Effects of passive hyperthermia versus exercise-induced hyperthermia on immune responses: hormonal implications

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ABSTRACT. Different stress hormones are released during prolonged exercise and passive hyperthermia. We hypothesized that these different hormonal responses could contribute to the different changes in the immune response during these two challenges.

Methods. Eight subjects completed three trials in a randomized order. In the control trial (C), the subjects remained in a sitting posture for three hours in thermoneutral conditions. In the exercise hyperthermia trial (E), they exercised for two hours on a treadmill at 65% VO2 max in thermoneutral conditions, followed by 1-h recovery in thermoneutral conditions; in the passive hyperthermia trial (PH), the subjects remained in a semi-recumbent position in a climatic chamber for two hours in hot conditions, followed by 1-h recovery in thermoneutral conditions. During the E and PH trials, wind speed and thermal conditions were modulated to reach a rectal temperature (Tre) of 38.5°C at 60 min and 39°C at 120 min. The subjects did not drink during the experiments. Blood samples (10 mL) were taken at 0, 60, 120 and 180 min of each trial. The total white cell count and its subsets were measured; plasma catecholamines, cortisol and prolactin were assayed. In a whole blood assay, blood leukocytes were stimulated by lipopolysaccharide (LPS) or phytohemagglutinin (PHA) for 24 and 48 hours, respectively. Cytokines, such as TNF-α, IL-10 and INF-γ were measured in the culture supernatant.

Results. The plasma levels of catecholamines were increased only during E, prolactin was increased only during PH, and cortisol was increased in both E and PH. Only the exercise caused a mobilization of blood leukocytes and leukocyte subsets. The INF-γ and TNF-α production by PHA- and LPS-stimulated blood, respectively, were inhibited in a substantial way in both E and PH compared to control when Tre reached 39°C. Only LPS-induced IL-10 production was enhanced during the exercise. The effects of the challenges were increased with 39°C compared to 38.5°C.

Conclusions. Catecholamines play a major role in the mobilization of immunocompetent cells and the production of IL-10 during exercise. Prolactin and catecholamines have adverse role on the immune response, whereas cortisol exerts similar effects during both trials. The consequence could be a protection against inflammatory overshooting.

Keywords: exercise, passive hyperthermia, catecholamine, cortisol, prolactin, immune response
the proviso of reproducing the same evolution of body temperature during the two tests. In addition, there are well-known tests to evaluate the functional response of immunocompetent cells: the stimulation with lipopolysaccharide (LPS) and phytohemagglutinin (PHA). LPS stimulates primarily monocytes and B lymphocytes [15], whereas PHA stimulates primarily T lymphocytes. This response causes the production of several cytokines. We chose to investigate the production INF-γ and TNF-α, which are the major pro-inflammatory cytokines [16], and IL-10 which is one of the major anti-inflammatory cytokines [16]. We also chose not to include water or carbohydrate intake during the recovery period.

METHODS

Eight subjects participated in the study. Age, weight and height (mean ± sem) were 23 ± 1.5 years, 69.2 ± 2.8 kg and 172 ± 2 cm respectively. The selection of the subjects was based on a normal clinical investigation that comprised detail medical history, physical examination and general blood screening. The subjects were required to be regularly trained for endurance, and unaccustomed to heat exposure. Procedures were carried out with the written informed consent of the subjects. The protocol was approved by the regional ethics committee (CCPPRB, Grenoble). Their physical fitness was estimated from the maximal O₂ uptake (VO₂ max), during a progressive treadmill test using a breath-by-breath automated gas exchange system (MedGraphics CPX/D, Medical Graphics Corporation, St Paul, MN, USA); the average result was 52.0 ± 2.2 mL.min⁻¹.kg⁻¹. The experiment took place in winter and spring.

Research design

A complete cross-over design was used in which each subject took part in three trials. Each of these trials was separated by at least 15 days. Three days before each trial the subjects were asked to refrain from strenuous exercise and to drink at least 2 L of water per day to ensure euhydration.

