To address the question of whether translocation of bacterial lipopolysaccharide (LPS) into the blood could be involved in the process of exercise-induced polymorphonuclear neutrophil (PMN) activation, 12 healthy male subjects who took part in a sprint triathlon (1.5 km river swim, 40 km bicycle race, 10 km road race) were studied. While there was no detectable amount of endotoxin in the blood samples drawn at rest, exercise was followed by the appearance of circulating endotoxin molecules at the end of competition in four subjects, and after one and 24 h recovery in three and seven athletes, respectively. The concentrations of plasma granulocyte myeloperoxidase ([MPO]) were significantly higher immediately after exercise and one hour later than baseline values ($P<0.001$). This variable returned to pre-race levels the day after exercise, despite the presence of detectable amounts of LPS, at that time, in seven athletes. The absence of significant correlation ($r=0.26; P=0.383$) and temporal association between [MPO] and plasma endotoxin levels led us to conclude that endotoxaemia was not involved in the process of exercise-induced PMN degranulation observed in our subjects.

**Key words:** Exercise, Endotoxin, Myeloperoxidase, Polymorphonuclear neutrophils

### Introduction

It is presently well admitted that long-term strenuous exercise is accompanied by transient endotoxaemia and activation of polymorphonuclear neutrophils (PMN). Because endotoxins [lipopolysaccharide (LPS) molecules] are potent activators of PMN, these findings raise the possibility that exercise-induced endotoxaemia could be involved in the process of PMN activation in exercising subjects. To test this hypothesis, we analyzed the changes in the plasma levels of LPS ([LPS]) and granulocyte myeloperoxidase (MPO) – taken as an *in vivo* marker of PMN activation – in 12 healthy male subjects who took part in a sprint triathlon.

### Materials and Methods

#### Subjects

Twelve male recreational triathletes aged 29±1 (SEM) years [mean body mass: 69±2 (SEM) kg] were studied. Ethical permission for the study was obtained from the Committee for Medical Ethics (Faculté de Médecine, Université de Liège). Written informed consent was obtained from all volunteers. They did not take any form of medication during the month preceding the study. The event included a 1.5 km river swim, a 40 km bike ride, and a 10 km road race.

#### Blood sampling and biochemical analyses

Two venous blood samples (10 ml) were collected in Vacutainers at the following time points: at rest, 3–5 days before the event (baseline); within 5–15 min after the end of the race; after 1 and 24 h recovery. Blood for the measurement of endotoxin was collected in pyrogen-free tubes (Endo tubes ET; Chromogenix AB, Mölndal, Sweden) containing sodium heparin (120 IU). Platelet-rich plasma was prepared by centrifugation at 2200 g for 15 min at 4°C, and samples were stored at –20°C. Blood used for the measurement of MPO was drawn on EDTA as anticoagulant. Plasma was removed after centrifugation for 10 min at 2500 g and stored immediately at –70°C until analysis. Plasma MPO and endotoxin concentrations were assessed according to the radioimmunological method described by

Possible *in vivo* tolerance of human polymorphonuclear neutrophil to low-grade exercise-induced endotoxaemia

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Pincemail et al.\textsuperscript{7} and a chromogenic \textit{Limulus} amebo-cyte lysate assay (Coatest plasma-endotoxin, Chromogenix AB; detection limit of 5 pg/ml), respectively. Plasma concentrations of MPO ([MPO]) and endotoxin ([LPS]) were adjusted for changes in plasma volume during and after exercise according to the method described by Dill and Costill.\textsuperscript{8}

**Statistical analysis**

Changes in MPO values as a function of time were assessed by Friedman’s test. Associations between endotoxin levels ranging from 5 to 23.3 pg/ml and corresponding MPO values were investigated using the Spearman rank correlation coefficient. The level of statistical significance was set at \( P<0.05 \).

**Results**

Time to complete the race averaged 150±5.4 (SEM) min. No trace of circulating endotoxin was found at rest. This substance was detected in eight subjects, most frequently the day after the race (7 cases out of 12) (Table 1). Immediately after the race and one hour later, detectable amounts of endotoxin were present in four and three samples, respectively. The plasma concentrations of this compound ranged between 5 and 23.3 pg/ml. The highest endotoxin value was detected in one subject after 1 h recovery (Table 1).

Mean plasma MPO concentrations measured at the end of competition and after one hour recovery were significantly higher than baseline (\( P<0.001 \)). Twenty-four hours later plasma MPO level had decreased to a level that did not differ significantly from the pre-exercise level (Table 1).

