text{AT}}$, % $HR_{\text{max}}$ | 88 ± 4
- Lactate$_{\text{AT}}$, mmol/l | 2.8 ± 0.7
- $V_{O_2}\text{max}$, ml·kg$^{-1}$·min$^{-1}$ | 68.1 ± 4.7

1-h Run

- Mean HR, % $HR_{\text{max}}$ | 94 ± 3
- $HR_{15}$, % $HR_{\text{max}}$ | 89 ± 3
- $HR_{60}$, % $HR_{\text{max}}$ | 96 ± 3
- Mean lactate, mmol/l | 3.2 ± 0.9
- Lactate$_{15}$, mmol/l | 3.0 ± 0.9
- Lactate$_{60}$, mmol/l | 3.3 ± 1.3
- Plasma volume change$^a$, % | $-6.9 ± 6.3$

Values are means ± SD. $HR$, heart rate; $HR_{\text{max}}$, maximal $HR$; subscript AT indicates measurements at the anaerobic threshold (33); subscript 15 and 60: HR/lactate after 15 and 60 min, respectively, of the 1-h run; $V_{O_2}\text{max}$, maximal oxygen consumption. $^a$ According to Dill and Costill (16).

Except for sampling immediately after exercise, all blood samples were drawn after subjects had rested for 30 min in a supine position. To minimize artificial activation of blood coagulation in vitro, a clean venipuncture (19-gauge needle) was performed from an antecubital vein under controlled venous stasis of 45 Torr by use of the Sarstedt system (Sarstedt, Nümbrecht, Germany).

**Determination of Hemostatic Variables**

Blood samples for hemostatic variables were collected first in the following sequence: 1) venous blood (4.5 ml) was added to 0.5 ml anticoagulant [stock solution containing 25 ml of citrate-theophylline-adenosine-dipyradimate (Becton Dickinson, Rutherford, NJ) plus 5 mg of phenyl prolyl arginine-chloromethylketone (Calbiochem, La Jolla, CA) giving a phenyl prolyl arginine-chloromethylketone concentration of 382 μM] for measurement of prothrombin fragment 1+2 (PTF1+2; Enzygnost-F1+2, Behring, Marburg, Germany), thrombin-antithrombin III complexes (TAT; Enzygnost-TAT, Behring), and fibrinopeptide A (FPA; radioimmuno reagents supplied by Imco, Stockholm, Sweden). The procedure of the latter assay has been reported in detail previously (22). 2) Blood (4.5 ml) was added to 0.5 ml of 0.106 M trisodium citrate for determination of factor VII concentration (FVII:Ag; Asserachrom VII: Ag, Diagnostica Stago, Asnières-sur-Seine, France), of factor VII clotting activity (FVII:ct) by a one-stage clotting assay using factor VII-deficient plasma and the prothrombin time reagent Thromborel S (Behring), and of circulating activated factor VII in plasma (Staclot VIIa-RTF, Diagnostica Stago).

Samples were rapidly put into melting crushed ice for 10 min and thereafter centrifuged at 4°C for 30 min at 2,000 g. Multiple aliquots of plasma were snap-frozen in liquid nitrogen and stored at −80°C until analysis. Changes in plasma volume were calculated according to the method of Dill and Costill (16). The results for proteins with a molecular weight >30,000 (21), i.e., all except for FPA, were corrected for changes of plasma volume occurring during and after exercise by the following factor: (100 + $\Delta$PV)/100, where $\Delta$PV is the change of plasma volume given in percent.

