# EXERCISE-INDUCED PROSTACYCLIN RELEASE POSITIVELY CORRELATES WITH $VO_2$max IN YOUNG HEALTHY MEN

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**Running title:** Prostacyclin and $VO_2$max in humans

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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 (x ± SD) 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 concentration 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 (*e.g.* minute ventilation, heart rate, plasma lactate, IL-6 and 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 whom 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

## 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 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 antecedent endothelial dysfunction 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 (see 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 ($PGI_2$) 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 the 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 ($\Delta PGI_2$) 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 ± SD: aged 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 6$^{th}$ minute prior to exercise until the test was stopped. Before and after each test, gas analysers 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 minutes 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 seconds 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-$PGF_{1\alpha}$ ($\Delta$ 6-keto-$PGF_{1\alpha}$) was considered to be a reliable index of the exercise-induced $PGI_2$ release. On theoretical ground $\Delta$6-keto-$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 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 (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 seconds and then centrifugated. The obtained samples of blood plasma (200 μl) were stored at a temperature of minus 32°C for further analysis of lactate concentration ($[La]_{pl}$) using an automatic analyser 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. Samples of plasma 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 Campany, 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 and standard deviation (x ± SD) 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 (x ± SD) power output at the end of the incremental exercise test (PO at $VO_2$max) was 260 ± 24 W. The mean oxygen uptake at rest was 374 ± 41 ml · $min^{-1}$ (see Table 1). The maximal oxygen uptake ($VO_2$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 prostacylin concentrations at rest and at $VO_2$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, tumour 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 $VO_2$max are presented in Table 1.

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

| | AT REST | | AT $VO_{2max}$ | |
|---|---|---|---|---|
| | **min-max** | $\bar{x} \pm SD$ | **min-max** | $\bar{x} \pm SD$ |
| **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 ± SD, minimal and maximal values (min-max).

*Exercise - induced prostacyclin release (ΔPGI$_2$) in relation to $VO_2$max 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-$PGF_{1\alpha}$ (Δ 6-keto-$PGF_{1\alpha}$ ) in 5 subjects with the lowest (A) and in 5 subjects (B) with the highest $VO_2$max. In the group B ($VO_2$max = 3813 ± 320 $ml \cdot min^{-1}$ ) the exercise-induced increase in plasma 6-keto-$PGF_{1\alpha}$ concentration was significantly higher ($p = 0.008$) than in the group A ($VO_2$max = 3251 ± 68 $ml \cdot min^{-1}$ ).

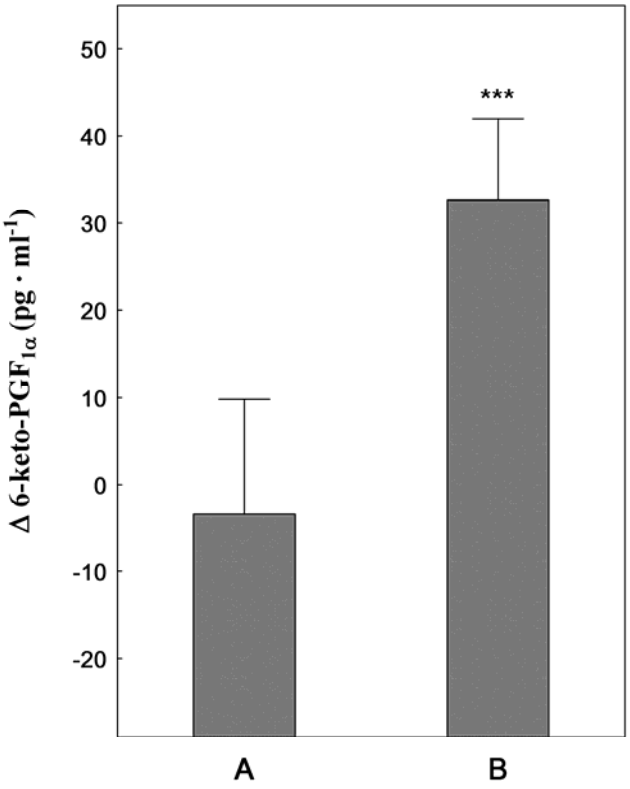


Figure 1. Exercise-induced changes in plasma 6-keto-$PGF_{1\alpha}$ concentration, expressed as the difference between the end-exercise minus pre-exercise concentration (Δ 6-keto-$PGF_{1\alpha}$) in 5 subjects with the lowest (A) and in 5 subjects (B) with the highest $VO_2$max.
*** - significantly different from A (p = 0.008).

*Correlations*

A significant correlation (r = 0.78, p = 0.004) between $VO_{2max}$ and Δ 6-keto-$PGF_{1\alpha}$ was observed (see Fig. 2 A). A significant correlation (r = 0.81, p = 0.003) between net $VO_2$ (expressed as the difference between pre-exercise $VO_2$ and $VO_2$max) and Δ6-keto-$PGF_{1\alpha}$ was found (see Fig. 2 B).

