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THE ROLE OF EPIDERMAL GROWTH FACTOR IN PLATELET-ENDOTHELIUM INTERACTIONS

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The objective of this study was to determine whether endogenous EGF released after submaximal physical exercise, affects platelet — endothelium interactions. Sixteen healthy male volunteers, aged 23—26 years, were submitted to a submaximal bicycle ergometry test. Blood for determination of EGF concentrations, platelet function studies (concentrations of β-TG, PF4 and TXB_2) and endothelium activity (LTC_4 and endothelin-1,2 concentrations) was taken *via* an intravenous catheter before starting exercise and 15, 30 and 60 min after. A similar scheme was followed to investigate changes in the same parameters induced by a slow intravenous infusion of 0.3 mg/kg b.w. phentolamine (an α-adrenergic blocker) before exercise. Plasma concentrations of EGF and the markers of platelet function — β-TG and PF4 as well as LTC_4 concentrations increased only 15 min following exercise. The concentrations of TXB_2 and endothelium-1,2 were almost unchanged 15 min after the submaximal bicycle ergometry test. Phentolamine markedly decreased the EGF concentrations in plasma (15 min following exercise) while at 30 and 60 min after exercise it had no effect on this parameter. No significant changes in concentrations of β-TG, PF4, LTC_4 and endothelin-1,2 after phentolamine infusion were found. These results show that increase of plasma EGF following exercise was accompanied with increase of β-TG, PF4 and LTC_4 concentrations. Inhibition of α-adrenergic receptors with phentolamine abolished the exercise — induced increase in plasma EGF concentration. The findings suggest that endogenous EGF may affect the platelet function and changes the reactivity of the vascular endothelium.

K e y w o r d s: *epidermal growth factor, platelet function, endothelium.*

INTRODUCTION

Epidermal growth factor (EGF) is a 6 kDA mitogenic polypeptide that is present in several organs and in nearly all the body fluids of humans (1—3). Platelets of rodents contain no EGF but in humans they are the main source of circulating EGF (4, 5). α-granules of human platelets contain several potent growth factors including platelet-derived growth factor (PDGF), insulin-like growth factor-I (IGF-I) as well as EGF (6—8). They are released during platelet

adhesion, aggregation or activation and affect proliferation of vascular endothelial and smooth muscle cells. According to the "response to injury" hypothesis, these growth factors play a crucial role in the pathogenesis of atherosclerosis (9). Plasma contains small amounts of EGF and it may be increased after prolonged submaximal exercise (10), a condition known to involve an increase in both α- and β-adrenergic agonists (11). In previous studies, we have shown that the endogenous EGF affects platelet function (12).

The present study was performed to determine whether endogenous EGF, released after submaximal exercise, affects platelet-endothelium interactions.

SUBJECTS AND METHODS

Subjects

Sixteen healthy male volunteers (aged 23—26 years, body weight 68—92 kg) non smokers, who were not on drug treatment, were screened for their ability to reach maximal heart rates for their age group (13) by submaximal exercise for 20 min on a bicycle ergometer. The investigation protocol was approved by the Ethics Committee of Białystok Medical Academy and informed consent was obtained from all volunteers. Each subject rested in a supine position for 30 min with a 19 gauge intravenous cannula in an antecubital vein prior to pedalling on the bicycle ergometer (Medicor, Hungary). Exercise started with a workload of 50 W and was increased by 25 W every 3 min up to submaximal standardised increase in heart rate. The electrocardiograms were simultaneously and continuously monitored. Blood pressure was intermittently monitored by an automatic blood pressure system. All subjects exercised for similar periods in accordance with the protocol (20.0 ± 1.1 min) and were studied between 9.00 and 12.^{AM}, 3 hours after their last meal.

Blood samples

Blood was taken from the antecubital vein before staring the exercise test and 15, 30 and 60 min after and collected into precooled tubes containing 0.05 M. EDTA or 4 ml collection tubes (Diatube-H, Diagnostica Stago, France).

Analytical procedures

1. Plasma concentration of EGF was assayed using the Human EGF Radioimmunoassay Kit (Biomedical Technologies Inc., Blood for EGF assay, was sampled into precooled tubes containing EDTA as well as a protease inhibitor — Trasylol (100 IU/ml) and centrifuged at 2000 g for 20 min at 4°C. After centrifugation plasma was stored at −20°C until assayed.