EMSAs

Peripheral blood mononuclear cells (PBMC) were separated from whole blood anticoagulated with 3.8% sodium citrate by density-gradient centrifugation on Ficoll Paque (Pharmacia, Freiburg, Germany). Binding activity of NF-κB was determined in nuclear extracts of PBMC by means of EMSA as described (23). Nuclear extracts of PBMC were harvested by the method of Andrews and Faller (5): 1 × 10$^6$ PBMC were lysed in 200 μl of cold buffer A (10 mM HEPES-KOH, pH 7.9 at 4°C, 1.5 mM MgCl$_2$, 10 mM KCl, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride) and incubated for 10 min on ice. Cells were centrifuged for 10 s at 15,000 rpm, and the supernatant was discarded. The pellet was resuspended in 100 μl of cold buffer C (20 mM HEPES-KOH, pH 7.9 at 4°C, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl$_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride) incubated for 20 min on ice, and centrifuged for 2 min at 15,000 rpm. The supernatant was quick-frozen at −80°C. Protein concentrations were determined by using a colorimetric assay (Stratagene, Heidelberg, Germany). Oligonucleotides were end labeled with [γ-32P]ATP using T4 polynucleotide kinase, and EMSA was performed as described (23).
to a specific activity $>5 \times 10^9$ cpm/µg DNA: NF-κB consensus: 5'-AGTTGAGGGACCTTTCCAGGC-3', TF-κB-like site: 5'-AGGGTCCCCGACCTTTCTTACCAGGA-3'. Binding of NF-κB was performed in 10 mM HEPES, pH 7.5, containing 0.5 mM EDTA, 100 mM KCl, 2 mM dithiothreitol, 2% glycerol, 4% Ficoll 400, 0.25% Nonidet P-40, 1 mg/ml BSA (DNase-free), and 0.1 µg/µl poly(dI/dC) in a total of 20 µl as described elsewhere (11). Nuclear extract (10 µg) was incubated for 20 min at room temperature in binding buffer in the presence of ~1 ng labeled oligonucleotide (~50,000 cpm (Cerenkov)).

Protein-DNA complexes were separated from the free DNA probe by electrophoresis through 5% native polyacrylamide gels containing 2.5% glycerol and 0.5 x Tris-borate-EDTA buffer. Gels were dried under vacuum on Whatmann DE-81 paper and exposed for 12-48 h to Amersham Hyperfilms at ~80°C with intensifying screens. Specific binding was ascertained by competition with a 160-fold molar excess of cold consensus oligonucleotides.

Assessment of the Glutathione Status in Monocytes

Monocytes were fractionated from PBMC by adherence to tissue culture plates (Greiner, Frickenhausen, Germany). After incubation at 37°C for 1 h, nonadherent lymphocytes were removed, yielding a cell preparation containing 90–95% monocytes (19) as evidenced by Pappenheim staining and fluorescence-activated cell sorting analysis. Proteins of adherent monocytes were precipitated by adding 40 µl of a solution of 5% sulfosalicyclic acid and 50 µM dithioerythritol before the cell pellet was harvested by means of a cell scraper. Intracellular reduced and oxidized glutathione concentrations were measured as previously described (28). Briefly, after derivatization with monobromobimane, the thiol-bimane adducts were quantified by reversed-phase ion-pair liquid chromatography and fluorescence detection. Reduced glutathione was used as a standard.

Determination of TF-mRNA by RT-PCR

RNA was extracted from 1 x 10^6 PBMC by using Trizol LS reagent (Life Technologies, Eggenstein, Germany) according to the manufacturer’s specifications. RT-PCR for TF and β-microglobulin as a positive control were performed basically as described by Potgens et al. (31). First-strand cDNA was synthesized with poly dT24 primer and 400 units of Superscript II transcriptase by the manufacturer’s specifications. RT-PCR for TF and NF-κB were performed in 10 mM HEPES, pH 7.5, containing 0.5 mM EDTA, 100 mM KCl, 2 mM dithiothreitol, 2% glycerol, 4% Ficoll 400, 0.25% Nonidet P-40, 1 mg/ml BSA (DNase-free), and 0.1 µg/µl poly(dI/dC) in a total of 20 µl as described elsewhere (11). Nuclear extract (10 µg) was incubated for 20 min at room temperature in binding buffer in the presence of ~1 ng labeled oligonucleotide (~50,000 cpm (Cerenkov)).

Protein-DNA complexes were separated from the free DNA probe by electrophoresis through 5% native polyacrylamide gels containing 2.5% glycerol and 0.5 x Tris-borate-EDTA buffer. Gels were dried under vacuum on Whatmann DE-81 paper and exposed for 12-48 h to Amersham Hyperfilms at ~80°C with intensifying screens. Specific binding was ascertained by competition with a 160-fold molar excess of cold consensus oligonucleotides.