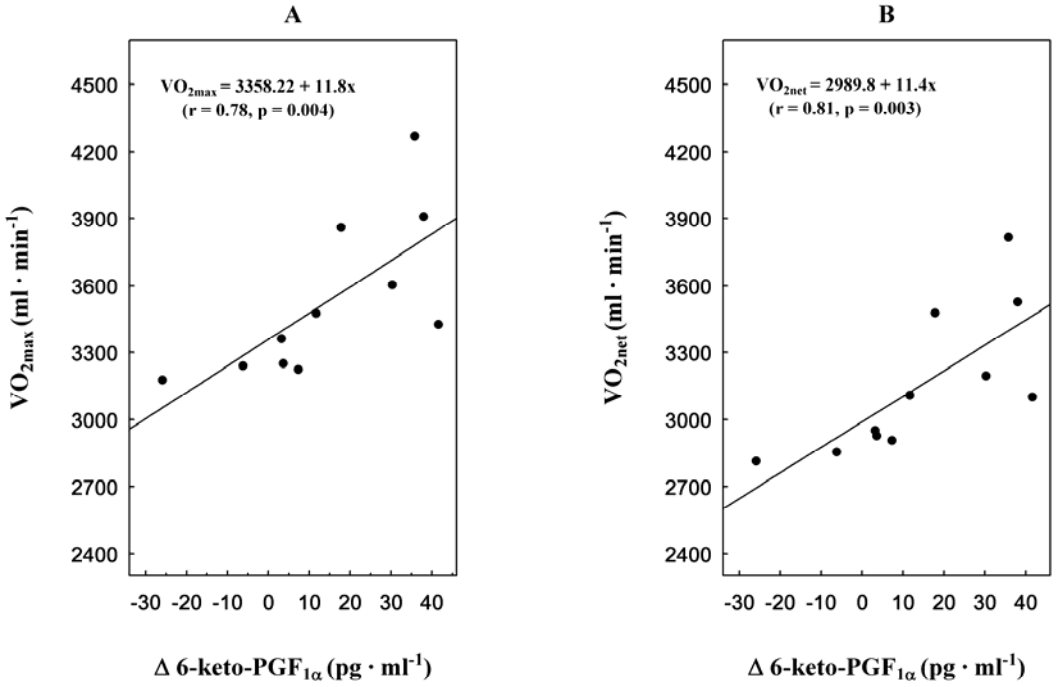


Figure 2. Correlation between maximal oxygen uptake ($VO_2$max) and the exercise-induced changes in plasma 6-keto-$PGF_{1\alpha}$ concentration, expressed as the difference between the end-exercise and pre-exercise concentration (Δ6-keto-$PGF_{1\alpha}$) (A) and the correlation between net $VO_2$ (expressed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

*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.