At the beginning of each experiment, the subjects arrived at the laboratory at 8:30, after a standard breakfast, and dressed themselves in shorts. The subjects emptied their bladder, were weighed and a polyethylene catheter (Angiocath 20GA 2in, Becton Dickinson, Sandy, UT, USA) was inserted in an antecubital vein of one arm. Throughout the experimental session, rectal temperature (Tre) was measured 2 every 30 min over a 10-min period.

During the E trial, the exercise intensity was checked by measuring VO₂ max in controlled thermal conditions (Tdb = 21-22°C, rH = 35-45%). The wind speed was modulated to ensure a rectal temperature = 38.5°C after 60 min of exercise, and 39°C after 120 min of exercise. Following the exercise, the subjects were dried, and stayed for 30 min at Tdb = 21-22°C in a sitting position, and 30 min in a standing position until the final blood sample was taken.

In the passive hyperthermia trial (PH), the subjects remained in a semi-recumbent position in a climatic chamber. The method used for the passive heating session has been described by Henane and Valatx [17]. Briefly, the subjects were asked to lie down on a balance (TESTUT 9009, Bethune, France; sensitivity = 3g) to measure the sweat loss. A copper-constantan thermocouple was insulated and inserted in the auditory canal. Climatic parameters were then adjusted (successively Tdb = 45°C, rH = 70% and Tdb = 50°C, rH = 30%) to reach a rectal temperature = 38.5°C at 60 min, and 39°C at 120 min. After the period of hyperthermia, the subjects stayed in a semi-recumbent position for a 60-min recovery period under controlled thermal conditions (Tdb = 21-22°C, rH = 35-45%), until the last blood sample was taken. During the three trials, a blood sample (10 mL) was taken at 60, 120 and 180 min. The subjects then emptied their bladder and were weighed. The subjects were not rehydrated during any of the experiments.

Physiological measurements

During the E trial, the exercise intensity was checked by measuring VO₂ max every 30 min over a 10-min period.

Plasma volume changes

Variations of PV changes (ΔVP) were calculated over time from hematocrit and hemoglobin concentration variations according to the Dill and Costill equation [18]. The hematocrit was multiplied by the factor (0.96*0.91) to correct for trapped plasma and to convert the venous hematocrit to a whole body hematocrit, and the hemoglobin was multiplied by the factor 0.92 to convert the venous hemoglobin to whole body hemoglobin according to Harrison et al. [19]. The calculations of Dill and Costill equations used the reference points adapted to the posture.

Hormonal analyses

After collection of blood into tubes containing lithium-heparin, samples were centrifuged at 3000 g for 10 min. The supernatant was removed and stored at −80°C until analysis. Plasma catecholamines were measured using high performance liquid chromatography with electrochemical detection. Plasma cortisol (ref TKC01), and plasma prolactin (IRMA Count ref IKPR1) were assayed using commercial kits (DPLC, La Garenne Colombes, France).

Leukocyte counts and hematocrit and hemoglobin measurements

Blood (3 mL) was placed in EDTA tubes and analyzed for differential white cell counts as routinely performed on PENTRA 120 Retic (ABX-France, Montpellier, France). This analysis included hematocrit, hemoglobin measure-
ments, determination of total white blood cell numbers and neutrophil, monocyte, and lymphocyte numbers in order to detect changes in circulating white blood cell populations.

**Whole blood assay**

Venous blood was collected in heparinized tubes (15 IU/mL blood; sodium heparin, ref 6541, Becton Dickinson, Rutherford, NJ, USA). One hundred μL of whole blood aliquots were diluted with 400 μL of RPMI 1640 (Sigma-Aldrich ref 50883, L’île d’Abeau, Chesnes, France), penicillin 100U.mL⁻¹ and streptomycin 100 μg.mL⁻¹ (Gibco ref 15140 and 15122), glutamine 4mM.mL⁻¹ and mercaptethanol 5x10⁻³ mol.mL⁻¹. Diluted blood was stimulated either by LPS 1μg.mL⁻¹ for 24h (E. coli, serotype, 055B11), either by PHA 50 μg.mL⁻¹ (Sigma-Aldrich ref L6143) for 48h. Twenty four h and 48h corresponded to the maximum cytokines production. Non-stimulated controls were examined under the same conditions.

During the incubation, the tubes were placed at 37°C + 5% CO₂. After the incubation, the tubes were spun for 10 min at 400 g, and the supernatants were collected and stored at -80°C until assayed.