Assessment of the Glutathione Status in Monocytes

Monocytes were fractionated from PBMC by adherence to tissue culture plates (Greiner, Frickenhausen, Germany). After incubation at 37°C for 1 h, nonadherent lymphocytes were removed, yielding a cell preparation containing 90–95% monocytes (19) as evidenced by Pappenheim staining and fluorescence-activated cell sorting analysis. Proteins of adherent monocytes were precipitated by adding 40 µl of a solution of 5% sulfosalicyclic acid and 50 µM dithioerythritol before the cell pellet was harvested by means of a cell scraper. Intracellular reduced and oxidized glutathione concentrations were measured as previously described (28). Briefly, after derivatization with monobromobimane, the thiol-bimane adducts were quantified by reversed-phase ion-pair liquid chromatography and fluorescence detection. Reduced glutathione was used as a standard.

Determination of TF-mRNA by RT-PCR

RNA was extracted from 1 x 10^6 PBMC by using Trizol LS reagent (Life Technologies, Eggenstein, Germany) according to the manufacturer’s specifications. RT-PCR for TF and β-microglobulin as a positive control were performed basically as described by Potgens et al. (31). First-strand cDNA was synthesized with poly dT24 primer and 400 units of Superscript II (Life Technologies) in a 40-µl reaction volume for 2 h at 45°C. For PCR, 1 µl of cDNA was used in a 20-µl master mix containing 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 2 mM MgCl2, 0.2 mM each dNTP, 1 U Taq DNA polymerase (Boehringer-Mannheim, Mannheim, Germany), and 10 pmol each of forward and reverse primer. Sequences of intron-spanning TF-specific primers were sense 5'-AGTGACGAACTTGTGACTTTGTCATC-3' and antisense 5'-CGATGACGTTTACGATATAACGTGAC-3' (Pharmacia Biotech, Roosendaal, The Netherlands), generating a 282-bp fragment. Amplification of part of the microglobulin gene was used as a positive control. Sequences of primers were sense 5'-CTCGCGCTACTCTCTCTTTCT-3' and antisense 5'-CTGGCAATGAG-3'. After initial denaturation at 94°C for 2 min, the following PCR profile was performed on a Genius thermal cycler (Techne, Cambridge, UK): 1 x (95°C, 300 s; 62°C, 90 s; 72°C, 240 s); 33 x (95°C, 60 s; 62°C, 90 s; 72°C, 240 s); and 1 x (95°C, 60 s; 62°C, 90 s, 72°C, 420 s). Amplification products were separated on a 1.5% agarose gel and stained with ethidium bromide.

Detection of TF by Flow Cytometry

TF expression on monocytes was examined by double immunofluorescence staining. Samples of 100 µl of heparinized whole blood were incubated for 15 min at room temperature in the dark with a murine FITC-conjugated monoclonal antibody against human TF (American Diagnostica, Greenwich, CT). Monocytes were identified by staining with a phycoerythrin-labeled mouse anti-human CD14 antibody (Becton Dickinson, Heidelberg, Germany). After incubation and washing of cells in PBS, erythrocytes were lysed (Optiprep, Becton Dickinson), and stained cells were fixed and stored at 4°C until measurement. Immunofluorescence analysis was performed within 4 h by flow cytometry on a FACScan using the LYSIS II software (Beckton Dickinson).

Measurement of Cellular and Plasmatic TF Concentrations

Cellular concentrations of TF were determined from PBMC (1 x 10^6) as described by Albrecht et al. (1). In brief, cell pellets were disrupted by repeated freezing and thawing, and TF was extracted by incubation with 60 µl of 0.05 M Tris·HCl, 0.1 M NaCl, 0.1% Triton X-100, pH 7.6, containing 5 mM EDTA for 30 min at 37°C. Cellular and plasmatic TF concentrations were determined by a sandwich ELISA using the antibody VIC12 as capture antibody and peroxidase-conjugated VIC7 as the detector antibody.

Statistical Analysis

Main effects of exercise were analyzed by nonparametric analysis of variance (Friedman). The Wilcoxon test was used for post hoc testing to examine the difference between values at baseline and those obtained after exercise. For these four multiple comparisons, a Bonferroni adjustment was made multiplying a particular P value by 4. The level of statistical significance was set at P < 0.05 (two-sided). Results are reported as means ± SE unless otherwise stated.

