Strenuous exercise causes systemic endotoxemia

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BOSENBERG, A. T., J. G. BROCK-UTNE, S. L. GAFFIN, M. T. B. WELLS, AND G. T. W. BLAKE. *Strenuous exercise causes systemic endotoxemia.* J. Appl. Physiol. 65(1): 106–108, 1988.— Eighteen triathletes were studied before and immediately after competing in an ultradistance triathlon. Their mean plasma lipopolysaccharide (LPS) concentrations increased from 0.081 to 0.294 ng/ml ($P < 0.001$), and their mean plasma anti-LPS immunoglobulin G (IgG) concentrations decreased from 67.63 to 38.99 μg/ml ($P < 0.001$). Both pretriathlon plasma LPS and anti-LPS IgG levels were directly related to the intensity of training ($P < 0.02$ and $P < 0.01$, respectively). It is possible that training-induced stress led to some leakage of LPS into the circulation, which, in turn, resulted in self-immunization against LPS. The effects on athletic performance in relation to exercise-induced changes in plasma LPS and anti-LPS IgG levels require further investigation.

lipopolysaccharide; anti-lipopolysaccharide; triathlon

WE HAVE PREVIOUSLY REPORTED that 81% of 89 exhausted runners requiring fluid resuscitation after an ultramarathon (90 km) had above-normal plasma lipopolysaccharide (LPS) endotoxin (1). A study in racehorses in which prerace levels of plasma LPS and anti-LPS immunoglobulin G (IgG) compared with those after the race revealed that the mean LPS level increased ($P < 0.02$) and the mean anti-LPS IgG level decreased ($P < 0.005$) as a result of the race (1a). The object of this study was to establish the extent to which systemic endotoxemia occurred in highly trained athletes after an ultratriathlon.

MATERIALS AND METHODS

Eighteen male volunteers were studied who participated in the Ohlssohns Ultratriathlon, which took place on April 26, 1987. The event consisted of a 3.2-km surf swim, followed by 140 km of cycling and then a 42.2-km standard marathon run. The race began at 0600 h, and all contestants included in the study arrived at the finish line between 1400 and 1500 h.

All participants were healthy both before and after the race. Venous blood samples were taken for LPS and anti-LPS IgG determination at the prerace meeting the day before the event, with the runners resting quietly. This sample coincided within 1–2 h of the postrace sampling time. The prerace training buildup for this event consisted of cross-training comprising swimming, cycling, and running over a 6-wk period. Weekly distances in each category were scored as shown in Table 1. The summed scores for each individual were taken as an index of overall training level and ranged from 3 to 11 (mean 6.3).

Within 0.25 h after the conclusion of the race, blood samples were taken and each runner was questioned about signs and symptoms of nausea, vomiting, or cramping. Times of completion of the race were also noted. The blood samples were centrifuged, and the plasma was removed under sterile conditions in a laminar flow hood and stored at −20°C. The samples were analyzed within a week. The chromogenic substrate modification of the *Limulus* amoebocyte lysate (LAL) (MA Bioproducts) technique was employed for the determination of LPS concentration in the plasma (3, 4). An enzyme-linked immunosorbent assay (ELISA) technique (5, 6) was used for measuring the relative plasma anti-LPS IgG concentrations.

Chromogenic LAL assay. During the quantitative chromogenic test, plasma was diluted 1:4 with sterile pyrogen-free water, heated for 10 min at 75°C, cooled on ice, and centrifuged as before (3, 4). Duplicate samples of 50 μl of heat-treated plasma and 50 μl of chromogenic LAL were incubated for 10 min in sterile depyrogenated glass tubes at 37°C. Thereafter, 100-μl aliquots of chromogen (1:1 solution of buffer and chromogenic substrate) (MA Bioproducts) were added and mixed. The tubes were incubated for a further 3–4 min. When adequate color had developed in the highest standard (optical density ≈ 1.0), the reaction was stopped with the addition of 100 μl of 25% acetic acid.

Plasma blanks were run for each samples (50 μl of heat-treated 1:4 dilution of plasma, 150 μl sterile water, and 100 μl 25% acetic acid) to account for the absorbance due to plasma alone. Optical density at 405 nm was read in a Titertek Multiskan plate reader. Results were standardized with calibration curves employing a reference LPS *Escherichia coli* 0111:B4. The calibration curves were sensitive to LPS from 0.02 ng/ml and were linear from 0.02 to 0.2 ng/ml LPS.

Recent studies have shown that when properly performed, the chromogenic substrate modification of the LAL test provides a quantitative measure of LPS in human blood (3). When this method was used to compare the recovery of LPS from spiked pyrogen-free saline and pooled plasma (measured simultaneously over a appropriate range of concentrations), no discrepancies in values obtained in the two solutions were found (12).

