

## 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%  $\text{VO}_2$  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  $\text{TNF-}\alpha$ , IL-10 and  $\text{INF-}\gamma$  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  $\text{INF-}\gamma$  and  $\text{TNF-}\alpha$  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

Many physiological and biological responses are different between exercise-induced hyperthermia and passive hyperthermia. Stress hormone responses are also different. Exercise increases catecholamine release to ensure the inflow of oxygen and metabolites towards the muscles [1-3], but not prolactin secretion [4]. Passive hyperthermia increases plasma prolactin [4], but not plasma catecholamines [2, 3]. Both challenges increase plasma cortisol [1]. In addition, it is well known that physical exercise affects the immune system. Intense exercise may be the origin of a transient immunosuppression [5]. This phenomenon is amplified during exercise in the heat. On the other hand, passive hyperthermia could also act on the immune system. Few studies are reported in the literature. Passive hyperthermia could enhance pro-inflammatory cytokines synthesis as described by Downing [6] and Ostberg [7], while Kappel [8] reported no effect. It seems that hyper-

thermia, *per se*, has an effect on the immune response. But are the effects of exercise-induced hyperthermia really different from those of passive hyperthermia? Stress hormones have an influence on the immune system [9-12]. Can they explain the differences in immune responses between exercise and hyperthermia? Most studies on immune responses during passive hyperthermia have been carried out by immersion in warm water. The problem is that immersion has its own effects on hormonal and hemodynamic responses, which are independent of those of hyperthermia, in particular, catecholamine levels are increased during immersion in warm water [13, 14]. It appears preferable to study the effect of passive hyperthermia in a climatic chamber. Therefore, the aim of this study was to compare the effect of an increase in body temperature on immune and hormonal responses during exercise *versus* during passive hyperthermia in a climatic chamber, with

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- $\gamma$  and TNF- $\alpha$ , 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  $\pm$  sem) were  $23 \pm 1.5$  years,  $69.2 \pm 2.8$  kg and  $172 \pm 2$  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 O<sub>2</sub> uptake (VO<sub>2</sub> 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  $52.0 \pm 2.2$  mL.min<sup>-1</sup>.kg<sup>-1</sup>. 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 recorded every min with thermistances (YSI series 400, Yellow Spring, OH, USA). They were then asked to stay in a sitting posture for the control (C) trial, in a standing posture for the exercise trial (E), and in a semi-recumbent position for the passive hyperthermia trial (PH) for 30 min to stabilize hemodynamic conditions. At the end of the 30 min, a reference blood sample (10 mL) was taken.

In the control trial (C), the subjects remained in a sitting position for three hours in controlled thermal conditions (Tdb = 21–22°C, relative humidity (rH) = 35–45%). There were dressed in shorts and a bathrobe in order to ensure thermoneutral conditions.

In the exercise trial (E), the subjects exercised for two hours on a treadmill, at 65% VO<sub>2</sub> max in controlled ther-

mal 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  $\dot{V}_O_2$  every 30 min over a 10-min period.

### Plasma volume changes

Variations of PV changes ( $\Delta VP$ ) 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  $\mu\text{L}$  of whole blood aliquots were diluted with 400  $\mu\text{L}$  of RPMI 1640 (Sigma-Aldrich ref 50883, L'isle d'Abeau, Chesnes, France), penicillin  $100\text{U}\cdot\text{mL}^{-1}$  and streptomycin  $100\text{ }\mu\text{g}\cdot\text{mL}^{-1}$  (Gibco ref 15140 and 15122), glutamine  $4\text{mM}\cdot\text{mL}^{-1}$  and mercaptoethanol  $5\times 10^{-3}\text{ mol}\cdot\text{mL}^{-1}$ . Diluted blood was stimulated either by LPS  $1\text{ }\mu\text{g}\cdot\text{mL}^{-1}$  for 24h (*E. coli*, serotype, 055B11), either by PHA  $50\text{ }\mu\text{g}\cdot\text{mL}^{-1}$  (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  $37^{\circ}\text{C} + 5\%$   $\text{CO}_2$ . After the incubation, the tubes were spun for 10 min at 400 g, and the supernatants were collected and stored at  $-80^{\circ}\text{C}$  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  $< 2\text{ pg}\cdot\text{mL}^{-1}$ .