RESULTS

Exercise-Induced Effects on Hemostatic Variables

One-hour running at maximal intensity resulted in the formation of thrombin and fibrin as indicated by significant increases of plasma levels of PTF 1+2 from 0.71 ± 0.05 to 0.95 ± 0.07 nmol/l (means ± SE) (P < 0.05), of TAT from 1.0 ± 0.1 to 3.2 ± 0.5 ng/ml (P = 0.01), and of FPA from 0.8 ± 0.1 to 1.9 ± 0.1 ng/ml (P < 0.01). Three hours after exercise, concentrations of TAT and FPA returned to baseline levels, whereas plasma levels of PTF 1+2 were still elevated at this time (Fig. 1).

Effects of Exercise on the Glutathione Status in Monocytes

Intracellular concentrations of total glutathione in monocytes were not significantly affected by the 1-h run. Exercise testing led to a significant decrease of reduced glutathione (GSH) by 20% on average (P < 0.05) accompanied by an apparent increase of oxidized glutathione (GSSG), which, however, was not statistically significant. The ratio GSH/GSSG was diminished by 32% on average immediately after exercise testing (P = 0.03), thus reflecting the generation of free radicals during exercise (Fig. 2).

Activation of NF-κB With Exercise

To investigate the effects of exercise on the transcription factor NF-κB, EMSAs were performed with nuclear extracts of PBMC isolated before and in intervals
after the 1-h run. In all individuals tested, exercise gave rise to a significant increase in NF-κB (p50/p65) binding activity to a NF-κB consensus sequence (Fig. 3). Densitometric analysis confirmed a 100% increase in binding activity during exercise that could still be detected 6 h after exercise but returned to baseline levels within 24 h (Fig. 3, left). A representative EMSA from one proband is shown on the right side of Fig. 3. When using the same nuclear extracts, however, no increase in NF-κB binding activity was detected at the nonconsensus-κB-like site in the TF promoter, known to selectively bind NF-κB p65/c-Rel-heterodimers (Fig. 4). This implicates that physical exercise might not induce significant formation of p65/c-Rel heterodimers and therefore might not result in an increased expression of TF.

Exercise-Induced Effects on the Expression of TF

Consistent with the lack of inducible NF-κB binding activity at the nonconsensus-κB-like site in the TF promoter, TF-specific RT-PCR demonstrated no induction of TF transcription after exercise (Fig. 5). Expression of TF was also not affected by exercise, as shown by measurements of cellular and plasmatic TF antigen by immunofluorescence staining and ELISA. In 6 of 12 subjects, trace amounts of TF were detectable in plasma (detection limit of the assay: 80 pg/ml), which, however, did not change after exercise (Table 2).

Effects of Exercise on Factor VII

Exercise testing had no influence on FVII concentrations (FVII:Ag), whereas FVII activity (FVII:c) was
slightly but significantly decreased immediately after exercise ($P < 0.05$). However, the ratio of FVII:c to FVII:Ag and plasma concentrations of circulating activated FVII remained essentially unchanged after exercise (Table 2).

**Correlations Between the Glutathione Status, NF-κB, and the Hemostatic Variables**

A significant correlation between the glutathione status (total GSH, GSH, GSSG, GSH/GSSG) and the...
activation of NF-κB (p50/p65) could not be established, either for absolute values or for exercise-induced changes (Δ values = difference between pre- and post-exercise values). There was also no significant correlation between the glutathione status and the hemostatic variables, i.e., PT, FVII, TAT, and FPA (absolute and Δ values). Likewise, binding activity of NF-κB (p50/p65) did not correlate with hemostatic variables (absolute and Δ values).

DISCUSSION

The data demonstrate that 1) intensive physical exercise gives rise to a considerable activation of the transcription factor NF-κB (p50/p65); 2) exercise-induced activation of NF-κB (p50/p65) is accompanied by a decrease of the ratio between intracellular reduced and oxidized glutathione (GSH/GSSG), indicating the generation of reactive oxygen species (ROS) during exercise; 3) the activation of NF-κB (p50/p65) does not induce the expression of TF, either on the transcriptional (TF mRNA) or on the protein level; and 4) accordingly, exercise does not lead to an activation of factor VII. Taken together, this study has shown that the TF-dependent pathway in PBMC does not account for activation of coagulation with exercise.