Cytokines were assayed in the supernatants with a Bio-Plex system analyzer (Luminex X Map technology, Biorad, Marnes la Coquette, France) and Human cytokine 2-plex for the detection of IL-10, TNF-alpha (ref X50000005H, Biorad, Marnes la Coquette, France) for the blood stimulated with LPS and Human cytokine 2-plex for the detection of IL-10, INF-gamma (ref X50000001V, Biorad, Marnes la Coquette, France) for the blood stimulated with PHA. The minimum detectable concentrations were < 2 pg.mL⁻¹.

**Statistical analysis**

Data analysis was performed with the Statistica® package (Statsoft Inc, Tulsa, Oklahoma, USA). Statistical differences were calculated with a two-way repeated-measures analysis of variance design; when an overall difference was found, individual stages were compared with the Tukey post hoc test. Data are presented as mean ± SEM, and the null hypothesis was rejected when p < 0.05 for all analyses.

**RESULTS**

**Changes in rectal temperature (Tre)**

In the PH and E trials, Tre increased gradually, reaching 38.5°C by the end of the 60-min challenge, and 39°C by 120 min, while the values remained steady around 37°C at the time of the test control (p < 0.001 compared to C) (figure 1).

**Plasma volume changes (ΔPV) and fluid losses**

Plasma volume (table 1) did not change significantly during the C and E trials, but showed an marked fall during PH (p < 0.001 compared to C and E). Because of this marked fall, all the values were corrected according to ΔPV. The percentage of dehydration was 0.6 ± 0.1%, 3.5 ± 0.1% and

![Figure 1](http://example.com/figure1.png)

**Figure 1** Changes in Tre (°C) over time in C (●), PH (■), and E (▲) trials. Values are means ± SEM.

ccc: significant difference from control trial (p < 0.001).

3.5 ± 0.1% of body weight at the end of the C, PH and E tests, respectively.

**Plasma epinephrine and norepinephrine**

Plasma catecholamines did not change significantly during the C and PH trials (figure 2). However, they rose markedly during the exercise (p < 0.001 compared to C and PH, p < 0.01 compared to time 0). The plasma rates returned to the reference values by the end of recovery (p < 0.01 compared to time 120 min). During the exercise, plasma epinephrine was at 3332 ± 517 nmoL.L⁻¹, 3932 ± 608 nmoL.L⁻¹ and 1447 ± 213 nmoL.L⁻¹, and plasma norepinephrine at 5843 ± 944 nmoL.L⁻¹, 11176 ± 1629 nmoL.L⁻¹ and 2981 ± 554 nmoL.L⁻¹ at time 60-min 120-min and 1-h recovery, respectively.

**Plasma cortisol and prolactin**

The circulating cortisol rates showed a progressive and significant fall during the C test, in relation to the circadian rhythm (p < 0.05 compared to time 0) (figure 3). The levels increased at 120 min during the E and PH tests (16 ± 1 μg.dL⁻¹, 15 ± 1 μg.dL⁻¹ respectively, p < 0.05 compared to the C trial). Plasma cortisol then continued to increase after the exercise (19 ± 2 μg.dL⁻¹, p < 0.001 compared to C and PH, p < 0.01 compared to time 120 min), while it returned to the basal values during the recovery of PH (12 ± 1 μg.dL⁻¹, p < 0.01 compared to time 120 min). Plasma prolactin did not change significantly during the C trial. The concentrations increased slightly but nonsignificantly during the exercise when Tre was at 39°C. It showed a large increase at time 60 and 120 min during PH (19 ± 4 mg.mL⁻¹ and 21 ± 2 mg.mL⁻¹ respectively, p < 0.001 compared to C and E, p < 0.01 compared to time 0 min), and returned to basal values by the end of the recovery (9 ± 1 mg.mL⁻¹, p < 0.01 compared to time 120 min).

