Physical exercise increases urinary excretion of lipoxin A₄ and related compounds

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rate approached the theoretical maximal heart rate (93–98%), which was calculated according to the formula 220 – age (yr). In two subjects, the test was interrupted, respectively, at 80 and 85% of the theoretical maximal heart rate because of muscular exhaustion. Maximal workload was 146.1 ± 44.9 W. Exercise duration was 13.3 ± 2.8 min. Urine and blood samples were collected after a recovery interval of 3 min from the interruption of the test and after 6 and 24 h.

All volunteers signed an informed consent form, and the study was approved by the local ethics committee.

**Extractions and reversed-phase-high-performance liquid chromatography.** LX were extracted from 5 ml of urine and suspended with 100 μl of methanol according to a recently published protocol (18). Extraction recovery was assessed by exogenously added prostaglandin (PG) B₂ measurements with a dual pump reversed-phase-HPLC gradient system equipped with a 996 photodiode array detector (Waters, Milford, MA) and a Waters Symmetry C₁₈, 3 μm, 2.1 ×150-mm column. Purun analysis was performed with the Millennium 32 chromatography manager. PGB₂ recovery was determined by comparing the peak area of the sample PGB₂ to a calibration curve constructed by injecting increasing amounts of authentic PGB₂ (Cascade Biochem, Reading, UK). Quantitation of the previously described urinary tetraene (18) was obtained by comparing the peak area of the tetraene with a standard curve constructed by injecting increasing amounts of authentic LXA₄.

**LXA₄ ELISA.** Immunoreactive LXA₄ (iLXA₄) levels were determined by using the LXA₄ ELISA kit, kindly provided by Neogen (Lexington, KY), as previously described (18). Briefly, 20 μl of extracted urine in methanol were taken to dryness and suspended with 150 μl of ELISA extraction buffer. Fifty microliters were used for iLXA₄ ELISA measurements in duplicate, as indicated by the manufacturer.

**Statistics.** Data are shown as means ± SD. The overall impact of exercise on iLXA₄ levels was evaluated by using one-way ANOVA with repeated measures. Comparisons between measurements at individual time points were analyzed with Wilcoxon’s signed rank test. P < 0.05 was considered statistically significant. All calculations were made with the computer program Stat-View II (Abacus Concepts, Berkeley, CA).

**RESULTS**

Urinary excretion of iLXA₄ during exercise was monitored by using a recently developed extraction method coupled to a commercially available ELISA kit (18). Exercise increased systolic blood pressure and heart rate from 115.6 ± 4.1 to 186.7 ± 5.3 mmHg and from 74.4 ± 11.6 to 171.2 ± 3.2 beats/min, respectively. It was also associated with a time-dependent variation in iLXA₄ excretion (P = 0.0016; 1-way ANOVA). In particular, a significant increment in urinary iLXA₄ was observed at the end of the exercise (0.061 ± 0.023 vs. 0.113 ± 0.057 ng/mg creatinine; P = 0.028; Wilcoxon’s test). These levels returned to baseline within 6 h to increase again, although at a lesser extent, after 24 h (P = 0.05; Wilcoxon’s test; Fig. 1). Notably, seven of the volunteers showed an increment that ranged from 17 to 383% above basal levels, whereas two of them did not display significant variations. No significant correlations were denoted between iLXA₄ levels at all time points and either exercise duration, maximal workload, or percentage of theoretical maximal heart rate.

Because in a recent report (18) our laboratory showed that a LXA₄-related material bearing the physical properties of a LXA₄ metabolite, including the typical tetraene structure, is excreted with human urine, we investigated the relationship between the ELISA-measured iLXA₄ and the amount of the urinary tetraene in the volunteers of this study. To this end, urinary tetraene was measured by RP-HPLC as previously described (18), and the values obtained were plotted as a function of the correspondent ELISA readings of iLXA₄, expressed as nanograms per milliliter. As shown in Fig. 2, a strong linear correlation (r = 0.988) was observed between the iLXA₄ ELISA readings and the RP-HPLC-based quantitations of the putative LXA₄ urinary metabolite, although individual ELISA measurements were ~60-fold lower than RP-HPLC quantitations of the tetraene in the corresponding sample. Also, materials eluted in the section of the chromatogram corresponding to the retention time of authentic LXA₄ were collected, concentrated, and subjected to ELISA. However, they did not give appreciable readings (results not shown). These results confirm the LXA₄-related nature of the urinary tetraene and indicate that urinary iLXA₄ is not native to LXA₄ but is rather a LXA₄-derived product.