ROS are produced in the course of the normal energy metabolism, and it is postulated that they are generated to a greater extent during intensive physical exercise, when oxygen consumption is elevated 10- to 15-fold above resting levels (2). Assessing the intracellular glutathione status as the major cellular antioxidant system, we found an exercise-induced decrease of the GSG-to-GSSG ratio in monocytes, demonstrating that the exercise test performed, i.e., a 1-h maximal run at the anaerobic threshold, led to the generation of free radicals. This finding is in accordance with former studies on the effects of intensive physical exercise on glutathione status, although these studies have applied whole blood assays that reflect erythrocytes predominantly (20, 35).

The 1-h run at the anaerobic threshold gave rise to the formation of thrombin and fibrin, as indicated by significant increases of plasma levels of prothrombin fragment 1+2, TAT, and FPA. Whether this exercise-induced activation at the final steps of the coagulation cascade is initiated by the intrinsic or the extrinsic pathway of coagulation is unknown. The extrinsic pathway is activated by TF, which is constitutively expressed by extravascular cells and inducibly expressed on monocytes and epithelial cells within the vasculature (27). On the transcriptional level, TF is regulated in part by members of the NF-κB family. It has been shown that ROS mediate the release of NF-κB from its cytoplasmatic inhibitor IκB, thus promoting the translocation of NF-κB into the nucleus, where NF-κB can bind to the promoter region of corresponding genes (7, 25, 32). EMSAs revealed that maximal running exercise gives rise to a considerable increase of the NF-κB (p50/p65) binding activity, on the order of 100%. It is suggestive that oxidative stress might have induced that activation of NF-κB (p50/p65) with intensive physical exercise. A statistically significant correlation between the binding activity of NF-κB (p50/p65) and the glutathione status, however, could not be established. The central role of ROS for the activation of NF-κB (p50/p65) has recently been questioned (14) because ROS-independent pathways to NF-κB have been postulated as for interleukin-1 and tumor necrosis factor-α (TNF-α) (6, 29). Thus it is conceivable that other inducers of NF-κB, in particular interleukin-1 and TNF-α, which both increase with exercise (15, 17), also contribute to the activation of NF-κB (p50/p65) with physical exercise.

Table 2. Exercise-induced effects on the expression of TF and on factor VII in 12 healthy young men before and in intervals after a 1-h maximal run on a treadmill