**Total leukocytes and subsets changes**

The exercise induced a significant mobilization of circulating leukocytes, neutrophils, lymphocytes and monocytes (p < 0.001 compared to C and PH) (table 1). The number of leukocytes, and subsets showed a 100% increase. A peak of neutrophils appeared at the end of the recovery during the PH (+39%) and E trials (+180%)
Table 1
Mean values (± SEM) of plasma changes (ΔPV), leukocytes (10^3.mL^-1), neutrophils (10^3.mL^-1), lymphocytes (10^3.mL^-1) and monocytes (10^3.mL^-1) changes corrected for ΔPV

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>end of recovery</th>
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<tr>
<td>ΔPV (%)</td>
<td></td>
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<tr>
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<tr>
<td>E</td>
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<tr>
<td>C</td>
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<tr>
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<td>0.63 ± 0.06</td>
<td>0.83 ± 0.08</td>
<td>0.58 ± 0.05</td>
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ccc: significant difference from C trial (p < 0.001); eee: significant difference from E trial (p < 0.001); hhh: significant difference from H trial (p < 0.001); *: significant difference from time 0 (p < 0.05); §: significant difference from time 0 (p < 0.05).

Figure 2
Changes in plasma epinephrine and norepinephrine (nmol.mL^-1) corrected for APV over time in C (●), PH (■), and E (▲) trials. Values are means ± SEM.
ccc: significant difference from control trial (p < 0.001).
hhh: Significant difference from H trial (p < 0.001).
§§: significant difference from Time 0 (p < 0.01).
++: significant difference from Time 60 min (p < 0.01).

Figure 3
Changes in plasma cortisol (µg.dL^-1) and prolactin (mg.mL^-1) corrected for APV over time in C (●), PH (■), and E (▲) trials. Values are means ± SEM.
ccc: significant difference from control trial (p < 0.001).
hhh: significant difference from H trial (p < 0.001).
eee: significant difference from E trial (p < 0.001).
*: significant difference from Time 0 (p < 0.05).
Relative changes in the release of TNF-α after stimulation by LPS over time in C (●), PH (▲), and E (▼) trials. Values are means ± SEM.
ccc: significant difference from control trial (p < 0.001).
ccc: significant difference from H trial (p < 0.001).
ccc: significant difference from control trial (p < 0.001).

Values are means ± SEM. (p < 0.05 compared to time 120 min). The monocytes remained high at the end of the recovery (+65%, compared to C and PH, p < 0.05). Except that, the concentrations of the leukocytes, lymphocytes and monocytes did not change during C and PH.

LPS-induced TNF-α and IL-10 production

The controls produced negligible quantities of cytokines in the supernatants (figure 4). As the absolute values were very different from one individual to another, the results of cytokines assays on cell culture supernatant are presented as relative changes. The response to LPS increased slightly, but not significantly during the C trial. We observed a reduction in TNF-α at the end of the PH and E trials when Tre = 39°C (ΔTNF-α = -16 ± 6% and -21 ± 8% for the PH and E trials, respectively, p < 0.001 compared to C). This reduction was clearly amplified at the end of recovery (ΔTNF-α = -32 ± 3% and -38 ± 6% for the PH and E trials, respectively, p < 0.001 compared to C, p < 0.001 compared to Time 60 min). The production of TNF-α during PH and E was similar in spite of a different number of monocytes. In addition, only the exercise stimulated IL-10 production after stimulation of blood with LPS (ΔIL-10: = +59 ± 10% and +79 ± 19 at time points 60 and 120 min respectively, p < 0.001 compared to C and PH, p < 0.001 compared to time 0 min).

PHA-induced INF-γ and IL-10 production

The controls produced negligible quantities of cytokines in the supernatants. E and PH induced a decrease in the production of INF-γ, as shown in figure 5, at the end of the 39°C challenge until the end of recovery. At 39°C, ΔINF-γ = -23 ± 5% and -23 ± 11% for the PH and E trials, respectively (p < 0.001 compared to C and PH, p < 0.001 compared to time point 60 min). The production of INF-γ during the PH and E trials was similar in spite of a different number of monocytes. At 1-h recovery, ΔINF-γ = -38 ± 7% and -66 ± 8% for the PH and E trials, respectively (p < 0.001 compared to C, p < 0.001 compared to time point 60 min). At this time, the difference between PH and E was not significant. We did not observe a significant effect of E and PH on the production of IL-10.