### 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  $\pm$  SEM, and the null hypothesis was rejected when  $p < 0.05$  for all analyses.

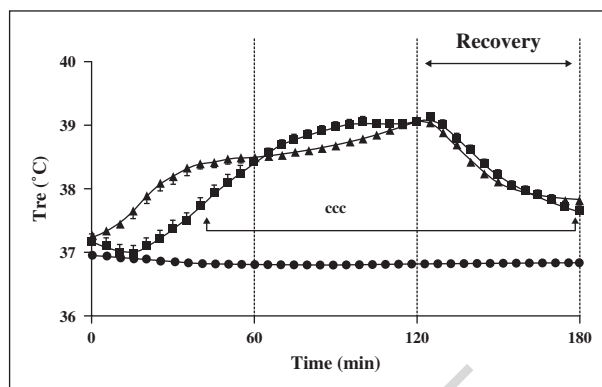
## RESULTS

### Changes in rectal temperature (Tre)

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

### Plasma volume changes ( $\Delta\text{PV}$ ) and fluid losses

Plasma volume (table 1) did not change significantly during the C and E trials, but showed a marked fall during PH ( $p < 0.001$  compared to C and E). Because of this marked fall, all the values were corrected according to  $\Delta\text{PV}$ . The percentage of dehydration was  $0.6 \pm 0.1\%$ ,  $3.5 \pm 0.1\%$  and



**Figure 1**

Changes in Tre ( $^{\circ}\text{C}$ ) over time in C (●), PH (■), and E (▲) trials. Values are means  $\pm$  SEM.

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

$3.5 \pm 0.1\%$  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  $3332 \pm 517\text{ nmoL}\cdot\text{L}^{-1}$ ,  $3932 \pm 608\text{ nmoL}\cdot\text{L}^{-1}$  and  $1447 \pm 213\text{ nmoL}\cdot\text{L}^{-1}$ , and plasma norepinephrine at  $5843 \pm 944\text{ nmoL}\cdot\text{L}^{-1}$ ,  $11176 \pm 1629\text{ nmoL}\cdot\text{L}^{-1}$  and  $2981 \pm 554\text{ nmoL}\cdot\text{L}^{-1}$  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 ( $16 \pm 1\text{ }\mu\text{g}\cdot\text{dL}^{-1}$ ,  $15 \pm 1\text{ }\mu\text{g}\cdot\text{dL}^{-1}$  respectively,  $p < 0.05$  compared to the C trial). Plasma cortisol then continued to increase after the exercise ( $19 \pm 2\text{ }\mu\text{g}\cdot\text{dL}^{-1}$ ,  $p < 0.001$  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 ( $12 \pm 1\text{ }\mu\text{g}\cdot\text{dL}^{-1}$ ,  $p < 0.01$  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  $39^{\circ}\text{C}$ . It showed a large increase at time 60 and 120 min during PH ( $19 \pm 4\text{ mg}\cdot\text{mL}^{-1}$  and  $21 \pm 2\text{ mg}\cdot\text{mL}^{-1}$  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 ( $9 \pm 1\text{ mg}\cdot\text{mL}^{-1}$ ,  $p < 0.01$  compared to time 120 min).

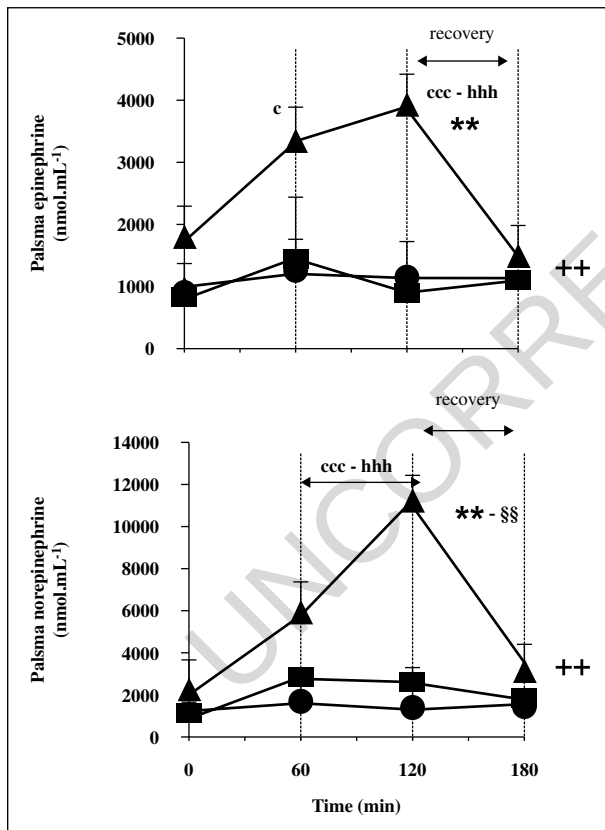
### Total leukocytes and subsets changes

