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Exercise-Induced Prostacyclin Release Positively Correlates with Vo_{2max} 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_{2max}) 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_{2max} (r=0.78, p=0.004) as well as with the net 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-α 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_{2max} when compared to the group of subjects with the lowest VO_{2max}, 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_{2max}, 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

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role of NO in the vascular adaptation to the exercise, far less is known, regarding the changes in prostacyclin (PGI_2) production during physical exercise. Some reports demonstrated that physical exercise was accompanied by an increased concentration of prostacyclin metabolite 6-keto-$PGF_{1\alpha}$ 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 PGI_2 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 PGI_2 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 PGI_2 release, the aim of this study was to evaluate the effect of maximal incremental exercise on the plasma PGI_2 concentration (assessed as plasma 6-keto-$PGF_{1\alpha}$ 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 PGI_2 concentration (ΔPGI_2) with the VO_{2max} – 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 PGI_2 production and VO_{2max}.

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}; VO_{2max} 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 PGI_2 metabolite (6-keto-$PGF_{1\alpha}$) 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-$PGF_{1\alpha}$ defined as the difference between the end-exercise minus pre-exercise plasma concentration of 6-keto-$PGF_{1\alpha}$ (Δ6-keto-$PGF_{1\alpha}$) was considered to be a reliable index of the exercise-induced PGI_2 release. On theoretical ground, Δ6-keto-$PGF_{1\alpha}$ could be determined not only by PGI_2 production but also by the rate of PGI_2 degradation and the rate of 6-keto-$PGF_{1\alpha}$ elimination. However, it seems unlikely that an alteration in the rate of degradation of PGI_2 or in the elimination of 6-keto-$PGF_{1\alpha}$ was responsible for the increase in 6-keto-$PGF_{1\alpha}$ during a single exercise of maximal intensity as applied in our experimental setting, so that the exercise-induced increase in 6-keto-$PGF_{1\alpha}$ concentration was attributed to the exercise-induced PGI_2 release.

Plasma lactate measurements

The samples for plasma lactate concentration

Table 1. The power output (PO), oxygen uptake (VO_2), minute ventilation (V_E), respiratory quotient (RQ), heart rate (HR), plasma concentration of lactate (La^-), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor (TNF-α) and 6-keto-$PGF_{1\alpha}$ at rest and at the VO_{2max}.

	REST		VO_{2max}	
	min-max	**Mean ± S.D.**	**min-max**	**Mean ± S.D.**
PO (W)	-	-	223 - 310	260 ± 24
VO_2 ($ml \cdot min^{-1}$)	318 - 452	374 ± 41	3178 - 4267	3527 ± 350
V_E ($l \cdot min^{-1}$)	10.0 - 13.0	11.9 ± 1.2	96.0 - 150.0	117.2 ± 17.8
RQ	0.82 - 1.01	0.93 ± 0.07	1.09 - 1.26	1.18 ± 0.06
HR ($1 \cdot min^{-1}$)	62 - 98	77 ± 11	178 - 205	193 ± 8
$[La^-]_{pl}$ ($mmol \cdot l^{-1}$)	1.3 - 2.7	2.0 ± 0.4	8.6 - 16.8	10.5 ± 2.4
IL-6 ($pg \cdot ml^{-1}$)	0.45 - 1.79	0.93 ± 0.39	0.31 - 4.20	1.92 ± 1.06
IL-10 ($pg \cdot ml^{-1}$)	0.00 - 0.10	0.22 ± 0.34	0.00 - 1.71	0.26 ± 0.51
TNF-α ($pg \cdot ml^{-1}$)	0.24 - 2.06	0.85 ± 0.55	0.65 - 5.31	1.46 ± 1.33
6-keto-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)	0.8 - 122.8	39.0 ± 35.0	16.4 - 101.8	53.3 ± 26.6

The date are presented as mean ± S.D., minimal and maximal values (min-max).

(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-$PGF_{1\alpha}$ measurements

For determination of 6-keto-$PGF_{1\alpha}$ 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-$PGF_{1\alpha}$ 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 $pg \cdot ml^{-1}$.

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 $pg \cdot ml^{-1}$, 0.05 $pg \cdot ml^{-1}$, 0.5 $ng \cdot ml^{-1}$, 5 $pg \cdot ml^{-1}$ and 1 $\mu I \cdot ml^{-1}$, 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_{2max}) was 260 ± 24 W. The mean oxygen uptake at rest was 374 ± 41 $ml \cdot min^{-1}$ (Table 1). The maximal oxygen uptake (VO_{2max}) in the studied subjects was 3527 ± 350 $ml \cdot min^{-1}$ (46.5 ± 3.9 $ml \cdot kg^{-1} \cdot min^{-1}$). Therefore the net VO_2 at the maximal power output amounted to 3153 ± 324 $ml \cdot min^{-1}$.

