Exercise-Induced Prostacyclin Release Positively Correlates with \( V_{O2\text{max}} \) in Young Healthy Men

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Summary
In this study we have evaluated the effect of maximal incremental cycling exercise (IE) on the systemic release of prostacyclin (PGI\(_2\)), assessed as plasma 6-keto-PGF\(_{1\alpha}\) concentration in young healthy men. Eleven physically active – untrained men (mean ± S.D.) aged 22.7 ± 2.1 years; body mass 76.3 ± 9.1 kg; BMI 23.30 ± 2.18 kg · m\(^{-2}\); maximal oxygen uptake (VO\(_{2\text{max}}\)) 46.5 ± 3.9 ml · kg\(^{-1}\) · min\(^{-1}\), performed an IE test until exhaustion. Plasma concentrations of 6-keto-PGF\(_{1\alpha}\), lactate, and cytokines were measured in venous blood samples taken prior to the exercise and at the exhaustion. The net exercise-induced increase in 6-keto-PGF\(_{1\alpha}\) concentration, expressed as the difference between the end-exercise minus pre-exercise concentration positively correlated with VO\(_{2\text{max}}\) (\(r=0.78\), \(p=0.004\)) as well as with the net \(\Delta VO_2\) increase at exhaustion (\(r=0.81\), \(p=0.003\)), but not with other respiratory, cardiac, metabolic or inflammatory parameters of the exercise (minute ventilation, heart rate, plasma lactate, IL-6 or TNF-\(\alpha\) concentrations). The exercise-induced increase in 6-keto-PGF\(_{1\alpha}\) concentration was significantly higher (\(p=0.008\)) in a group of subjects (\(n=5\)) with the highest VO\(_{2\text{max}}\) when compared to the group of subjects with the lowest VO\(_{2\text{max}}\), in which no increase in 6-keto-PGF\(_{1\alpha}\) concentration was found. In conclusion, we demonstrated, to our knowledge for the first time, that exercise-induced release of PGI\(_2\) in young healthy men correlates with VO\(_{2\text{max}}\), suggesting that vascular capacity to release PGI\(_2\) in response to physical exercise represents an important factor characterizing exercise tolerance. Moreover, we postulate that the impairment of exercise-induced release of PGI\(_2\) leads to the increased cardiovascular hazard of vigorous exercise.

Key words
Exercise • Maximal oxygen uptake • Power output • Prostacyclin

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Introduction
Endothelial function is essential for maintenance of health of the cardiovascular system, while endothelial dysfunction leads to cardiovascular disease (Bonetti et al. 2003, Chlopicki and Gryglewski 2005). Physical exercise has been shown, both in animal and humans studies, to be an important factor affecting the endothelial function (Green et al. 2004). In this respect the relationship between physical exercise and nitric oxide (NO) has been widely studied and it was repeatedly demonstrated that exercise training augment endothelial NO-dependent vasodilatation (Green et al. 2004). Importantly, exercise improves endothelial function in subjects in whom endothelial dysfunction already exists and the improvement of endothelial NO-dependent function independent on changes in risk factors, may translate into the better cardiovascular outcome of these patients (Green et al. 2004). Although PGI\(_2\) and NO seem to be released from the endothelium in a coupled manner (Gryglewski et al. 1986), it is NO-cGMP but not PGI\(_2\)-cAMP pathway that controls basal vascular tone. Accordingly, in contrast to the abundant literature on the...
role of NO in the vascular adaptation to the exercise, far less is known, regarding the changes in prostacyclin (PGI2) production during physical exercise. Some reports demonstrated that physical exercise was accompanied by an increased concentration of prostacyclin metabolite 6-keto-PGF1α in blood (Mehta et al. 1983, Feng et al. 1999, Frandsen et al. 2000) as well as in muscle interstitial fluid (Frandsen et al. 2000, Karamouzis et al. 2001), but the significance of these findings remains obscure. Interestingly, it was shown that exercise-induced PGI2 release was reduced in patients with coronary heart disease (Mehta et al. 1983, Wennmalm et al. 1990, Rasmanis et al. 1991, Kishi et al. 1992, Lang et al. 1997), but again the cardiovascular consequences of the impaired PGI2 response in the exercise have not been clearly described so far.