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 ( $\pm$  SEM) of plasma volume changes ( $\Delta$ PV), leukocytes ( $10^3 \cdot \text{mL}^{-1}$ ), neutrophils ( $10^3 \cdot \text{mL}^{-1}$ ), lymphocytes ( $10^3 \cdot \text{mL}^{-1}$ ) and monocytes ( $10^3 \cdot \text{mL}^{-1}$ ) changes corrected for  $\Delta$ PV

|                                            |    | 0 min           | 60 min                             | 120 min                            | end of recovery                    |
|--------------------------------------------|----|-----------------|------------------------------------|------------------------------------|------------------------------------|
| $\Delta$ PV (%)                            | C  | 0               | $0.1 \pm 0.6$                      | $-1.4 \pm 1.0$                     | $-1.5 \pm 1.2$                     |
|                                            | HP | 0               | $-12.3 \pm 1.1$ <sup>ccc-eee</sup> | $-16.9 \pm 1.7$ <sup>ccc-eee</sup> | $-13.3 \pm 0.9$ <sup>ccc-eee</sup> |
|                                            | E  | 0               | $-0.7 \pm 1.1$                     | $-4.0 \pm 1.4$                     | $-1.6 \pm 1.1$                     |
| Leukocytes<br>$10^3 \cdot \text{mL}^{-1}$  | C  | $4.4 \pm 0.4$   | $4.7 \pm 0.4$                      | $5.1 \pm 0.5$                      | $5.1 \pm 0.5$                      |
|                                            | HP | $4.3 \pm 0.3$   | $4.6 \pm 0.4$                      | $5.3 \pm 0.9$                      | $5.7 \pm 0.7$                      |
|                                            | E  | $5.3 \pm 0.7$   | $8.1 \pm 0.9$ <sup>ccc-hhh</sup>   | $10.9 \pm 1.1$ <sup>ccc-hhh</sup>  | $10.0 \pm 0.8$ <sup>ccc-hhh</sup>  |
| Neutrophils<br>$10^3 \cdot \text{mL}^{-1}$ | C  | $2.3 \pm 0.2$   | $2.5 \pm 0.2$                      | $2.9 \pm 0.4$                      | $2.8 \pm 0.4$                      |
|                                            | HP | $2.2 \pm 0.2$   | $2.4 \pm 0.2$                      | $3.3 \pm 0.8$                      | $3.9 \pm 0.6$ <sup>□§</sup>        |
|                                            | E  | $2.3 \pm 0.2$   | $4.4 \pm 0.8$ <sup>ccc-hhh</sup>   | $7.0 \pm 1.1$ <sup>ccc-hhh</sup>   | $8.0 \pm 0.5$ <sup>ccc-hhh</sup>   |
| Lymphocytes<br>$10^3 \cdot \text{mL}^{-1}$ | C  | $1.5 \pm 0.2$   | $1.6 \pm 0.1$                      | $1.6 \pm 0.1$                      | $1.7 \pm 0.1$                      |
|                                            | HP | $1.5 \pm 0.1$   | $1.6 \pm 0.2$                      | $1.5 \pm 0.1$                      | $1.3 \pm 0.1$                      |
|                                            | E  | $1.8 \pm 0.2$   | $2.4 \pm 0.2$ <sup>ccc-hhh</sup>   | $3.0 \pm 0.3$ <sup>ccc-hhh</sup>   | $1.7 \pm 0.1$                      |
| Monocytes<br>$10^3 \cdot \text{mL}^{-1}$   | C  | $0.36 \pm 0.03$ | $0.39 \pm 0.04$                    | $0.40 \pm 0.04$                    | $0.35 \pm 0.03$                    |
|                                            | HP | $0.37 \pm 0.03$ | $0.36 \pm 0.04$                    | $0.32 \pm 0.05$                    | $0.33 \pm 0.04$                    |
|                                            | E  | $0.39 \pm 0.04$ | $0.63 \pm 0.06$ <sup>ccc-hhh</sup> | $0.83 \pm 0.08$ <sup>ccc-hhh</sup> | $0.58 \pm 0.05$ <sup>ccc-hhh</sup> |