Minute ventilation, plasma lactate, cytokines and prostacylin concentrations at rest and at VO_{2max}

The values of minute ventilation (V_E),

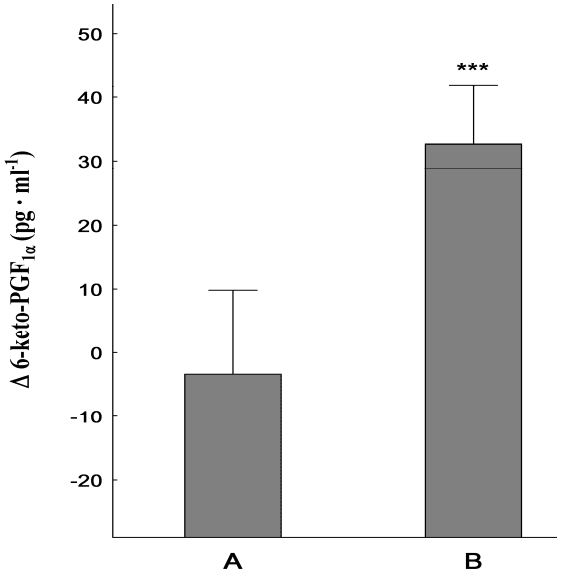

Fig. 1. Exercise-induced changes in plasma 6-keto-$PGF_{1\alpha}$ concentration, expressed as the difference between the end-exercise minus pre-exercise concentration (Δ6-keto-$PGF_{1\alpha}$) in 5 subjects with the lowest (A) and in 5 subjects (B) with the highest VO_{2max}. *** - significantly different from A (p=0.008).

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 (PGI_2) assessed as plasma 6-keto-$PGF_{1\alpha}$ concentration, measured at rest and at the VO_{2max} are presented in Table 1.

Exercise-induced prostacyclin release (ΔPGI_2) in relation to VO_{2max} 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 of plasma 6-keto-$PGF_{1\alpha}$ (Δ6-keto-$PGF_{1\alpha}$) in 5 subjects with the lowest (A) and in 5 subjects (B) with the highest VO_{2max}. In the group B (VO_{2max} = 3813 ± 320 ml · min^{-1}) the exercise-induced increase in plasma 6-keto-$PGF_{1\alpha}$ concentration was significantly higher (p=0.008) than in the group A (VO_{2max} = 3251 ± 68 ml · min^{-1}).

Correlations

A significant correlation (r=0.78, p=0.004) between VO_{2max} and Δ6-keto-$PGF_{1\alpha}$ was observed (Fig. 2 A). We also found significant correlation (r=0.81, p=0.003) between net VO_2 (expressed as the difference between pre-exercise VO_2 and VO_{2max}) and Δ6-keto-$PGF_{1\alpha}$ (Fig. 2 B).

Table 2. Non-parametric correlations between the exercise-induced increase in minute ventilation (ΔV_E), respiratory quotient (ΔRQ), heart rate (ΔHR), plasma lactate concentration (ΔLa^-) and plasma cytokines concentrations (interleukin–6; ΔIL-6), interleukin–10; ΔIL-10, tumor necrosis factor; ΔTNF-α) and Δ6-keto-$PGF_{1\alpha}$. The exercise-induced changes in those variables (Δ) were expressed as the difference between the end-exercise minus pre-exercise values.

Correlation variables			Spearman rank correlations	
			r	***P***
Δ6-keto-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)	:	ΔV_E ($l \cdot min^{-1}$)	0.27	p>0.05
Δ6-keto-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)	:	ΔRQ	0.21	p>0.05
Δ6-keto-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)	:	ΔHR ($1 \cdot min^{-1}$)	0.20	p>0.05
Δ6-keto-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)	:	ΔLa^- ($mmol \cdot l^{-1}$)	0.08	p>0.05
Δ6-keto-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)	:	ΔIL-6	0.29	p>0.05
Δ6-keto-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)	:	ΔIL-10	0.30	p>0.05
Δ6-keto-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)	:	ΔTNF-α	0.10	p>0.05

a) Correlation between power output at maximal oxygen uptake (PO at VO_{2max}) and the Δ6-keto-$PGF_{1\alpha}$

A significant correlation (r=0.69, p=0.02) between PO at VO_{2max} and the Δ6-keto-$PGF_{1\alpha}$ was found.