Taking into consideration that little is known, as regards the relationship between physical exercise capacity and the vascular PGI2 release, the aim of this study was to evaluate the effect of maximal incremental exercise on the plasma PGI2 concentration (assessed as plasma 6-keto-PGF1α concentration) in relationship with respiratory, cardiac, metabolic or inflammatory parameters of the exercise in young healthy men. In particular, we compared the exercise-induced increase in PGI2 concentration (ΔPGI2) with the VO2max – that is considered as an index of physical capacity. To our best knowledge up to date there are no reports regarding the relationship between exercise-induced PGI2 production and VO2max.

Subjects and Methods

Subjects characteristics

Eleven non-smoking men (mean ± S.D.: age 22.7 ± 2.1 years; body mass 76.3 ± 9.1 kg; height 180.8 ± 5.8 cm; BMI 23.30 ± 2.18 kg · m⁻²; VO2max 46.5 ± 3.9 ml · kg⁻¹ · min⁻¹) participated in this study. All procedures were approved by the Local Ethic Committee and performed according to the declaration of Helsinki. Subjects gave informed written consent and were aware of the aims of the study.

Exercise protocol

The incremental exercise test was performed on the cycloergometer Ergo-Line GmbH & Co KG 800s (Bitz, Germany). Before the test, a 6-min resting period was allowed to determine the resting stage of the cardio-respiratory parameters, as well as to withdraw the blood samples. The exercise test started at power output 30 W, followed by gradual increase amounting to 30 W every 3 min and it was continued until exhaustion. The incremental test was performed at 60 rev · min⁻¹ (for details see Zoladz et al. 1998).

Gas exchange variables

Gas exchange variables were measured continuously breath-by-breath using the Oxycon Champion (Mijnhardt BV, Bunnik, The Netherlands), starting from 6th minute prior to exercise until the test was stopped. Before and after each test, gas analyzers were calibrated with certificated calibration gases as previously described by Zoladz et al. (1995).

Blood sampling

Blood samples were taken using an Abbot Int-Catheter, Ireland (18G/1.2 x 45 mm), inserted into the antecubital vein about 15 min prior to the onset of the exercise. The catheter was connected to an extension set using a “T” Adapter SL Abbot, Ireland (the tube 10 cm in length). Immediately before taking each blood samples, 1 ml of blood volume was taken in order to eliminate blood from the catheter and the T-set. Blood samples for plasma lactate concentrations were taken prior to the exercise test, at the end of each step of the incremental exercise (the last 15 s before increase power output) and at the moment of ending the exercise protocol. Blood samples for measurement of PGI2 metabolite (6-keto-PGF1α) and cytokines concentrations were taken prior to the exercise at rest and at the end of the exercise protocol (at the exhaustion). The magnitude of exercise-induced increase in plasma 6-keto-PGF1α defined as the difference between the end-exercise minus pre-exercise plasma concentration of 6-keto-PGF1α (Δ6-keto-PGF1α) was considered to be a reliable index of the exercise-induced PGI2 release. On theoretical ground, Δ6-keto-PGF1α could be determined not only by PGI2 production but also by the rate of PGI2 degradation and the rate of 6-keto-PGF1α elimination. However, it seems unlikely that an alteration in the rate of degradation of PGI2 or in the elimination of 6-keto-PGF1α was responsible for the increase in 6-keto-PGF1α during a single exercise of maximal intensity as applied in our experimental setting, so that the exercise-induced increase in 6-keto-PGF1α concentration was attributed to the exercise-induced PGI2 release.

Plasma lactate measurements

The samples for plasma lactate concentration
(0.5 ml each) were placed in 1.8 ml Eppendorf tubes containing 1 mg ammonium oxalate and 5 mg sodium fluoride and mixed for about 20 s and then centrifugated. The obtained samples of blood plasma (200 μl) were stored at -32 °C for further analysis of lactate concentration ([La]pl) using an automatic analyzer Vitros 250 Dry Chemistry System, Kodak (Rochester, NY, USA).