2. Levels of β-thromboglobulin (β-TG) and platelet factor 4 (PF4) were determined by ELISA methods (Asserachrom β-TG and Asserachrom PF4, Diagnostica Stago, France). Blood for β-TG and PF4 assays was sampled into precooled 4 ml collection tubes. The specimens were immediately capped and gently inverted, placed on melting ice for 15 min and centrifuged at 2600 g for 30 min at 4°C. 0.5 ml mid-layer plasma was collected by a plastic pipette and stored at −20°C until analysis.

3. Plasma concentration of TXB_2 was assayed using the Thromboxane B_2 [^{3}H] assay system (Amersham, UK). Blood for TXB_2 measurement was sampled into tubes containing 7.5 mM EDTA and 0.04 M indomethacin solution and centrifuged at 2000 g for 20 min at 4°C.

4. Plasma concentration of leukotriene C_4 (LTC_4) was measured using the Leukotriene C_4 [^{3}H] RIA Kit (NEN Research Products, USA). Blood for LTC_4 assay was sampled into tubes

containing EDTA and 0.01 mM nordihydroguajaretic acid solution and centrifuged at 2000 g at 4°C.

5. Plasma endothelin-1,2 concentration was measured by radioimmunoassay (Endothelin-1,2 [^{125}J] assay system — Amsterdam, UK). Blood for endothelin-1,2 assay was collected into tubes containing 7.5 mM EDTA and centrifuged at 2000 g for 20 min at 4°C.

TXB_2, LTC_4 and endothelin-1,2 were extracted from plasma using Amprep extractions (Amersham, UK). The samples were dried under nitrogen and stored at −20°C until the assays were conducted.

A similar scheme was followed to investigate changes in the same parameters (concentrations of EGF, β-TG and PF4, TXB_2, LTC_4 and endothelin-1,2) induced by slow intravenous infusion (20 min) of 0.3 mg/kg b.w. phentolamine (Regitine — Ciba Geigy) before starting the exercise.

All values were expressed as means ± SD. p. values < 0.05 were considered significant. All statistics were calculated with the assistance of a computer program (CSS-Statistica).

RESULTS

As shown in *Fig. 1*, under resting conditions the plasma concentrations of EGF was 0.51 ± 0.11 ng/ml. 15 min after exercise, the EGF concentration significantly increased to 1.95 ± 0.65 ng/ml. There were no significant changes in plasma EGF concentrations at 30 and 60 min after submaximal exercise. Phentolamine markedly lowered the concentration of plasma EGF 15 min after exercise, while at 30 and 60 min after exercise it had no effect on this parameter.