ELISA procedure. Plasma was centrifuged for 5 min at 16,000 *g* before analysis. Triplicate samples were di-

TABLE 1. *Prerace training and respective scoring of subjects*

Swim per week, km	3	3–5	5–8	8–10	10
Score	1	2	3	4	5
Cycle per week, km	100	150	200	250	300
Score	1	2	3	4	5
Run per week, km	50	75	100	125	150
Score	1	2	3	4	5

TABLE 2. *Lipopolysaccharides and anti-lipopolysaccharide IgG before and after the race*

	Before	After
	Lipopolysaccharides, ng/ml	
Mean	0.081	0.294*
Range	0.057–0.115	0.063–0.510
SE	0.004	0.042
	Anti-lipopolysaccharide IgG, μg/ml	
Mean	67.63	38.99*
Range	11.32–324.36	7.773–142.97
SE	13.22	9.29

IgG, immunoglobulin G. * $P < 0.001$.

luted 1:100 in 0.1 M NaCl containing 0.05 M tris(hydroxymethyl)aminomethane, adjusted to pH 8.0, and 2% each of sheep and bovine plasma. The LPS-precipitable IgG was then assayed with plates coated with 14 endotoxins at a concentration of 10 μg/ml in a carbonate-bicarbonate buffer, pH 9.6. The 14 endotoxins were obtained from Difco (Detroit, MI). They were from *Salmonella typhimurium, Salmonella minnesota, Salmonella abortus equi, Salmonella enteritidis, Salmonella typhosa, Shigella flexneri, Serratia marscens, E. coli* 0127:B8, *E. coli* 0111:B4, *E. coli* 026:B6, *E. coli* 055:B5, *E. coli* 0128:B12, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Microtitration plates (Dynatech M129B) were coated with a mixture of endotoxins for 2 h at room temperature. Conjugate of antibody to human IgG with horseradish peroxidase was employed. Results were measured by absorption at 495 nm and calibrated by an immunoprecipitin reaction (8) with human anti-LPS IgG standard. With the latter standard, anti-LPS IgG was quantitated as described.

Statistical analysis was performed with the use of Student's paired t test and Pearson's linear correlation coefficient. Significance was accepted at the 5% level.

RESULTS

The mean age of the competitors was 33 yr (range 22–51 yr), and their mean weight was 76 kg (range 60–100 kg). The environmental conditions of the race were fair, with a moderate northeasterly wind. The vast majority of the triathletes ran through the halfway point in an ambient temperature of 23.1°C, whereas the temperature at the finish line was 22.3°C.

The mean LPS concentration increased from a mean of 0.081 to 0.294 ng/ml ($P < 0.001$), whereas the mean antibody IgG fraction decreased from 67.63 to 38.99 μg/ml ($P < 0.001$) (Table 2).

Muscle cramps predominantly involving the calves, quadriceps, and gluteals were more likely to occur in heavier runners ($P < 0.01$). Although the levels of LPS rose in all runners during the race, the increase tended to be greater in runners who cramped than in runners who did not cramp ($P < 0.10$). All cramps were relieved by stretching, massaging, and application of ice. There was no statistical significant correlation between endotoxemia and nausea and vomiting in this small study. However, in a previous study there was such a correlation (1).

Prerace anti-LPS IgG and LPS levels were both directly related to the extent of training ($P < 0.02$) ($r = 0.61$, $t = 2.81$; $r = 0.59$, $t = 2.64$ respectively). Cramping tendency appeared to be inversely related to both intensity of training and prerace anti-LPS IgG, although the associations did not attain statistical significance.

DISCUSSION

Large amounts of gram-negative bacteria and endotoxins are normally present in the intestines. Endotoxins are highly toxic LPS that form part of the outer cell wall of the gram-negative bacteria. Occlusion of or reduction in splanchnic blood flow (10) hypoxia (7) or elevated core temperature (9) damages the intestinal wall and leads to excessive leakage of LPS into the portal circulation, overwhelming the ability of the liver's reticuloendothelial system (RES) to remove LPS from the circulation. When splanchnic flow is restored to normal, RES function returns the LPS to normal base-line values in 1–2 h (7).

Our findings of a significant increase in LPS after the race seem to confirm the work by Cannon and Kluger (2), who found an increased endogenous pyrogen activity in human plasma after exercise.

The reason for the significant ($P < 0.001$) increase in LPS as a result of the race may be that endurance athletes probably have a reduced splanchnic blood flow, due to the combination of adrenergic activation and deviation of a high percentage of blood volume to the muscles as well as an elevated core temperature compounded by dehydration, all leading to an increased rate of breakdown of the gut barrier to LPS (1a). This in turn leads to an increase in plasma LPS level together with a fall in anti-LPS IgG. Our results seem to lend credibility to the hypothesis that well-trained athletes have a higher than normal anti-LPS IgG, possibly due to the repeated leakage of small amounts of gut-derived LPS during intensive endurance training, which leads to specific antibody production.

There may also have been alterations in specific IgM and IgA plasma concentrations, but these were not measured because IgG was considered to be more important (11, 13).

The finding that a raised prerace anti-LPS IgG level and a high level of training appear to reduce cramping tendency has already been mentioned. It is possible that each of these two factors confers a measure of protection from cramping. However, prerace anti-LPS IgG levels and training intensity are themselves highly intercorrelated ($r = 0.61$; $P < 0.02$). An analysis of covariance based on the results from our runners ($P < 0.25$) suggests that, for any given level of training, the circulating level

of anti-LPS IgG in runners who cramp is less than in runners who do not cramp.

It can be concluded that endotoxemia occurs in humans after strenuous exercise. The importance of the plasma LPS and anti-LPS IgG in relation to endurance athletes' performance requires further investigation.

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