Physical exercise increases urinary excretion of lipoxin A_4 and related compounds

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Gangemi, Sebastiano, Graziella Lucchi, Etrusca D’Urbano, Agostino Mallamace, Domenico Santoro, Guido Bellinghier, Giovanni Davi, and Mario Romano. Physical exercise increases urinary excretion of lipoxin A_4 and related compounds. J Appl Physiol 94: 2237–2240, 2003. First published February 14, 2003; 10.1152/japplphysiol.01004.2002.—Lipoxins (LX) are lipoxygenase-derived eicosanoids with potent anti-inflammatory activities and vascular bed-dependent vasodilatory actions. LX can be formed in vitro and in vivo in a number of conditions, and we have reported that immunoreactive LX₄ (iLX₄) is physiologically excreted with human urine. Using a recently developed LX extraction method coupled to an ELISA, we examined whether iLX₄ excretion was modified by strenuous exercise, which is known to trigger potential LX-forming events. Maximal exertion significantly increased iLX₄ urinary excretion in nine healthy volunteers (0.061 ± 0.023 vs. 0.115 ± 0.057 ng/mg creatinine; P = 0.028). iLX₄ levels returned to baseline after 6 h and increased, although at a smaller extent, after 24 h. A significant correlation (r = 0.988) was denoted between iLX₄ ELISA measurements and reversed-phase high-performance liquid chromatography quantitation of a previously described urinary tetraene, confirming its LX₄-related nature. These findings show for the first time that an increase in excretion of LX₄-related compounds can be observed in response to strenuous exercise. This may be the reflection of an enhanced LX biosynthesis, which may represent a safeguard mechanism that keeps the inflammatory reaction triggered by physical stress under control.

arachidonic acid; lipoxygenase; inflammation; metabolism

LIPOXINS (LX) ARE TETRAENE-CONTAINING EICOSANOIDs that are generated by lipoxygenase transformation of arachidonic acid. Multiple routes of LX biosynthesis have been recognized that involve single cell types or cell-to-cell interactions (reviewed in Ref. 22). LX possess potent bioactions that may regulate key mechanisms of the immunoinflammatory reaction. In particular, LX₄ inhibits polymorphonuclear neutrophil (PMN) adhesion to human endothelial cells by reducing P-selectin expression (14) and blocks IL-6, IL-8, and metalloproteinase release (9, 23). Both LX₄ and B₃ stimulate monocytic cell migration and adhesion to laminin by using distinct signal transduction pathways (12, 19). LX and their recently discovered 15R epimers, aspirin-triggered LX (4), possess potent anti-inflammatory activity in vivo (25, 26), and temporal biosynthesis of LX, concurrent with spontaneous resolution, has been observed during exudate formation (10). LX₄ may contribute to the resolution of the inflammatory response by stimulating phagocytosis of apoptotic neutrophils (8).

Release of inflammatory mediators and multicellular activation can be observed during strenuous exercise. In particular, increased production of cytokines, chemokines, and arachidonic acid metabolites, as well as platelet-PMN activation and interactions, have been documented during maximal exertion (11, 13, 15, 27). However, no appreciably clinical signs of inflammation are commonly denoted after exercise, suggesting that homeostatic mechanisms may limit the extent and duration of the exercise-induced inflammatory response.

Because LX are potently anti-inflammatory and because potential LX-forming multicellular interactions occur during strenuous exercise, we evaluated urinary excretion of LX₄ and related compounds in healthy volunteers.

In this report, we show that maximal physical exercise is accompanied by a rapid increase in the urinary excretion of iLX₄, which is likely to reflect in vivo cell-to-cell interactions and to represent a defense mechanism against stress-induced inflammation.

METHODS

Subjects and study design. Nine healthy subjects, six men and three women, aged 29.3 ± 3.5 yr, were recruited for this study. They were all nonsmokers and free from drugs for at least 2 wk. Urine samples were collected after an overnight fast. Volunteers were then asked to rest for at least 30 min in supine position. Exercise was started on an electrically braked cycle ergometer (Cardioline ERG 602) with an initial workload of 25 W for 2 min that was incremented by 25 W every 2 min. Heart rate and blood pressure were constantly monitored, and the exercise was terminated when the heart rate reached 85% of age-predicted maximum. Volunteers were then allowed to rest for at least 30 min. Urine samples were collected after 6, 12, 24, and 48 h. Urine samples were stored at −80°C until analysis.

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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. Purophan analysis was performed with the Millennium 32 chromatography manager. PGB2 recovery was determined by reporting 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 StatView 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.

**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 LXA₄. We observed a significant increase in iLXA₄ urinary excretion after strenuous exercise in nine healthy volunteers (Fig. 1). Moreover, we established that the material recognized by the anti-LXA₄ antibody of the ELISA assay is a tetraene, which is likely to represent a LXA₄ 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 coinubcations of platelet with PMN, because platelet 12-lipoxygenase functions as a LX-synthase, being able to convert PMN-derived leukotriene A₄ into LXA₄ and B₄ (7, 17). This biosynthetic pathway also occurs in vivo after atherosclerotic plaque rupture by coronary angioplasty, when nanogram amounts of LXA₄ are formed (2). Thus the increase in urinary excretion of iLXA₄ 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 iLXA₄ observed 24 h postexercise has a similar cellular and regional origin as that denoted immediately after the exercise.

That a LXA₄-derived material (i.e., metabolite) appears in urine immediately at the end of strenuous exercise may be justified by the fact that in vivo LXA₄ formation can be very rapid, because it can be observed within 10 s from angioplasty (2). Moreover, >60% of LXA₄ 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 B₂ 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 LXA₄ 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 exercise-induced urinary LXA₄ increase in response to exercise, independently from exercise duration, maximal workload, or percentual increase of in vivo LXA₄ formation (16, 28).

In conclusion, this study represents the first in vivo evidence of an increment in iLXA₄ excretion in a nonpathological 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 LXA₄ 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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