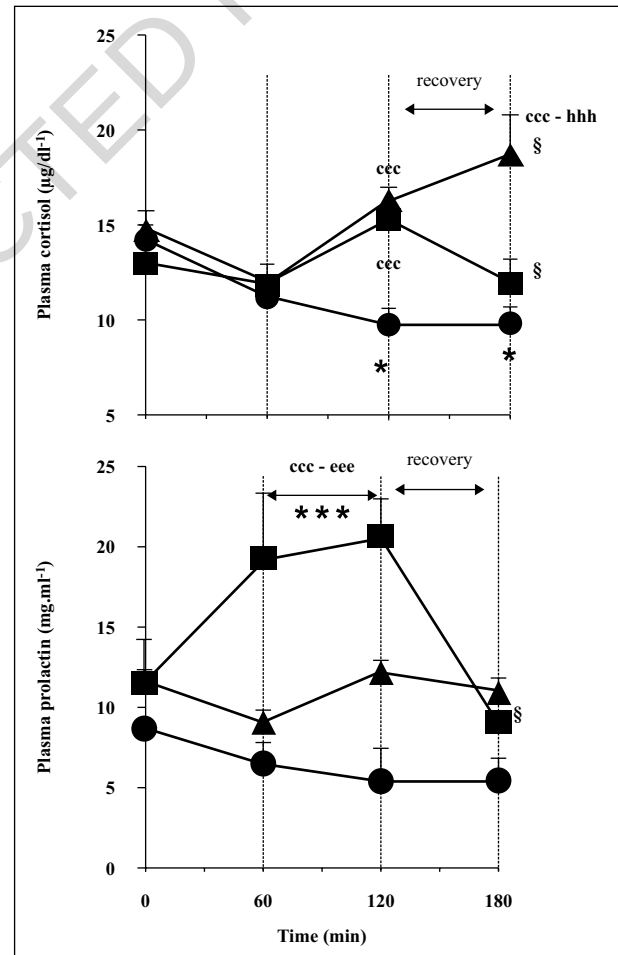
ccc: significant difference from C trial ( $p < 0.001$ ); eee: significant difference from E trial ( $p < 0.001$ ); hhh: significant difference from H trial ( $p < 0.001$ ); \*: significant difference from time 0 ( $p < 0.05$ ); §: significant difference from time 0 ( $p < 0.05$ ).



**Figure 2**

Changes in plasma epinephrine and norepinephrine ( $\text{nmol} \cdot \text{mL}^{-1}$ ) corrected for  $\Delta$ PV over time in C (●), PH (■), and E (▲) trials. Values are means  $\pm$  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 ( $p < 0.01$ )  
++: significant difference from Time 120 min ( $p < 0.01$ ).



**Figure 3**

Changes in plasma cortisol ( $\mu\text{g} \cdot \text{dL}^{-1}$ ) and prolactin ( $\text{mg} \cdot \text{mL}^{-1}$ ) corrected for  $\Delta$ PV over time in C (●), PH (■), and E (▲) trials. Values are means  $\pm$  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 ( $p < 0.05$ )  
□□□: significant difference from time 0 ( $p < 0.001$ )  
§: significant difference from time 60 min ( $p < 0.05$ ).