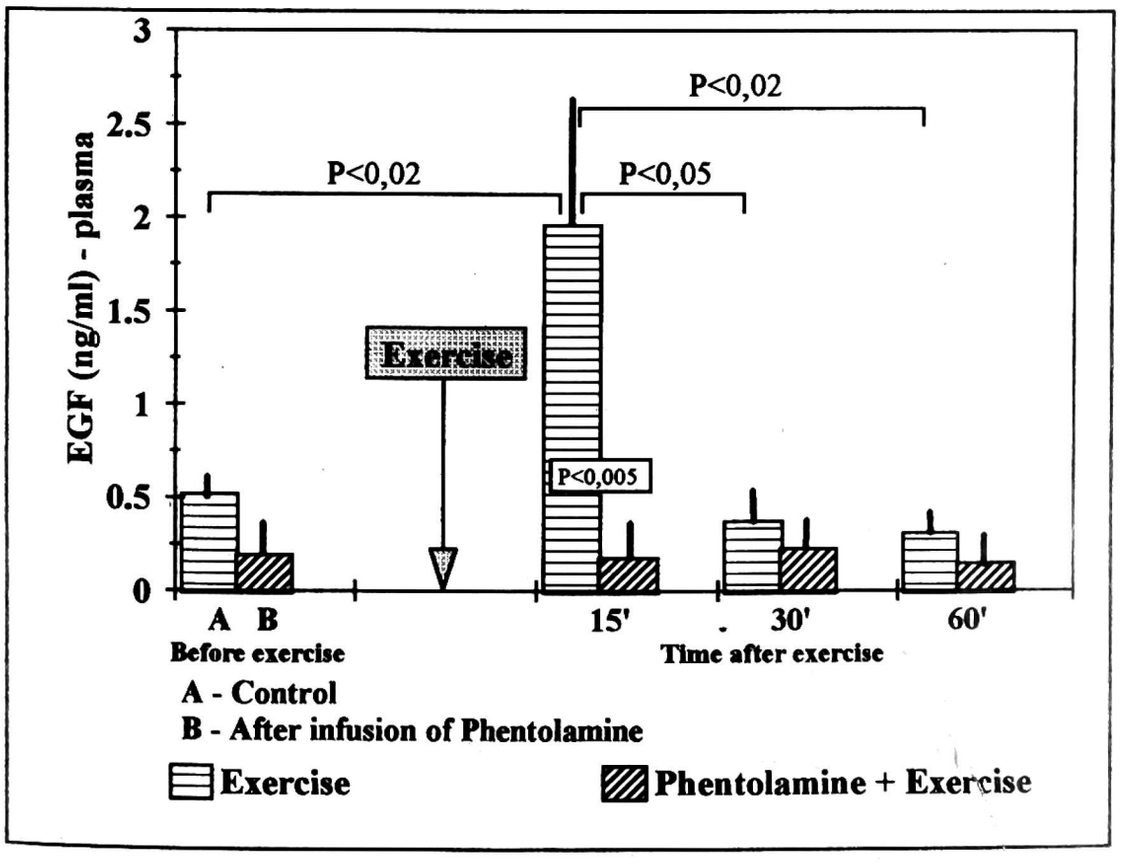

Fig. 1. Plasma concentrations of epidermal growth factor (EGF) in healthy volunteers before and after submaximal exercise (A) and after administration of phentolamine (B).

The levels of plasma β-TG and PF4 concentrations in response to exercise are shown in *Fig. 2* and *Fig. 3*.

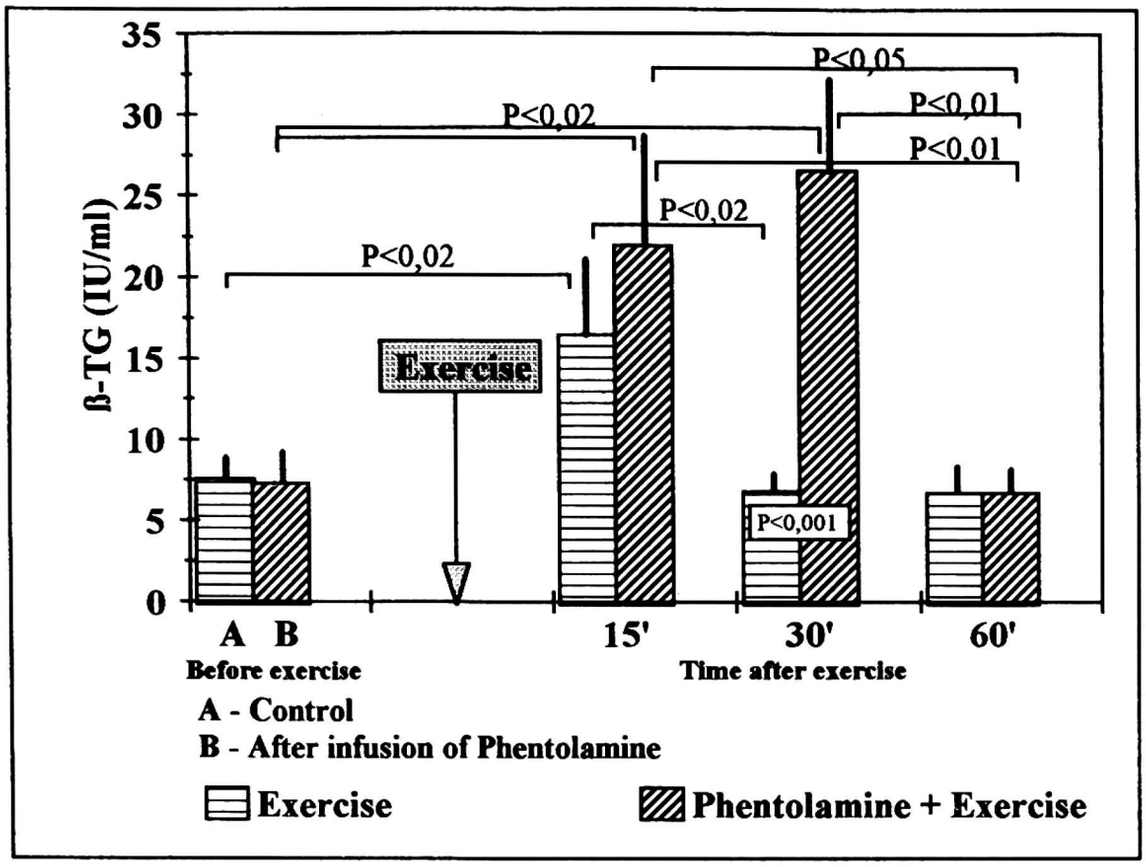

Fig. 2. Plasma concentrations of β-thromboglobulin (β-TG) in healthy volunteers before and after submaximal exercise (A) and following administration of phentolamine (B).