b) Correlations between the exercise-induced increase in V_E, RQ, HR, La, IL-6, IL-10, TNF-α and Δ6-keto-$PGF_{1\alpha}$ concentrations

No significant correlation was found between the exercise-induced increase in minute ventilation (ΔV_E), respiratory quotient (ΔRQ), heart rate (ΔHR), plasma lactate concentration (ΔLa^-) and exercise-induced prostacyclin release, expressed by Δ6-keto-$PGF_{1\alpha}$ concentration. Similarly, no significant correlation was observed between the exercise-induced increase in plasma concentrations of IL-6 (ΔIL-6), IL-10 (ΔIL-10), TNF-α (ΔTNF-α) and Δ6-keto-$PGF_{1\alpha}$ (Table 2).

Discussion

In the present study we have evaluated the effect of an incremental cycling exercise on the systemic release of prostacyclin (PGI_2) in young healthy men in relation to

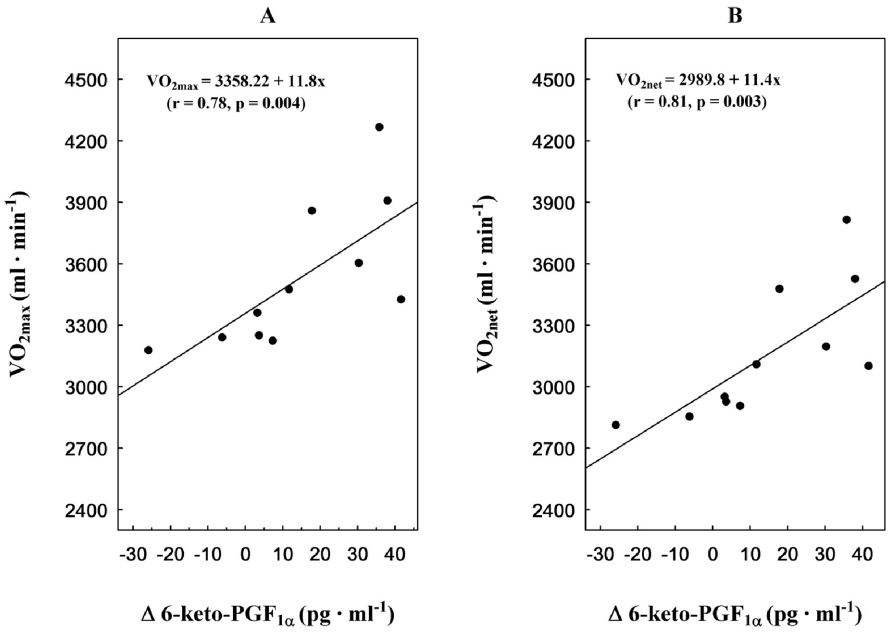

Fig. 2. Correlation between maximal oxygen uptake (VO_{2max}) and the exercise-induced changes in plasma 6-keto-$PGF_{1\alpha}$ concentration, expressed as the difference between the end-exercise and pre-exercise concentration (Δ6-keto- $PGF_{1\alpha}$) (**A**) and the correlation between net VO_2 (expressed as the difference between pre-exercise VO_2 and the VO_{2max}) and the (Δ6-keto-$PGF_{1\alpha}$) (**B**).

the maximal oxygen uptake (VO_{2max}).

The main and original finding of this study is that the exercise-induced release of prostacyclin (ΔPGI_2), detected as the difference between pre-exercise 6-keto-$PGF_{1\alpha}$ plasma concentration and its value reached at VO_{2max}, displays significant positive correlations with the maximal oxygen uptake, with the net VO_2 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 PGI_2 concentration at the end of the incremental exercise test ($p=0.06$) was due to the increase in PGI_2 metabolite in the group of subjects with the highest VO_{2max} (Fig. 2A). No increase in 6-keto-$PGF_{1\alpha}$ concentration was found in the group of subjects (n=5) with the lowest VO_{2max} (Fig. 1 note the difference ($p=0.008$) in ΔPGI_2).

The exercise-induced increase in PGI_2 metabolites measured in urine (Koivisto *et al.* 1989, Wennmalm *et al.* 1990, Rasmanis *et al.* 1991, Ronni-Sivula *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 PGI_2 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 PGI_2 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 PGI_2. Both COX-1 and COX-2 are linked to systemic PGI_2 production, however, the latter seems to be the major enzymatic source of PGI_2 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 PGI_2 production as well as its possible pulmonary origin (Gryglewski 1980b). Although inflammatory stimuli like TNF-α increases PGI_2 production (Moore *et al.* 1991), in the present study we did not find significant correlations between the exercise-induced release of PGI_2 and the exercise-induced increase in IL-6, IL-10 and TNF-α (Table 2). This suggests that the exercise-induced release of PGI_2 is independent on exercise-induced release of these cytokines (IL-6, IL-10 and TNF-α).