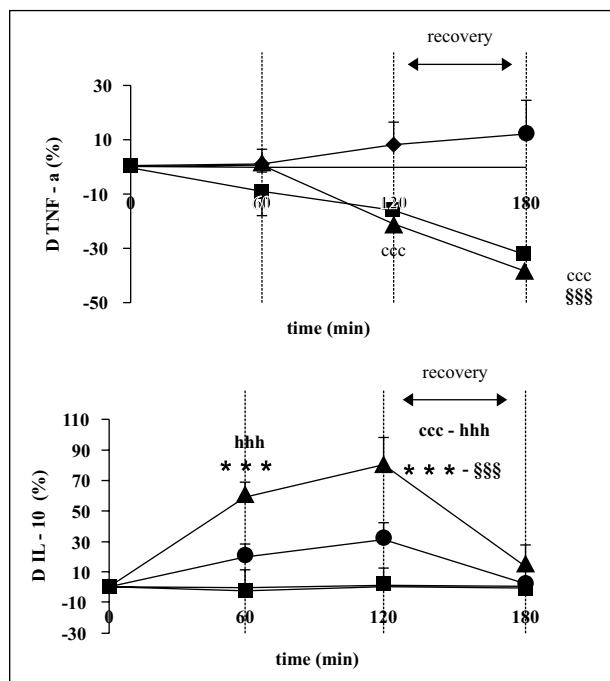


Figure 4

Relative changes in the release of TNF- $\alpha$  and IL-10 by whole blood after stimulation by LPS over time in C (●), PH (■), and E (▲) trials. Values are means  $\pm$  SEM.

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

hhh: significant difference from H trial ( $p < 0.001$ ).

□ □ □: significant difference from time 0 ( $p < 0.001$ ).

\$\$\$ : significant difference from time 60 min ( $p < 0.001$ ).

( $p < 0.05$  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- $\alpha$ 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- $\alpha$  at the end of the PH and E trials when  $T_{re} = 39^{\circ}\text{C}$  ( $\Delta\text{TNF-}\alpha = -16 \pm 6\%$  and  $-21 \pm 8\%$  for the PH and E trials, respectively,  $p < 0.001$  compared to C). This reduction was clearly amplified at the end of recovery ( $\Delta\text{TNF-}\alpha = -32 \pm 3\%$  and  $-38 \pm 6\%$  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- $\alpha$  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 ( $\Delta\text{IL-10} = +59 \pm 10\%$  and  $+79 \pm 19\%$  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- $\gamma$ 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- $\gamma$ , as shown in figure 5, at the end of the

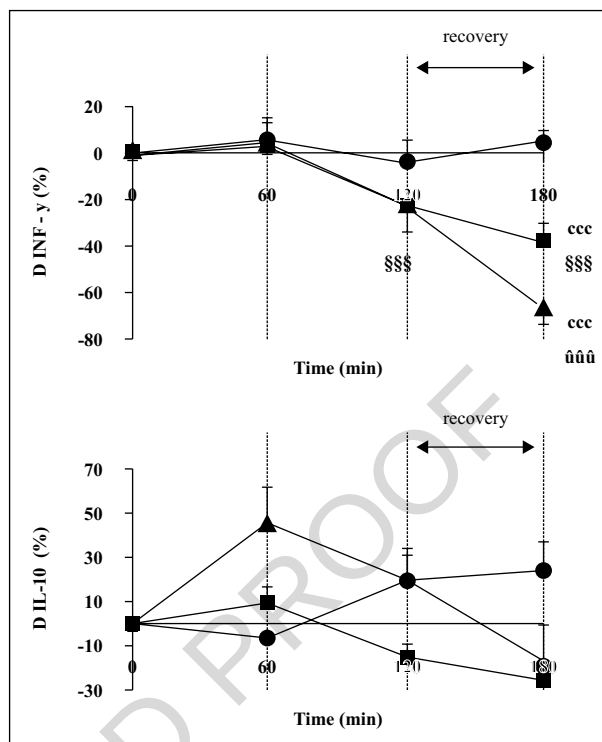


Figure 5

Relative changes in the release of INF- $\gamma$  and IL-10 by whole blood after stimulation by PHA over time in C (●), PH (■), and E (▲) trials. Values are means  $\pm$  SEM.

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

\$\$\$ : significant difference from time 60 min ( $p < 0.001$ ).

$39^{\circ}\text{C}$  challenge until the end of recovery. At  $39^{\circ}\text{C}$ ,  $\Delta\text{INF-}\gamma = -23 \pm 5\%$  and  $-23 \pm 11\%$  for the PH and E trials, respectively ( $p < 0.001$  compared to C,  $p < 0.001$  compared to time point 60 min). The production of INF- $\gamma$  during the PH and E trials was similar in spite of a different number of monocytes. At 1-h recovery,  $\Delta\text{INF-}\gamma = -38 \pm 7\%$  and  $-66 \pm 8\%$  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  $T_{re}$  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- $\alpha$  and INF- $\gamma$  production by stimulated blood was inhibited in a substantial way in both E and PH compared to the control when  $T_{re}$  reached  $39^{\circ}\text{C}$ . IL-10 production was enhanced only during exercise when blood was stimulated with LPS. Both challenges raised  $T_{re}$ , to  $39^{\circ}\text{C}$  compared to  $38.5^{\circ}\text{C}$ , 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  $\Delta$ PV.