**Plasma 6-keto-PGF\(_{1α}\) measurements**

For determination of 6-keto-PGF\(_{1α}\) blood samples were collected to Eppendorf tubes with indomethacin 10 μM and EDTA 1 mM (final concentrations), and immediately spun for 5 min at 2000 x g to obtain plasma. Plasma samples were stored at -70 °C. The concentrations of 6-keto-PGF\(_{1α}\) in plasma prior to the exercise test, and at the end of the exercise protocol were assayed using commercially available enzyme immunoassay kits (Cayman Chemical Co., MI, USA or R&D Systems, Inc., MN, USA) and expressed in pg · ml\(^{-1}\).

**Plasma cytokines measurements**

IL-6, IL-10 (R&D System, USA), (DSL, USA), TNF-α (BioSource, Belgium) were measured by IRMA. Analytical sensitivity for these measurements were 0.04 pg · ml\(^{-1}\), 0.05 pg · ml\(^{-1}\), 0.5 ng · ml\(^{-1}\), 5 pg · ml\(^{-1}\) and 1 μl · ml\(^{-1}\), respectively. Intra- and interassay CV were < 8.0 % and < 8.5 % for IL-6 and for IL-10, < 3.4 % and < 5.1 % for IGFBP3, < 5.2 % and 6.8 % for TNF-α and < 2.4 % and 6.8 % for insulin. For RIA and IRMA methods the radioactivity of the samples were measured by using gamma scintillation counter (Wallac, Finland).

**Statistics**

The presented results are expressed as mean ± S.D. as well as minimum (min) and maximum (max). Statistical significance was tested using Wilcoxon-signed-rank test (for paired samples) and Wilcoxon-Mann-Whitney test (for two independent samples). Non-asymptotic, exact, two-sided p-values are presented (see the Results section). Correlation between two variables was tested with Spearman’s correlation analysis. The statistics was done using the statistical packet StatXact 6.1 and STATISTICA 7.1.

**Results**

**Power output and maximal oxygen uptake**

The mean power output at the end of the incremental exercise test (PO at VO\(_{2\text{max}}\)) was 260 ± 24 W. The mean oxygen uptake at rest was 374 ± 41 ml · min\(^{-1}\). The maximal oxygen uptake (VO\(_{2\text{max}}\)) in the studied subjects was 3527 ± 350 ml · min\(^{-1}\) (46.5 ± 3.9 ml · kg\(^{-1}\) · min\(^{-1}\)). Therefore the net VO\(_2\) at the maximal power output amounted to 3153 ± 324 ml · min\(^{-1}\).

**Minute ventilation, plasma lactate, cytokines and prostacyclin concentrations at rest and at VO\(_{2\text{max}}\)**

The values of minute ventilation (V\(_E\)),...
respiratory quotient (RQ), heart rate (HR), plasma concentration of lactate (La—), cytokines (interleukin-6; IL-6, interleukin-10; IL-10, tumor necrosis factor; TNF-α) as well as prostacyclin (PGI2) assessed as plasma 6-keto-PGF1α concentration, measured at rest and at the VO2max are presented in Table 1.

**Exercise-induced prostacyclin release (ΔPGI2) in relation to VO2max and power output (PO).**

Figure 1 illustrates the magnitude of exercise-induced prostacyclin release assessed as the difference between the end-exercise minus pre-exercise concentration (Δ6-keto-PGF1α) in 5 subjects with the lowest (A) and in 5 subjects (B) with the highest VO2max. *** - significantly different from A (p=0.008).

**Correlations**

A significant correlation (r=0.78, p=0.004) between VO2max and Δ6-keto-PGF1α was observed (Fig. 2 A). We also found significant correlation (r=0.81, p=0.003) between net VO2 (expressed as the difference between pre-exercise VO2 and VO2max) and Δ6-keto-PGF1α (Fig. 2 B).

**Discussion**

In the present study we have evaluated the effect of an incremental cycling exercise on the systemic release of prostacyclin (PGI2) in young healthy men in relation to
the maximal oxygen uptake (VO_{2max}).

The main and original finding of this study is that the exercise-induced release of prostacyclin (ΔPGI2), detected as the difference between pre-exercise 6-keto-PGF1α plasma concentration and its value reached at VO_{2max}, displays significant positive correlations with the maximal oxygen uptake, with the net VO2 increase at maximal power output (Figs 2A and 2B) as well as with the power output reached at VO_{2max} (the maximal power output reached during the incremental exercise test) (Fig. 3). Moreover, we have found that a substantial increase in PGI2 concentration at the end of the incremental exercise test (p=0.06) was due to the increase in PGI2 metabolite in the group of subjects with the highest VO_{2max} (Fig. 2A). No increase in 6-keto-PGF1α concentration was found in the group of subjects (n=5) with the lowest VO_{2max} (Fig. 1 note the difference (p=0.008) in ΔPGI1).