**DISCUSSION**

Evidence indicates that physical stress is associated with the release of inflammatory mediators and with potentially prothrombotic cell-to-cell interactions (11, 13, 15, 27). However, it is generally accepted and
proved by a number of clinical studies that physical exercise is beneficial for the cardiovascular system, particularly in pathological conditions (24). To identify novel mechanisms that may counterbalance the effects of inflammatory mediators released during exercise, we measured urinary levels of the potent anti-inflammatory LXA_{4}. We observed a significant increase in iLXA_{4} urinary excretion after strenuous exercise in nine healthy volunteers (Fig. 1). Moreover, we established that the material recognized by the anti-LXA_{4} antibody of the ELISA assay is a tetraene, which is likely to represent a LXA_{4} metabolite (Fig. 2). Together, these results indicate that strenuous exercise may induce LX biosynthesis and further metabolism. In fact, LX bear the typical trait of autacoids, because they are rapidly formed on cell stimulation, act locally, and are rapidly metabolized and inactivated. Thus it is unlikely that exercise-induced urinary excretion of LX-related compounds may originate by an enhanced release of preformed, stored material.

Platelet and PMN may be the cellular sources of LX during exercise. Indeed, strenuous physical exertion is associated with the rapid formation of in vivo platelet/PMN aggregates and activation of both cell types (11). Earlier studies have shown that LX are formed during incubations of platelet with PMN, because platelet 12-lipoxygenase functions as a LX-synthase, being able to convert PMN-derived leukotriene A_{4} into LXA_{4} and B_{4} (7, 17). This biosynthetic pathway also occurs in vivo after atherosclerotic plaque rupture by coronary angioplasty, when nanogram amounts of LXA_{4} are formed (2). Thus the increase in urinary excretion of iLXA_{4} after exercise may be consistent with transcellular metabolic exchanges between activated platelets and PMN, although the contribution of additional sources cannot be excluded, because transcellular exchange-generating LX may also occur in the urinary tract (1). Along these lines, from the present results, it is difficult to firmly establish the anatomic district(s) involved in LX biosynthesis during physical exercise. Whether it reflects renal production, muscle release, or the summation of whole body vascular stress remains to be determined. Also, it is not clear whether the smaller increment in urinary iLXA_{4} observed 24 h postexercise has a similar cellular and regional origin as that denoted immediately after the exercise.

That a LXA_{4}-derived material (i.e., metabolite) appears in urine immediately at the end of strenuous exercise may be justified by the fact that in vivo LXA_{4} formation can be very rapid, because it can be observed within 10 s from angioplasty (2). Moreover, >60% of LXA_{4} is metabolized by peripheral blood monocytes within 30 s (21). Consistently, a rapid postexercise increment in the urinary levels of metabolites of other arachidonic acid-derived eicosanoids, namely thromboxane B_{2} and prostacyclin, has been documented (16, 28).

An increase in LX biosynthesis during exercise may have relevant pathophysiological implications. LX function as stop signals during inflammation, and their role in the resolution of the inflammatory response has been recently elucidated (10). Moreover, LX possess vasodilatory properties (3, 5, 6) and inhibit PMN adherence to microvasculature by downregulating P-selectin expression (20). Thus LX production in the course of physical exercise may, on one side, counterbalance the action of exercise-induced proinflammatory mediators and, on the other, may represent one of the mechanisms of the long-term beneficial effect of physical exercise on the cardiovascular system. In this respect, it has to be pointed out that LX generated on physical exercise are also potentially proresolving in local cellular damage; thus they may contribute to limit the extent of exercise-associated microtrauma. Whether the capability to produce higher LXA_{4} levels should be regarded as predictive of a lower incidence of vascular diseases remains to be established. In this respect, the variable extent among our volunteers of evidence of an increment in iLXA_{4} excretion in a non-strenuous exercise may, on one side, counterbalance the action of exercise-induced proinflammatory mediators and, on the other, may represent one of the mechanisms of the long-term beneficial effect of physical exercise on the cardiovascular system. In this respect, it has to be pointed out that LX generated on physical exercise are also potentially proresolving in local cellular damage; thus they may contribute to limit the extent of exercise-associated microtrauma.

In conclusion, this study represents the first in vivo evidence of an increment in iLXA_{4} excretion in a non-pathological state in humans. The present results may contribute to a better understanding of the inflammatory reaction during maximal exercise and confirm that the methodology recently developed in our laboratory (18) can be successfully applied to human studies.

The authors thank Neogen (Lexington, KY) for providing the LXA_{4} ELISA kits used in this study and Dr. Tommaso Virga for technical assistance.

This work was supported in part by grants from the Italian Ministero dell’Università e della Ricerca Scientifica (ex. 60%) (to M. Romano).
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