### 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 60<sup>th</sup> 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 Møller *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 immunocompetent cells can also depend on the Tre. Dubose *et al.* [25] have observed leucocytosis 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- $\alpha$  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- $\alpha$  production by monocytes is higher during PH than during E. To our knowledge, there are very few studies on this subject. Starkie *et al.* [27, 28] found by flow cytometry, that there was a decrease in the amount of TNF- $\alpha$  per cell post-compared 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- $\alpha$  secretion [11, 16]. The higher TNF- $\alpha$  production by monocytes with PH could be an effect of prolactin [10]. Catecholamines may be implicated in the low production of TNF- $\alpha$  by monocytes, since, in the blood of healthy volunteers, epinephrine reduced the TNF- $\alpha$  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  $\beta$ -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- $\gamma$  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- $\gamma$  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

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- $\gamma$ , produces as much as 10-fold more INF- $\gamma$  than cells taken at basal temperatures from the same individuals. In our study, both exercise and passive hyperthermia suppress the production of INF- $\gamma$ , probably by a direct effect of cortisol [11, 16]. The higher TNF- $\alpha$  production by monocytes with PH could be an effect of prolactin, which would have an inductive role on the secretion of INF- $\gamma$  [10]. At 1-h recovery, the suppression of the production of TNF- $\alpha$  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 catecholamines assay. The excellent technical assistance of A. Roux, J. Denis, L. Vachez-Collomb and N. Clerc is also gratefully acknowledged. The authors extend their thanks to the volunteers whose participation made this study possible.

## REFERENCES

- Galbo H. The hormonal response to exercise. *Diabetes Metab Rev* 1986; 1: 385.
- Brenner IKM, Zamecnik J, Shek PN, Shephard RJ. The impact of heat exposure and repeated exercise on circulating stress hormones. *Eur J Appl Physiol* 1997; 76: 445.
- Melin B, Koulmann N, Jimenez C, *et al.* Comparison of passive heat or exercise-induced dehydration on renal water and electrolyte excretion: the hormonal involvement. *Eur J Appl Physiol* 2001; 85: 250.
- Christensen SE, Jørgensen O, Møller J, *et al.* Body temperature elevation, exercise and serum prolactin concentrations. *Acta Endocrinol (Copenh)* 1985; 109: 458.
- Nieman DC, Pedersen BK. Exercise and immune function. Recent developments. *Sports Med* 1999; 27: 73.
- Downing JF, Martinez-Valdez H, Elizondo RS, *et al.* Hyperthermia in humans enhances interferon-gamma synthesis and alters the peripheral lymphocyte population. *J Interferon Res* 1988; 8: 143.
- Ostberg JR, Taylor SL, Baumann H, *et al.* Regulatory effects of fever-range whole body hyperthermia on the LPS-induced acute inflammatory response. *J LeukBiol* 2000; 68: 815.
- Kappel M, Tvede N, Hansen MB, *et al.* Cytokine production ex vivo: effect of raised body temperature. *Int J Hyperthermia* 1995; 11: 329.
- Ensor JE, Wiener SM, Viscardi RM, *et al.* Differential effects of hyperthermia on macrophage interleukin-6 and tumor necrosis alpha expression. *Am J Physiol Cell Physiol* 1994; 266: C967.
- Breidhardt T, Frohn C, Luhm J, *et al.* Prolactin induces enhanced interferon gamma release in peripheral whole blood after stimulation with either PHA or LPS. *Immunobiology* 2002; 206: 424.
- DeRijk R, Michelson D, Karp B, *et al.* Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin 1 $\beta$ , IL- and tumor necrosis factor- $\alpha$  production in humans: high sensitivity of TNF- $\alpha$  and resistance of IL-6. *J Clin Endocrinol Metab* 1997; 82: 2182.
- Rhind S, Gannon GA, Shek PN, *et al.* Contribution of exertional hyperthermia to sympathoadrenal-mediated lymphocyte subset redistribution. *J Appl Physiol* 1999; 87: 1178.
- Møller N, Beckwith R, Butler PC, *et al.* Metabolic and hormonal responses to exogenous hyperthermia in man. *Clin Endocrinol (Oxf)* 1989; 30: 651.
- Kappel M, Stadeager C, Tvede N, *et al.* Effects of in vivo hyperthermia on natural killer cell activity, in vitro proliferative responses and blood mononuclear cell subpopulations. *Clin Exp Immunol* 1991; 84: 175.
- Belge KU, Dayyani F, Horelt A, *et al.* The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. *J Immunol* 2002; 168: 3536.
- Elenkov IJ. Glucocorticoids and the Th1/Th2 balance. *Ann N Y Acad Sci* 2004; 966: 290.
- Henane R, Valatx JL. Thermoregulatory changes induced during heat acclimatization by controlled hyperthermia in man. *J Physiol* 1973; 230: 255.
- Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974; 37: 247.
- Harrison MH. Effects of thermal stress and exercise on blood volume in humans. *Physiol Rev* 1985; 65: 149.
- Jimenez C, Melin B, Koulmann N, *et al.* Plasma volume changes during and after acute variations of body hydration level in humans. *Eur J Appl Physiol* 1999; 80: 1.
- Won S-J, Lin M-T. Thermal stresses reduce natural killer cell cytotoxicity. *J Appl Physiol* 1995; 73: 732.
- Utter AC, Kang J, Nieman DC, *et al.* Effect of carbohydrate ingestion and hormonal responses on ratings of perceived exertion during prolonged cycling and running. *Eur J Appl Physiol* 1999; 80: 92.
- Bridge MW, Allen T, Sharma A, *et al.* Effect of ambient temperature on exercise-induced prolactinaemia. *J Physiol* 1999; 521: 100.
- Pedersen BK, Bruunsgaard H, Klokke M, *et al.* Exercise-induced immunomodulation - possible roles of neuroendocrine and metabolic factors. *Int J Sports Med* 1997; 18: S2.
- Dubose DA, Wenger CB, Flinn SD, *et al.* Distribution and mitogen response of peripheral blood lymphocytes after exertional heat injury. *J Appl Physiol* 2003; 95: 2381.