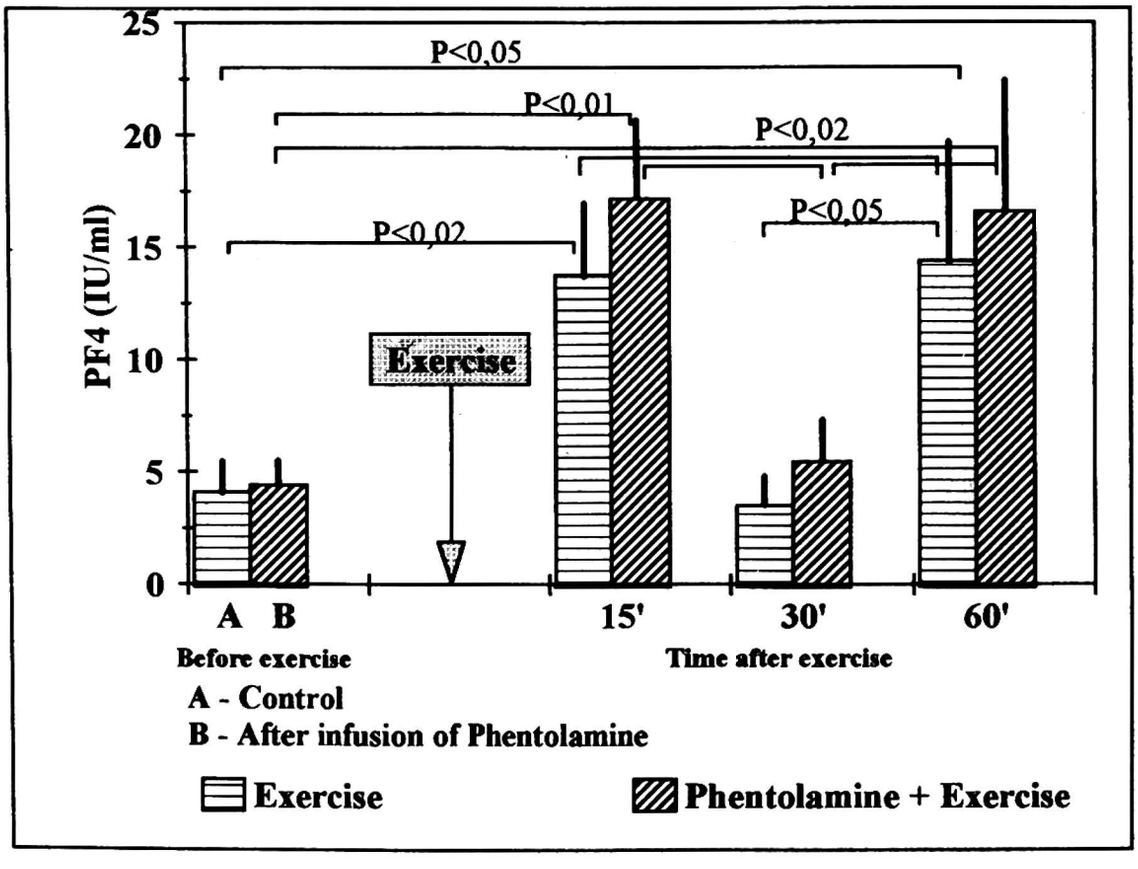

Fig. 3. Plasma concentrations of platelet factor 4 (PF4) in healthy volunteers before and after submaximal exercise (A) and following the administration of phentolamine (B).

It was noticed that plasma B-TG concentration significantly increased to 16.48 ± 3.83 IU/ml. 15 min after exercise and tended to decrease at 30 and 60 min following exercise. The infusion of phentolamine caused further increases in β-TG concentrations at 15 min after exercise (21.97 ± 6.75 IU/ml) and 30 min after exercise (26.56 ± 5.89 IU/ml), whereas no significant changes were observed 60 min after the exercise test.