DISCUSSION

The Tre time courses were similar during E and PH. However, the stress hormones responses were different according to the nature of the challenge. The plasma levels of catecholamines were increased only during E, prolactin was increased only during PH, whereas cortisol was increased during both E and PH. In the same period, the main results of the immune responses showed that only the exercise caused a marked mobilization of blood leukocytes and leukocyte subsets. The TNF-α and INF-γ production by stimulated blood was inhibited in an substantial way in both E and PH compared to the control when Tre reached 39°C. IL-10 production was enhanced only during exercise when blood was stimulated with LPS. Both challenges raised Tre, to 39°C compared to 38.5°C, and were still effective 1 h after the end of the challenges. As has been previously shown [20], the present results confirm that, for the same level of dehydration, plasma
volume is better preserved during exercise than during passive heat exposure. The marked fall in PV during PH justifies the correction of hormonal values according to ΔPV.

**Hormonal changes**

The differences that we observed in the hormonal responses are in good agreement with the current state of knowledge. Exercise increases catecholamines, but passive hyperthermia does not. The lack of increase in plasma catecholamines during passive hyperthermia versus exercise has already been described [2, 3], with a rise in core temperature of 0.7°C for Brenner et al. [1997] [2], and 2°C, for Melin et al. [3], as in our study.

During prolonged, submaximal exercise, increases in cortisol levels coincide with a decrease in blood glucose concentration [1]. It is probably for this reason that it increased from the 60th min of exercise in our study, but not in the study of Brenner et al. [2], where the exercise was shorter and less intense. The increase in cortisol concentrations during passive heating seems however, to have had more attention. For Möller et al. [13], a 3-h warm bath induced a peak of circulating cortisol, while for Brenner et al. [2] two bouts of 30-min in a climatic chamber at 40°C, 30% relative humidity did not alter the concentrations of this hormone. In our study, the increase in plasma cortisol appeared between 60 and 120-min of passive heating. Won and Lin [21] have shown in mice that passive thermal stress produced marked accumulation of adrenal cortisol. One can suppose that the exposure time to heat and the level of relative humidity (which intensifies the challenge with heat) are factors that can explain the differences between our results and Brenner’s. Plasma cortisol returned to basal levels during PH recovery, because the heat challenge had disappeared. However, during recovery from the exercise, plasma cortisol was significantly increased compared to the levels observed at the end of the exercise. Utter et al. [22] have shown that lack of carbohydrate substrate availability could induce higher levels of cortisol. In our study, the long duration of the exercise and the lack of water and carbohydrate supply could explain the cortisol peak at 1-h of recovery.

Plasma prolactin concentrations increased slightly, but nonsignificantly during exercise when Tre was at 39°C, but showed a clear increase during PH. Our results are in agreement with the current state of knowledge [4]. Circulating plasma prolactin concentrations increases during exercise are largely in response to the ambient temperature, with lower rises for a thermoneutral ambient temperature [23]. However, an increase in body temperature at rest induced by external heating is a stimulus for prolactin secretion [4]. Thus, these different hormonal responses may have a role in the differences in immune responses observed during E and PH.