The exercise-induced increase in PGI2 metabolites measured in urine (Koivisto et al. 1989, Wennmalm et al. 1990, Rasmanis et al. 1991, Ronnivuori et al. 1993, Boger et al. 1995), blood (Ritter et al. 1983, Barrow et al. 1986) or in the interstitial fluid of muscles, was previously reported (Frandsen et al. 2000, Karamouzis et al. 2001). Moreover, it was reported that the magnitude of the increase in PGI2 concentration in the interstitial fluid of the working muscles was dependent on the exercise intensity (Karamouzis et al. 2001).

Endothelium is considered as the major site of PGI2 production (Gryglewski et al. 1988). It was claimed that smooth muscles (Schildknecht et al. 2005), as well as peritendinous tissue (McLennan and Macdonald 1991, Langberg et al. 2002) and fibroblasts (Yu et al. 1997) may also contribute to the systemic production of PGI2. Both COX-1 and COX-2 are linked to systemic PGI2 production, however, the latter seems to be the major enzymatic source of PGI2 in healthy humans (Grosser et al. 2006). We and others did not discriminate the tissue (Boushel et al. 2000, Kjaer et al. 2006), enzymatic origin, mechanism of exercise-induced PGI2 production as well as its possible pulmonary origin (Gryglewski 1980b).

Although inflammatory stimuli like TNF-α increases PGI2 production (Moore et al. 1991), in the present study we did not find significant correlations between the exercise-induced release of PGI2 and the exercise-induced increase in IL-6, IL-10 and TNF-α (Table 2). This suggests that the exercise-induced release of PGI2 is independent on exercise-induced release of these cytokines (IL-6, IL-10 and TNF-α).

Whatever is the mechanism of the exercise-induced PGI2 release, our results indicate for the first time, that PGI2 may represent a key factor regulating the exercise capacity as determined by VO_{2max} in healthy men. At the current state of knowledge, we can only speculate on the mechanisms by which exercise-induced release of PGI2 regulates physical capacity and maximal oxygen uptake. It is generally accepted that the maximal oxygen uptake during whole body exercise in humans (e.g. cycling – as in the present study) is not constrained by the mitochondrial oxygen consumption capacity but by the magnitude of oxygen delivery to the working muscle (for review see Bassett and Holewy 2000,
Andersen and Saltin (1985) demonstrated that a mass of 2-3 kg of knee extensor muscles during maximal exercise can accommodate blood flow of 5-7 l · min⁻¹ and consume about 0.8 l O₂ · min⁻¹ (i.e. about 320 ml O₂ · kg muscle mass⁻¹). They postulated that in sedentary men the involvement of only about 30 % of muscle mass during intense exercise results in the maximal cardiac output. Therefore, any factor(s) that improves oxygen delivery to the working muscle during exercise can contribute to the increased maximal oxygen uptake and physical capacity. Accordingly, VO₂max depends on the oxygen delivery to the working muscle that is regulated not only by cardiac output but also by blood oxygenation and peripheral muscle flow. Quite surprisingly, all these parameters may be regulated by PGI₂.

Endogenous PGI₂-dependent regulation of vascular tone during exercise may however occur indirectly, through the intermediation of erythrocytes. Indeed, Sprague et al. (2003, 2005) demonstrated that erythrocytes express IP receptors, stimulation of which activates adenylate cyclase and the release of ATP that determines vascular resistance. Furthermore, PGI₂ appears as a safeguard of coronary, pulmonary and peripheral microcirculation endangered by the exercise-induced activation of platelets, somewhat similarly to the role of PGI₂ in maintaining perfusion of the microcirculation in cardiovascular pathologies (Muller et al. 1988, Pasqualini et al. 2002, Ciuffetti et al. 2003) and in prophylaxis of reperfusion-induced edema during organ transplantation (Hill and Pearl 1999, Rocca et al. 2001).