Under resting conditions PF4 concentration was 4.1 ± 1.22 IU/ml. In response to exercise, plasma PF4 concentration increased significantly to 13.73 ± 3.21 IU/ml at 15 min after exercise and decreased significantly to 3.49 ± 1.16 IU/ml at 30 min after exercise. It is noteworthy, that 60 min following exercise, it was again found that a significant increase in PF4 concentration occurs to 14.38 ± 5.20 IU/ml. Phentolamine caused no changes in plasma PF4 concentrations before and after physical exercise.

The results of TXB_2 levels following submaximal exercise are summarised in *Fig. 4*. The level of TXB_2 under control conditions was 226.65 ± 68.60 pg/ml. At 15 and 30 min after exercise there were no changes in the levels of TXB_2, whereas 60 min after exercise the TXB_2 level increased significantly to 429.4 ± 143.81 pg/ml. Phentolamine did not affect the level of TXB_2 before, as well as 15 and 30 min, after physical exercise but markedly lowered this parameter at 60 min after exercise (273.32 ± 113.01 pg/ml).

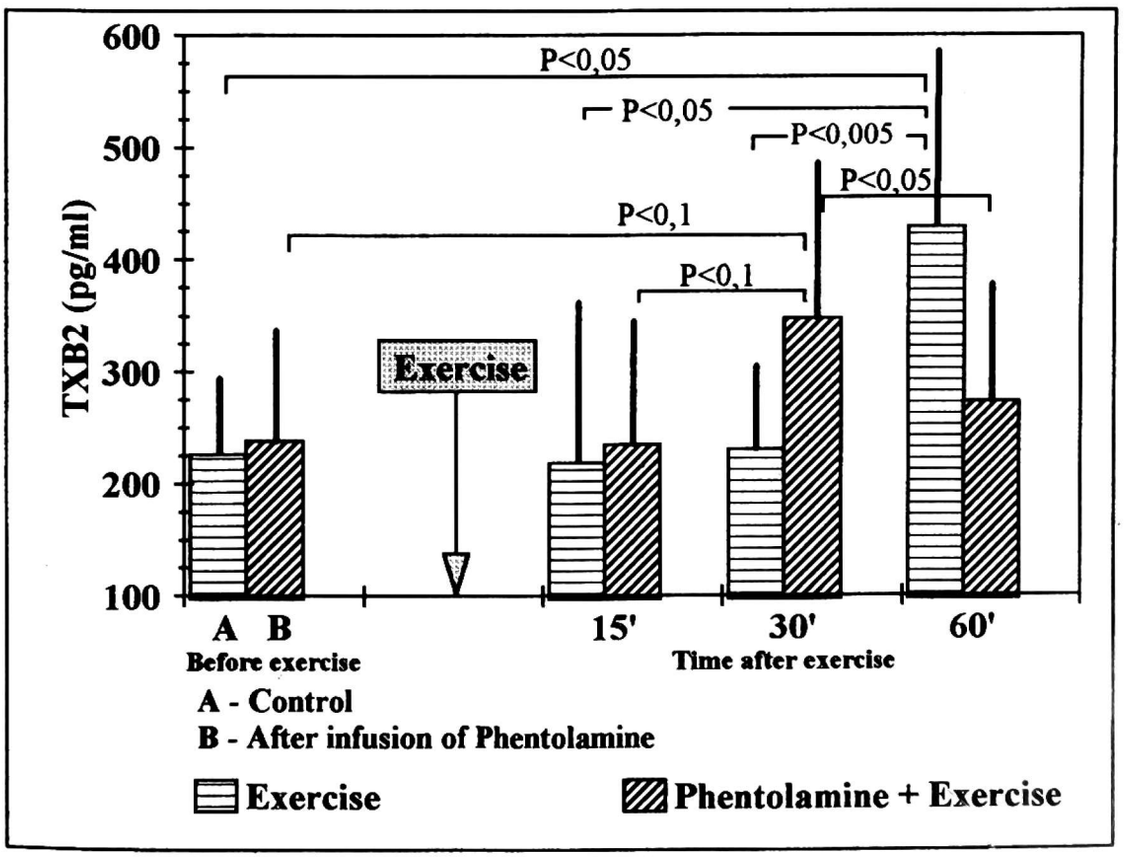

Fig. 4. Plasma concentrations of thromboxane B_2 (TXB_2) in healthy volunteers before and after submaximal exercise (A) and following the administration of phentolamine (B).

The levels of markers of endothelial cell damage- LTC_4 and endothelin-1,2 in response to exercise are shown in *Fig. 5* and *Fig. 6*.