Whatever is the mechanism of the exercise-induced PGI_2 release, our results indicate for the first time, that PGI_2 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 PGI_2 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,

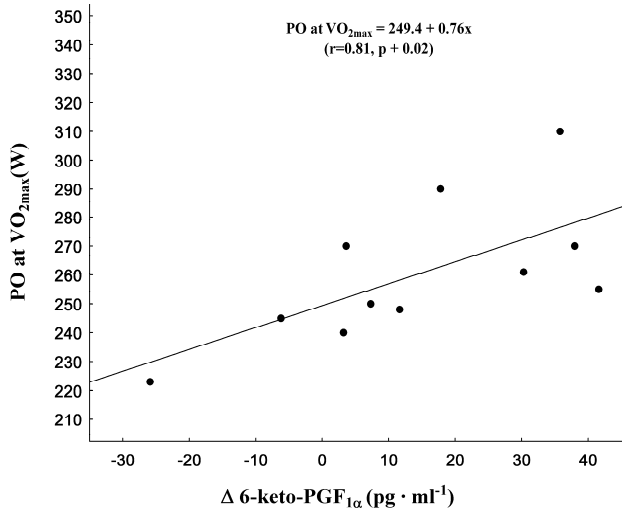

Fig. 3. Correlation between power output reached at maximal oxygen uptake (PO at VO_{2max}) (the maximal power output during the incremental exercise) and the exercise-induced changes in plasma 6-keto-$PGF_{1\alpha}$ concentration, expressed as the difference between the end-exercise and pre-exercise concentration (Δ6-keto-$PGF_{1\alpha}$).

Richardson and Saltin 1998, Saltin and Calbet 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 O_2 · min^{-1} (i.e. about 320 ml O_2 · 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, VO_{2max} 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 PGI_2.

Indeed, coronary vessels are extremely sensitive to the vasodilating effects of PGI_2 (Raczka and Quintana 1999) and endogenous PGI_2 may be involved in the exercise-induced coronary vasodilation (Nosaka *et al.* 1997, Merkus *et al*. 2006). Assuming that maximal cardiac output is one of the most important determinant of maximal oxygen uptake (for review see Richardson and Saltin 1998, Bassett and Holewy 2000, Saltin and Calbet 2006), it could well be that the exercise-induced PGI_2-dependent coronary vasodilatation determines the cardiac output that is reached at the time of exhaustion. Moreover, PGI_2 may improve the right heart chamber working condition by lowering pulmonary arterial pressure that increases during maximal exercise. PGI_2 may also improve gaseous exchange in the lungs during exercise by limiting alveolar edema formation (Sakuma *et al.* 2004).

In contrast, it seems unlikely that PGI_2 determines exercise capacity by direct vasodilator action in the peripheral blood flow. Indeed, the involvement of PGI_2 in exercise-induced hyperemia in the skeletal muscle blood flow was not unambiguously demonstrated (Lang *et al*. 1997, Merkus *et al.* 2004, Schrage et *al.* 2004, Saunders *et al.* 2005, Schrage *et al*. 2007). On the other hand, COX products, most likely PGI_2 (Karamouzis *et al.* 2001), released by working muscle, sensitize muscle mechanoreceptors that are involved in reflex sympathetic activation (Middlekauff and Chiu 2004). Obviously this response may contribute to exercise-induced increase in cardiac output and hence may regulate maximal oxygen uptake.

Endogenous PGI_2-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, PGI_2 is the most potent endogenous inhibitor of platelet activity, and its antiplatelet activity may be of paramount importance during exercise. Indeed, vigorous exercise causes platelet activation, increases platelet-platelet and platelet-leukocyte aggregates (Kestin *et al.* 1993, Li *et al.* 2007). Exercise also enhances the responsiveness of platelets and leukocytes to agonist stimulation examined *in vitro* (Streiff and Bell 1994). It is increasingly appreciated that microcirculation perfusion may be hampered by platelets aggregates. Thus, PGI_2 appears as a safeguard of coronary, pulmonary and peripheral microcirculation endangered by the exercise-induced activation of platelets, somewhat similarly to the role of PGI_2 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 PGI_2 or PGI_2 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.*

1986). 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 know 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_{2max} 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_{2max} (n=5 in each group). However, in some subjects, with the lowest VO_{2max}, 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 maladaptative 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_{2max}, 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.

Acknowledgements

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