**Immune responses**

Only exercise induced a significant mobilization of circulating leukocytes and leukocyte subsets. It is now well known that catecholamines are responsible for this effect [24]. This is why Kappel et al. [14] observed leukocytosis during passive hyperthermia by immersion in warm water; they also saw an increase in plasma catecholamines. In our study, there were no plasma catecholamine changes and no leukocytosis during PH. However, these changes in immuno-competent cells can also depend on the Tre. Dubose et al. [25] have observed leukocytosis in subjects presenting exertional heat injury, with Tre > 40°C. During the recovery, the neutrophils were increased in both E and PH trials. This could be an effect of cortisol according to Rhind et al. [12], and also of granulocyte colony stimulating factor (G-CSF), according to Ellis et al. [26]. E and PH induced a marked decrease (40%) in the production of TNF-α by immune blood cells stimulated with LPS from the end of the 39°C challenge to 1-h recovery, while there was no change during the C trial. However, if these results are compared against the monocyte count changes, the TNF-α production by monocytes is higher during PH than during E. To our knowledge, there are very few studies on this subject. Starkle et al. [27, 28] found by flow cytometry, that there was a decrease in the amount of TNF-α per cell post-comapt with pre-exercise, which is in good agreement with our results. For Kappel et al. [8], hyperthermia after immersion did not influence the production of cytokines from stimulated blood cells. In vitro, when cells are stimulated with LPS and submitted to a pre-stress with a mild heat shock, cytokine production is decreased compared with controls [7, 9, 29, 30]. Cortisol plays an important role in the inhibition of TNF-α secretion [11, 16]. The higher TNF-α production by monocytes with PH could be an effect of prolactin [10]. Catecholamines may be implicated in the low production of TNF-α by monocytes, since, in the blood of healthy volunteers, epinephrine reduced the TNF-α mRNA concentration after stimulation by LPS, as reported by Bergmann [31]. They play a part, nevertheless in the monocyte count increase. Exercise strongly enhanced LPS-stimulated IL-10 production (+70%), while there was no variation in the IL-10 production during PH. This increase could be due to the increase in the number of monocytes induced by catecholamines [24]. Moreover, they enhance the production of IL-10 in human whole blood cultures stimulated with LPS [31]. These effects are mediated by stimulation of β-adrenoceptors [32]. In the blood of healthy volunteers, epinephrine increased the LPS-stimulated IL-10 blood production by 77.8%. The absence of an increase in the plasma catecholamines and so, the absence of change in the monocyte count, explains the absence of IL-10 production during PH.

E and PH induced a marked decrease (60%) in the production of INF-γ by immune blood cells stimulated with PHA from the end of the 39°C challenge to 1-h recovery, while there was no change during the C trial. If these results are set against the lymphocyte count changes, the INF-γ production by lymphocyte is higher during PH than during E. It is acknowledged that PHA-stimulated cell responses decrease during exercise, as estimated by flow cytometry [33-35] and fluorescence intensity methods [25]. Mitchell et al. [35] observed, as we did, a greater decrease during the recovery period, with complete restoration 24 h after the end of the exercise. To our knowledge, there are few data dealing with the effect of passive hyperthermia on the response to mitogens. According to Dubose et al. [25], T lymphocyte subsets showed reduced PHA responses both...
Effect of body temperature on cytokine production by blood cells

in exertional heat injury subjects and exercised subjects. However, the reduction was significantly greater in heat injury subjects compared to exercise subjects without heat illness. In this study, the Tre of exertional heat injury subjects was above 40°C. Another experiment using immersion in warm water [6], showed that the stimulation of lymphocytes of hyperthermic men which would have an inductive role on the secretion of INF-γ, produces as much as 10-fold more INF-γ than cells taken at basal temperatures from the same individuals. In our study, both exercise and passive hyperthermia suppress the production of INF-γ, probably by a direct effect of cortisol [11, 16]. The higher TFN-α production by monocytes with PH could be an effect of prolactin, which would have an inductive role on the secretion of INF-γ [10]. At 1-h recovery, the suppression of the production of TFN-α was less for the PH trial. This could be explained by the lower cortisol levels and also by an effect of prolactin. We did not observe any significant effect on IL-10.

CONCLUSION

We screened only a part of the immune response. After stimulation of monocytes and T-lymphocytes, cortisol and catecholamines inhibit the production of pro-inflammatory cytokines, while prolactin stimulates it. So prolactin and catecholamines have conflicting roles and this might explain the less marked inhibition of pro-inflammatory cytokines during PH compared to E, whereas cortisol exerts similar effects during both trials. These effects became major when Tre reached 39°C. Consequently, exercise and passive hyperthermia, via the stress hormones, promote Th2 responses, since they decrease the production of pro-inflammatory cytokines and enhance the production of anti-inflammatory cytokines [16]. It could induce immunosuppression or a protection of the organism from systemic “overshooting” with Th1/pro-inflammatory cytokines, as suggested by Elenkov [16] and Petersen [36]. However, these authors propose this beneficial effect only during exercise. In our study, this effect also applied to passive hyperthermia. Nevertheless, the trend towards protection against inflammation is stronger for exercise than for passive hyperthermia, because of the hormones involved.

Acknowledgements. The authors thank D. Guicherd for the volunteers whose participation made this study possible.

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