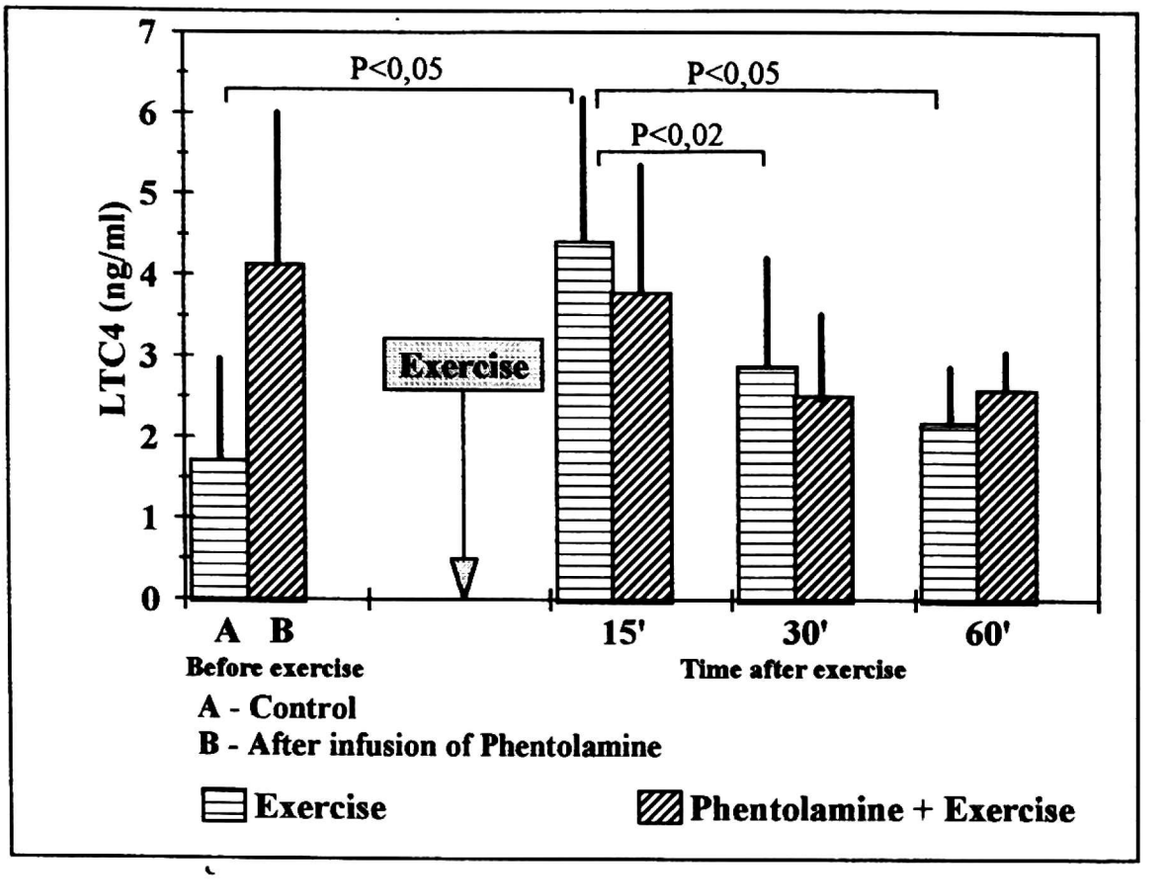

Fig. 5. Plasma concentrations of leukotriene C_4 (LTC_4) in healthy volunteers before and after submaximal exercise (A) and following administration of phentolamine (B).

At rest the level of LTC_4 was 1.70 ± 1.19 ng/ml. 15 min after exercise the LTC_4 level increased significantly to 4.41 ± 1.76 ng/ml, whereas at 30 and 60 min after exercise normalisation of LTC_4 levels was observed, The infusion of phentolamine markedly increased the level of LTC_4 prior to submaximal exercise test (B group — 4.12 ± 1.89 ng/ml), but did not exert any effect on the LTC_4 level at 15, 30 and 60 min after exercise.

From *Fig. 6* it can be seen, that the endothelin-1,2 level at rest was 26.01 ± 16.55 pg/ml and had a tendency to increase at 15 min after exercise (41.09 ± 10.55 pg/ml). At 30 and 60 min following exercise the endothelin-1,2 level tended to decrease. After administration of phentolamine there were no statistically significant changes in endothelin-1,2 levels at 15 and 60 min after the exercise test, while at 30 min after exercise, a significant increase in this parameter was noted (78.01 ± 10.50 pg/ml vs. 17.97 ± 6.04 pg/ml).