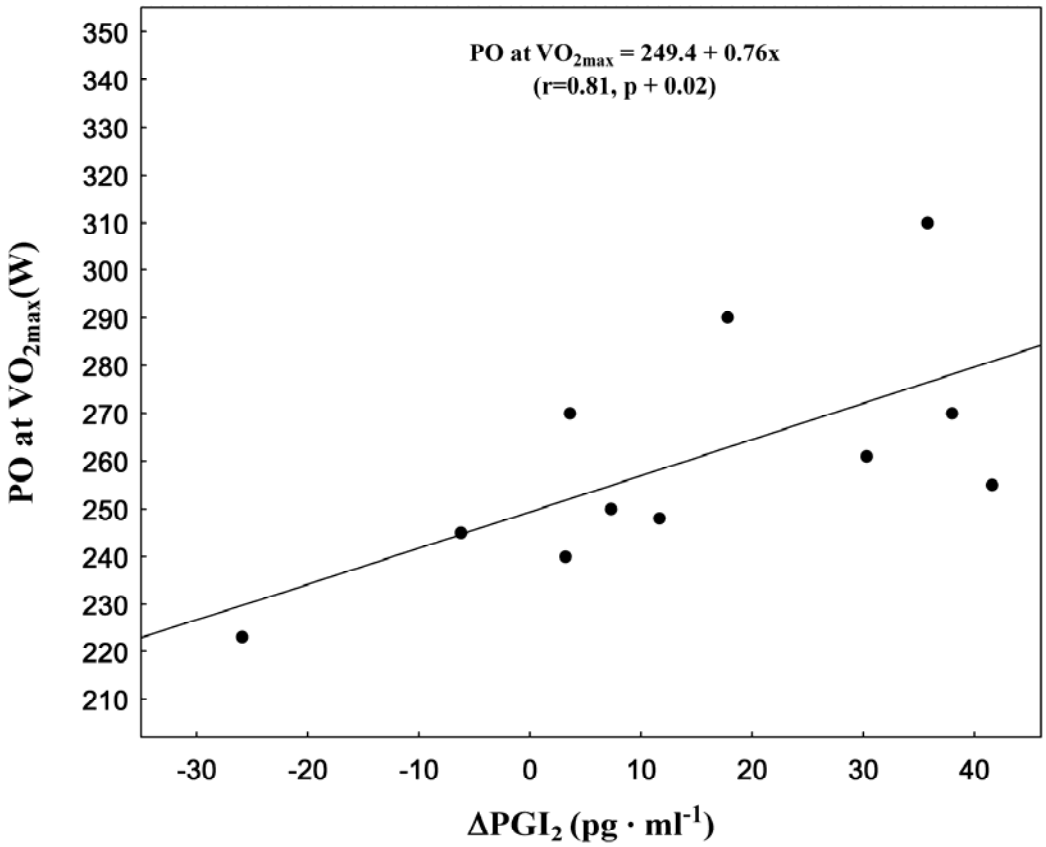


Figure 3. Correlation between power output reached at maximal oxygen uptake (PO at $VO_{2max}$) (the maximal power output during the incremental exercise) and the exercise-induced changes in plasma 6-keto-$PGF_{1\alpha}$ concentration, expressed as the difference between the end-exercise and pre-exercise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

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

No significant correlation was found between the exercise-induced increase in minute ventilation ($\Delta V_E$), respiratory quotient ($\Delta$RQ), heart rate ($\Delta$HR), plasma lactate concentration ($\Delta La^-$) and exercise-induced prostacyclin release, expressed by $\Delta$6-keto-$PGF_{1\alpha}$ concentration. Similarly, no significant correlation was found between the exercise-induced increase in plasma concentrations of IL-6 ($\Delta$IL-6), IL-10 ($\Delta$IL-10), TNF-$\alpha$ ($\Delta$TNF-$\alpha$) and $\Delta$6-keto-$PGF_{1\alpha}$ (see Table 2).

Table 2. Non-parametric correlations between the exercise-induced increase in minute ventilation ($\Delta V_E$), respiratory quotient (ΔRQ), heart rate (ΔHR), plasma lactate concentration ($\Delta La^-$) and plasma cytokines concentrations (interleukin – 6; ΔIL-6), interleukin – 10; ΔIL-10, tumour 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-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)** | **:** | **Δ$V_E$ ($l \cdot min^{-1}$)** | 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-$PGF_{1\alpha}$ ($pg \cdot ml^{-1}$)** | **:** | **Δ$La^-$ ($mmol \cdot l^{-1}$)** | 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 |

## DISSCUSION

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 the maximal oxygen uptake ($VO_2max$).

The main and original finding of this study is that the exercise-induced release of prostacyclin ($\Delta PGI_2$), detected as the difference between pre-exercise 6-keto-$PGF_{1\alpha}$ plasma concentration and its value reached at $VO_2max$, displays the significant positive correlation with the maximal oxygen uptake, with the net $VO_2$ increase at maximal power output (see

Fig. 2 A and B) as well as with the power output reached at $VO_2$max (the maximal power output reached during the incremental exercise test) (see 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 $VO_2$max (see Fig. 2 A). No increase in 6-keto-$PGF_{1\alpha}$ concentration was found ($p = 1.00$) in the group of subjects ($n = 5$) with the lowest $VO_2$max (see Fig. 1. note the difference ($p = 0.008$) in $\Delta PGI_2$).

The exercise-induced increase in $PGI_2$ metabolites measured in urine (see *e.g.* Koivisto *et al.* 1989, Wennmalm *et al.* 1990, Rasmanis *et al.* 1991, Ronni-Sivula *et al.* 1993, Boger *et al,* 1995), blood (see e.g. 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), yet 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). Of note, inflammatory stimuli like TNF-$\alpha$ increases $PGI_2$ production (Moore *et al.* 1991), however 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-$\alpha$

(see 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-$\alpha$ ).

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 $VO_2$max 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, 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 $\cdot$ $min^{-1}$ and consume about 0.8 l $O_2$ $\cdot$ $min^{-1}$ (*i.e.* about 320 ml $O_2$ $\cdot$ kg muscle $mass^{-1}$). They postulated that in sedentary men 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, $VO_2$max 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 determinates of maximal oxygen uptake (for review see *e.g.* 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 (see also 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, involvement of $PGI_2$ in the exercise-induced hyperaemia 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.,* demonstrated that erythrocytes express IP receptors, stimulation of which activates adenylate cyclase and the release of ATP that determines vascular resistance (Sprague *et al*. 2003, Sprague *et al.* 2005).

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 in a relatively small experimental group two subgroups of apparently healthy subjects with the lowest and highest $VO_2$max that correlated with lowest and highest release of $PGI_2$ (see Fig. 2 A). It could also well be 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 $PGI_2$ determine 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 in patients with heart failure compensatory increase in $PGI_2$ release and its augmented contribution to exercise-induced peripheral vasodilation 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 $VO_2$max (n = 5 in each group). However, in some subjects, with the lowest $VO_2$max, even a decrease in the $PGI_2$ concentration during exercise was observed (see Fig. 1 and Fig. 2 A). This findings seem 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 anti-atherogenic 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 $VO_2$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.

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