Couple of studies demonstrated that PGI₂ or PGI₂ analogues increased exercise capacity not only in patients with pulmonary hypertension (Wax et al. 1999, Wensel et al. 2000, Blumberg et al. 2002) but also in patients with stable angina pectoris (Bugiardini et al. 1999, Zoladz et al. 2006). Andersen and Saltin (1985) demonstrated that a mass of 2-3 kg of knee extensor muscles during maximal exercise can accommodate blood flow of 5-7 l · min⁻¹ and consume about 0.8 l O₂ · min⁻¹ (i.e. about 320 ml O₂ · kg muscle mass⁻¹). They postulated that in sedentary men the involvement of only about 30 % of muscle mass during intense exercise results in the maximal cardiac output. Therefore, any factor(s) that improves oxygen delivery to the working muscle during exercise can contribute to the increased maximal oxygen uptake and physical capacity. Accordingly, VO₂max depends on the oxygen delivery to the working muscle that is regulated not only by cardiac output but also by blood oxygenation and peripheral muscle flow. Quite surprisingly, all these parameters may be regulated by PGI₂.
Quite surprisingly short intravenous infusion of iloprost consistently prolonged exercise duration and reduced platelet aggregation at peak exercise in these patients, suggesting that antiplatelet effect of PGI$_2$ may account for myocardial or skeletal blood perfusion at vigorous exercise and thus determine the exercise capacity in these patients.

On the other hand, the magnitude of exercise-induced release of PGI$_2$ may determine the cardiovascular hazard of strenuous exercise. Indeed, it is well known that physical exertion in an individual unaccustomed to habitual physical activity is associated with 100-fold increase in the risk of acute myocardial infarction due to excessive platelets activation (Bartsch et al. 1999). Here we were able to identify within a relatively small experimental group two subgroups of apparently healthy subjects with the lowest and highest VO$_{2\text{max}}$ that correlated with lowest and highest release of PGI$_2$ (Fig. 2A). It could also be that they represent subgroups of healthy subjects with different relative hazard to cardiovascular risk of vigorous exercise due to differential level of activation of platelets. This hypothesis is currently under investigation. Altogether, we are tempted to speculate that exercise-induced release of PGI$_2$ determines not only exercise capacity but also cardiovascular hazard of vigorous exercise. This is in line with the early findings showing that the individuals with poor physical capacity such as elderly people, as well as patients after heart infarction and diabetics are characterized by poor ability to release PGI$_2$ during exercise (Koivisto et al. 1989, Rasmanis et al. 1991, Vanhoutte 2002, Woodman et al. 2005) as well as the high cardiovascular risk of vigorous exercise (Bartsch 1999). On the other hand, compensatory increase in PGI$_2$ release and its augmented contribution to exercise-induced peripheral vasodilatation may counterbalance the limitation of exercise incapacity in patients with heart failure (Lang et al. 1997). In the present study the basal concentration of PGI$_2$ was similar in the group of subjects (n=5) with the lowest and the highest VO$_{2\text{max}}$ (n=5 in each group). However, in some subjects, with the lowest VO$_{2\text{max}}$, even a decrease in the PGI$_2$ concentration during exercise was observed (Figs 1 and 2A). This finding seems to pin-point the subjects with the poorest physical capacity and maladaptive response to maximal exercise that could pose the risk of cardiovascular events.

Summing up, there is overwhelming evidence today that PGI$_2$ affords antiplatelet, vasculoprotective, cardioprotective and antiatherogenic activity (Gryglewski 1980a, Dowd et al. 2001, Grosser et al. 2006). Our results points out to the important physiological role of endogenous PGI$_2$ in the setting of vigorous physical exercise that opens new perspectives to exercise physiology and pharmacology and warrants further studies.

In conclusion, we demonstrated, to our knowledge for the first time that exercise-induced release of PGI$_2$ in young healthy men correlates with VO$_{2\text{max}}$, suggesting that vascular capacity to release PGI$_2$ in response to physical exercise represents an important factor characterizing exercise tolerance. Moreover, we postulate that the impairment of exercise-induced release of PGI$_2$ leads to the increased cardiovascular hazard of vigorous exercise.

**Conflict of Interest**

There is no conflict of interest.

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**References**