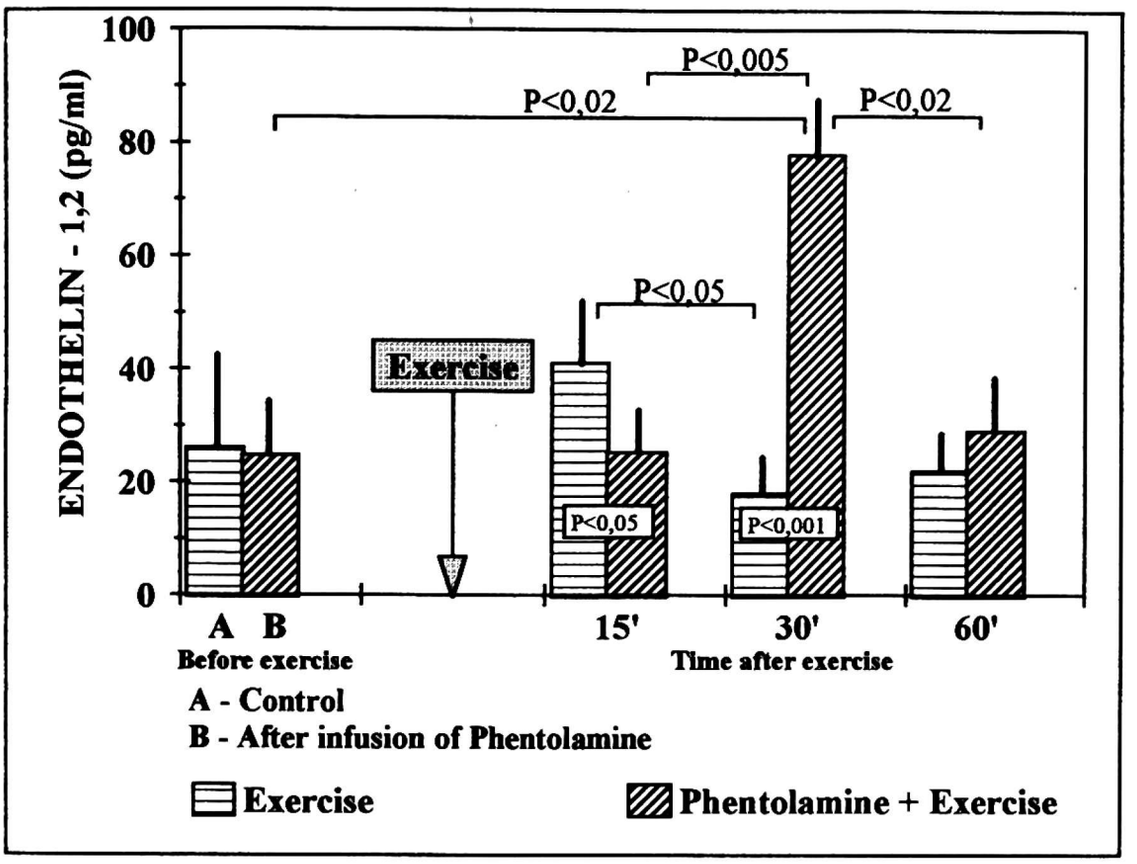

Fig. 6. Plasma concentration of endothelin-1,2 in healthy volunteers before and after submaximal exercise (A) and following the administration of phentolamine (B).

Continuos ECG monitoring and measurement of blood pressure did not reveal ischemic changes in the volunteers after exercise as well as after the injection of phentolamine.

DISCUSSION

Although 30 years have passed since EGF was first discovered, its physiological roles are far from clear. In addition to being a mitogenic, EGF can act as a growth inhibitor (14), as a promotor of cell migration and as a regulator of differentiated cell functions in various systems (15). Oka *et al.* (4) have suggested that human EGF appears to be liberated from platelets during coagulation and it may be involved in normal vascular and tissue repair and in the pathogenesis of atherosclerotic lesions. Moreover, EGF stimulates prostanoid synthesis of many cultured animal cells (16—18) as well as being the only growth factor found in platelets known to increase human endothelial cell prostacyclin (PGI_2) production (19, 20).