26. Ellis GS, Carlson DE, Hester L, et al. G-CSF, but not corticosterone, mediates circulating neutrophilia induced by febrile-range hyperthermia. *J Appl Physiol* 2005; 98: 1799.
27. Starkie RL, Rolland J, Angus DJ, et al. Circulating monocytes are not the source of elevations in plasma IL-6 and TNF- $\alpha$  after prolonged running. *Am J Physiol Cell Physiol* 2001; 280: C769.
28. Starkie RL, Hargreaves M, Rolland J, et al. Heat stress, cytokines, and the immune response to exercise. *Brain Behav Immun* 2005; 19: 404.
29. Singh IS, Viscardi RM, Kalvakolanu I, et al. Inhibition of tumor necrosis factor- $\alpha$  transcription in macrophages exposed to febrile range temperature. *J Biol Chem* 2000; 275: 9841.
30. Ferlito M, De Maio A. Enhanced LPS-induced TNF- $\alpha$  production in heat-shocked human promonocytic cells: regulation at the transitional/post translational level. *Biochim Biophys Acta* 2005; 1743: 20.
31. Bergmann M, Gornikiewicz A, Sautner T, et al. Attenuation of catecholamine-induced immunosuppression in whole blood from patients with sepsis. *Shock* 1999; 12: 421.
32. Elenkov IJ, Chrousos GP. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Trends Endocrinol Metab* 1999; 9: 359.
33. Severs Y, Brenner I, Shek PN, et al. Effects of heat and intermittent exercise on leukocyte and subpopulation cell counts. *Eur J Appl Physiol* 1996; 74: 234.
34. Nielsen HB, Pedersen BK. Lymphocyte proliferation in response to exercise. *Eur J Appl Physiol* 1997; 75: 375.
35. Mitchell JB, Dugas JP, Mcfarlin BK, et al. Effect of exercise, heat stress, and hydration on immune cell number and function. *Med Sci Sports Exerc* 2002; 34: 1941.
36. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol* 2005; 98: 1154.