The relationship between [MPO] and [LPS] was not statistically significant (\( r=-0.261; \ P=0.383 \)), and the patterns of change of these variables as a function of time were fairly different (Table 1). For example, in subject 2, [MPO] rose from a resting value of 150 ng/ml to 1200 ng/ml at the end of exercise and one hour later. By the time these MPO concentrations were reached, circulating LPS levels were equal to 14.5 and 5 pg/ml, respectively. The day after the race, [LPS] reached its highest value of 17.5 pg/ml while [MPO] had decreased to 380 ng/ml. A similar conclusion can be drawn by analyzing the data of subject 9. In this case, a marked increase of [MPO] from 140 to 1190 ng/ml was observed despite the absence of any detectable amount of circulating LPS.

These findings led us to conclude that endotoxaemia, at least within the range of LPS concentration measured in the present study, was not involved in the process of exercise-induced PMN activation observed in our subjects.

**Discussion**

To our knowledge, the present study demonstrated for the first time that strenuous exercise lasting, on average, less than three hours can initiate the appearance of detectable amounts of endotoxin in the blood. Among the mechanisms thought to be involved in exercise-induced endotoxaemia are; (1) reduced splanchnic blood flow, and (2) hyperthermia, both leading to damage to the intestinal wall and subsequent release of endotoxin into the circulatory system.\textsuperscript{1,2}

Taken overall, the present data demonstrated that the magnitude of endotoxaemia was greater in triathletes than in subjects who took part in a

| Subjects | Rest | Immediately after | 1 h recovery | 24 h recovery |
|----------|------|------------------|--------------|--------------|
|          | LPS (pg/ml) | MPO (ng/ml) | LPS (pg/ml) | MPO (ng/ml) | LPS (pg/ml) | MPO (ng/ml) | LPS (pg/ml) | MPO (ng/ml) |
| 1        | 0    | 240             | 6.7          | 420          | 0            | 380         | 0            | 190          |
| 2        | 0    | 150             | 14.2         | 1200         | 5            | 1200        | 17.5         | 380          |
| 3        | 0    | 180             | 12.5         | 420          | 0            | 280         | 16.7         | 160          |
| 4        | 0    | 200             | 8.3          | 290          | 0            | 680         | 18.3         | 96           |
| 5        | 0    | 110             | 0            | 600          | 20           | 680         | 18.3         | 96           |
| 6        | 0    | 84              | 0            | 460          | 23.3         | 200         | 5            | 150          |
| 7        | 0    | 180             | 0            | 500          | 0            | 420         | 20           | 185          |
| 8        | 0    | 340             | 0            | 600          | 0            | 230         | 5            | 140          |
| 9        | 0    | 140             | 0            | 800          | 0            | 1190        | 0            | 250          |
| 10       | 0    | 150             | 0            | 520          | 0            | 180         | 0            | 84           |
| 11       | 0    | 130             | 0            | 220          | 0            | 150         | 0            | 64           |
| 12       | 0    | 240             | 0            | 280          | 0            | 180         | 0            | 200          |
| Mean     | 178.7| 525.8*          | 465.8*       | 189.9        |
| SEM      | 20.1 | 76.8            | 108.1        | 29.8         |

*Significantly different from resting values, \( P<0.001 \).
marathon competition. Furthermore, traces of endotoxin ≥5 pg/ml were detected more frequently in triathletes (in 29 versus 12% of the blood samples drawn according to the same protocol in triathletes and marathon runners, respectively).

The absence of temporal association and significant correlation between [MPO] and [LPS] strongly suggested that endotoxaemia was not the underlying mechanism of exercise-induced PMN activation. Furthermore, the return of MPO concentration to baseline, shown by the time detectable amounts of LPS molecules were found in seven subjects after 24 h recovery, indicated that the presence of this compound in the blood had no effect, per se, on the process of PMN degranulation, at least in our subjects, and within the range of LPS concentration measured. This apparent unresponsiveness of PMN to LPS could be considered as a further manifestation of the endotoxin tolerance phenomenon associated with repeated endotoxin challenge, but the extent to which its underlying mechanisms are similar to those responsible for the suppression of the plasma tumor necrosis factor response to LPS by prior exercise (thereby reflecting the beneficial effect of training on the host’s resistance to the detrimental effects of endotoxaemia) is an attractive hypothesis that should be experimentally verified.

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