The present study was performed to determine whether endogenous EGF, released during submaximal physical exercise, affects platelet — endothelium interactions. Sixteen healthy male volunteers were submitted to a submaximal

bicycle ergometry test. The following parameters before and 15, 30 and 60 minutes after exercise were evaluated: plasma concentrations of EGF, platelet specific proteins being released from α-granules during platelet activation, e.g. β-TG and PF4, plasma TXB_2 as well as endothelium derived substances — endothelin-1,2 and LTC_4. The concentration of immunoreactive EGF in plasma was about 0.5 ng/ml and increased significantly only at 15 min following exercise while at 30 and 60 min after exercise normalisation of plasma EGF concentration was observed. These results show that plasma EGF increases following exercise but it is a short-lived phenomenon. Konradsen *et al.* (10) found a similar increase in the concentration of plasma and urine EGF following exercise in humans. There is evidence of both hypercoagulability and increased fibrinolysis following exercise as well as an increase in the circulating platelet count which varies with the severity and duration of the exercise performed (21—23). Despite the increase in the platelet count, most studies regarding the effects of exercise on platelet functional behaviour, mainly aggregation and secretion, have been either controversial or incomplete (24, 25). The results of our study show an evident increase of both markers of the increase of platelet activation and release reaction *in vivo* — β-TG and PF4. This effect was largely limited to 15 min after exercise. We found a close correlation between the temporary increase of plasma concentration EGF, limited to 15 min after exercise, and the concentrations of β-TG and PF4 in plasma. Submaximal exercise did not stimulate statistically significant alterations in TXB_2 concentrations 15 min following exercise. The increase in TXB_2 concentration only appeared at 60 min after exercise. Taniguchi *et al.* (26) also reported no change in plasma concentrations in response to exercise. However, Todd *et al.* (27) suggested that exercise — induced increases in TXB_2 may be related to intensity — exercise at higher intensities may lead to significant increases in plasma TXB_2. Thus, it seems that platelet activation following exercise is connected with EGF release. Platelet activation enhances the production of endothelin-1, an extremely potent vasoconstrictor and stimulator of vascular smooth muscle proliferation made by endothelial cells (28). Increased levels of circulating endothelin reflect endothelial activation/dysfunction or damage. Both the endothelium and platelets also generate LTC_4 — contracting factor, which regulates vascular tone (29). The submaximal exercise stimulates a greater increase in LTC_4 than in endothelin-1,2 concentrations 15 min following exercise. It appears, therefore, that endothelial activation in response to exercise is also connected to EGF release.

The origin of the circulating EGF is unclear. Hwang *et al.* (6) concluded that different organs contribute to plasma EGF fractions. Nexo *et al.* (30) reported that the salivary concentration of EGF increased with a median factor of 1.8 after prolonged submaximal exercise, a condition known to

involve an increase in both α- and β-adrenergic agonists (11). Phentolamine is an α-adrenergic receptor blocking agent (31, 32). In the present study we have demonstrated that infusion of phentolamine before exercise markedly decreased the EGF concentrations in plasma but this effect was limited only to 15 min after exercise. In contrast, we did not observe any changes in β-TG and PF4 or TXB_2 and LTC_4 concentrations after phentolamine infusion. It seems that inhibition of the α-adrenergic receptor with phentolamine does not influence post exercise platelet and endothelium functions. This effect was also limited to 15 min after exercise. However, considering the increase in β-TG and endothelin-1,2 concentrations 30 min following exercise and following phentolamine infusion, as well as an increase in PF4 concentration 60 min following exercise, other factors may influence this phenomenon finding. The endothelium is an important regulator of vascular growth producing both growth factors and growth inhibitors. Inhibitors such as heparin, heparin sulphates, NO and TGF-β normally predominate to keep vascular smooth muscle cell proliferation under control. Platelets have been found to release highly active mediators such a serotonin, TXA_2, NO and platelet — derived growth factors (33, 34). Several of these platelet — derived mediators can interact with endothelial receptors. Thus, the mechanisms involved in the observed hyperactivity of the platelets and endothelium during exercise are not fully understood to date.

Nevertheless, our findings show that plasma EGF and the plasma markers of platelet and endothelium activation *in vivo* transitory increase in response to exercise. Inhibition of α-adrenergic receptors with phentolamine abolished the exercise-induced increase in plasma EGF and endothelin-1,2 concentrations. Therefore, the endogenous EGF appears to affect platelet-endothelium interactions.

These findings also give a new insight into the possible effects to submaximal exercise on platelet and endothelium functions in healthy volunteers.

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