<table>
<thead>
<tr>
<th></th>
<th>Before Exercise</th>
<th>0 h</th>
<th>3 h</th>
<th>6 h</th>
<th>24 h</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF Ag (FACS) fluorescence</td>
<td>1.1 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>TF Ag, cellular pg/ml</td>
<td>102 ± 9</td>
<td>104 ± 11</td>
<td>108 ± 13</td>
<td>113 ± 10</td>
<td>97 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>TF Ag, plasma pg/ml (n = 6)</td>
<td>205 ± 43</td>
<td>218 ± 47</td>
<td>213 ± 50</td>
<td>203 ± 44</td>
<td>203 ± 39</td>
<td>NS</td>
</tr>
<tr>
<td>FVII Ag, %</td>
<td>64 ± 3</td>
<td>65 ± 5</td>
<td>64 ± 6</td>
<td>68 ± 7</td>
<td>68 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>FVII c, %</td>
<td>96 ± 2</td>
<td>85 ± 3</td>
<td>98 ± 4</td>
<td>91 ± 4</td>
<td>100 ± 4</td>
<td>0.01</td>
</tr>
<tr>
<td>FVII c/FVII:Ag</td>
<td>1.55 ± 0.09</td>
<td>1.37 ± 0.06</td>
<td>1.58 ± 0.10</td>
<td>1.41 ± 0.07</td>
<td>1.51 ± 0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>cFVIIa, mU/ml</td>
<td>40.3 ± 3.3</td>
<td>44.3 ± 3.2</td>
<td>35.4 ± 2.2</td>
<td>39.4 ± 3.7</td>
<td>45.9 ± 4.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. *ANOVA (Friedman). Cellular tissue factor (TF) antigen (Ag) was analyzed by flow cytometry (FACS) and ELISA technique. Trace amounts of TF were detectable in the plasma of 6 subjects (ELISA). Activation of factor VII was assessed by measuring FVII antigen (FVII:Ag), FVII coagulant activity (FVII:c), and concentrations of circulating activated FVII (cFVIIa) in plasma. †P < 0.05 for significant differences between pre- and postexercise values (Wilcoxon test with Bonferroni adjustment).
Exercise-induced activation of NF-κB (p50/p65), however, did not enhance transcription of the TF gene, because expression of TF mRNA remained unchanged after exercise. Also, at the level of protein synthesis, intensive physical exercise had no effect on the expression of TF, either on the expression of surface antigen on monocytes or on the cellular and plasmatic concentrations of TF. Recently, Osterud and co-workers (24, 26) evaluated the activation of monocytes with exercise under ex vivo in vitro conditions by measuring the TF activity in preparations of mononuclear cells after stimulation with lipopolysaccharides (LPS). The authors reported a slight increase of LPS-induced TF activity after 30 min of running at 80% VO2 max in untrained but no significant changes in trained subjects (26). It is unclear what impact these findings on the in vitro reactivity of monocytes have on the situation in vivo. Nevertheless, our data have consistently demonstrated that intensive physical exercise does not affect the expression of TF in vivo.

A number of transcription factors that bind to distinct regions of the TF promoter are involved in the complex regulation of TF (27). The LPS- and TNF-α-induced TF expression in human endothelial and mononuclear cells, for instance, depends on the interaction of both NF-κB and AP-1 (Jun/Fos) binding sites (12, 27). Parry and Mackman (30) have demonstrated that the NF-κB heterodimer p65/c-Rel selectively binds to the TF-specific κB-sequence in mononuclear cells. Exercise-induced upregulation of mononuclear NF-κB p50/p65 heterodimers therefore might favor formation of NF-κB complexes that do not recognize the TF-specific κB-site. From the study presented here, however, it cannot be decided whether the stimulus itself mediates selective NF-κB activation to specifically regulate the pattern of gene expression or whether mononuclear cells respond differently to a stimulus capable of inducing TF in other cells.

On the TF-dependent pathway, TF activates factor VII, which acts as a cofactor for the activation of factor X. Maximal running exercise did not lead to an activation of factor VII with respect to unchanged plasma levels of circulating FVIIa and an even decreased FVII coagulant activity after exercise. In line with our data, a slight decrease of FVIIa after intensive exercise has also been reported by van den Burg et al. (36, 37).

Within the vasculature, TF is inducibly expressed on monocytes and endothelial cells (27). Whether effects of exercise on the activation of NF-κB and the expression of TF observed in PBMC can also be generalized to endothelial cells is uncertain. The lacking increase of factor VII coagulant activity with exercise, however, might argue against differential effects of physical exercise on endothelial cells compared with monocytes, at least with respect to the expression of TF.

Several mechanisms have been proposed to be relevant for exercise-induced activation of coagulation. First, the large increase in sheer stress and the release of catecholamines both might play a crucial role in particular for the activation of platelets with exercise (9, 22). Also, acidosis resulting from anaerobic metabolism and traumatic hemolysis due to mechanical strain have been discussed as potentially contributing factors (22, 39). Our study has evaluated the impact of oxidative stress, demonstrating that the generation of ROS with exercise does not affect the TF-dependent pathway. Even though the exact mechanisms of exercise-induced hemostatic activation remain unclear, our data have shown that the mechanisms involved relate to the intrinsic pathway of coagulation.

In summary, the formation of thrombin and fibrin after intensive physical exercise is not initiated by the TF-dependent pathway. Exhaustive exercise gives rise to a considerable activation of NF-κB (p50/p65) and thus represents a novel physiological stimulus of NF-κB (p50/p65). The role of ROS for the pathway to exercise-induced activation of NF-κB (p50/p65) has still to be clarified.

REFERENCES