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Expression of intracellular cytokines, HSP72, and apoptosis in monocyte subsets during exertional heat stress in trained and untrained individuals

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Selkirk GA, McLellan TM, Wright HE, Rhind SG. Expression of intracellular cytokines, HSP72, and apoptosis in monocyte subsets during exertional heat stress in trained and untrained individuals. *Am J Physiol Regul Integr Comp Physiol* 296: R575–R586, 2009. First published January 21, 2009; doi:10.1152/ajpregu.90683.2008.—This study examined intracellular cytokine, heat shock protein (HSP) 72, and cellular apoptosis in classic and inflammatory CD14⁺ monocyte subsets during exertional heat stress (EHS). Subjects were divided into endurance-trained [TR; $n = 12$, peak aerobic power ($\dot{V}O_{2peak}$) = 70 ± 2 ml·kg lean body mass (LBM)⁻¹·min⁻¹] and sedentary-untrained (UT; $n = 11$, $\dot{V}O_{2peak}$ = 50 ± 1 ml·kg LBM⁻¹·min⁻¹) groups before walking at 4.5 km/h with 2% elevation in a climatic chamber (40°C, 30% relative humidity) wearing protective clothing until exhaustion (Exh). Venous blood samples at baseline and 0.5°C rectal temperature increments (38.0, 38.5, 39.0, 39.5, and 40.0°C/Exh) were analyzed for cytokines (TNF- α , IL-1 β , IL-6, IL-1ra, and IL-10) in CD14⁺⁺CD16⁻/CD14⁺CD16⁺ and HSP72/apoptosis in CD14^{Brij}/CD14^{Dim} subsets. In addition, serum levels of extracellular (e)HSP72 were also examined. Baseline and Exh samples were separately stimulated with LPS (1 μ g/ml) or heat shocked (42°C) and cultured in vitro for 2 h. A greater temperature-dependent increase in CD14⁺CD16⁺ cells was observed in TR compared with UT subjects as well as a greater LPS tolerance following in vitro LPS stimulation. TNF- α and IL-1 β cytokine expression was elevated in CD14⁺CD16⁺ but not in CD14⁺⁺CD16⁻ cells. A greater induction of intracellular HSP72 and eHSP72 was observed in TR compared with UT subjects, which coincided with reduced apoptosis at Exh and following in vitro heat shock. Induced HSP in vitro was not uniform across CD14⁺ subsets. Findings suggest that circulating CD14⁺CD16⁺, but not CD14⁺⁺CD16⁻ monocytes, contribute to the proinflammatory cytokine profiles observed during EHS. In addition, the enhanced HSP72 response in endurance-trained individuals may confer improved heat tolerance through both anti-inflammatory and anti-apoptotic mechanisms.

immune function; cardiovascular/thermoregulatory strain; flow cytometry; heat shock protein

PERIPHERAL BLOOD MONOCYTES play an important role in protection against invading pathogens and activation of innate immunity (46). Based on differential expression of antigenic markers CD14 [part of the LPS receptor, CD14/Toll-like receptor (TLR-4)/MD-2] and CD16 (Fc γ RIII), monocytes can be divided into two phenotypically and functionally distinct subsets (82, 90). The bulk of monocytes are defined as classic monocytes and are strongly positive for surface receptor CD14 (CD14⁺⁺CD16⁻), whereas the minor subset are referred to as inflammatory monocytes (CD14⁺CD16⁺) because of their high capacity to express proinflammatory

cytokines such as TNF- α (6, 62, 64, 90). Inflammatory monocytes have the ability to respond directly with antimicrobial activity, whereas the classic subset of monocytes act as scavengers, removing apoptotic cells and aiding in the resolution of inflammation (46).

Normally comprising <10% of all circulating monocytes, the inflammatory subset in the peripheral blood has been shown to expand with exercise (31, 78) and is associated with various inflammatory disorders (29, 30, 82). Elevations in body temperature (32) and secreted levels of TNF- α (71) also have been correlated with inflammatory subset expansion; however, to our knowledge, no data exist relative to exertional heat stress (EHS). Many studies have reported changes in circulating proinflammatory cytokines during EHS (54, 59, 76), and the potential that these mediators originate from circulating leukocytes has been under considerable debate (21, 47, 58, 74–76, 83). Several studies have examined monocytic protein content using a variety of methods including mRNA transcript expression (47, 83) and intracellular flow cytometry (58, 74, 76, 77, 84, 89) in circulating monocytes, without distinction between subsets (21). We recently reported that increases in circulating TNF- α and endotoxin during EHS are accompanied by nuclear factor (NF)- κ B translocation in peripheral blood mononuclear cells, suggestive of peripheral blood inflammatory cell activation (68). NF- κ B DNA binding in the liver (11) and intestinal epithelial/mucosal cells (56) is an important source of inflammatory mediators during endotoxemia (11, 57) before spillover into the systemic circulation (28). It is possible, however, given the findings by Belge et al. (6) and others (62, 64) regarding CD14⁺CD16⁺ capacity for LPS-induced TNF- α production in vivo, that circulating inflammatory monocytes may be contributing to the changes in circulating cytokine profiles observed during EHS (68).

Induction of the stress response plays a pivotal role in the regulation of the inducible transcription factor NF- κ B during inflammatory insult (10, 11, 56, 79), improving survival during hyperthermia (70) and influencing both innate and adaptive immunity (41). Intracellular heat shock protein (HSP) 72 accumulation improves heat tolerance through cytoprotective effects (22, 37) such as the reduction of heat-induced cellular apoptosis (60) and protection against ischemia-reperfusion injury (42), yet limited data exist examining differences in HSP72 expression within monocyte subsets (4, 53). In contrast with intracellular expression, extracellular (e)HSPs have been suggested to act as a “danger signal,” activating immune-competent cells (33) through similar LPS TLR-4/CD14-depen-

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dent signaling (2, 3, 8, 17, 44). Reduced CD14⁺CD16⁺ inflammatory profiles (81) as well as a greater increase in HSP72 expression (60) and increased heat shock factor (HSF)-1 DNA binding affinity (43) have been related to training adaptations. These anti-inflammatory effects of training may contribute not only to an improved heat tolerance but also to reduced apoptosis sensitivity (48, 60), NF- κ B translocation, and circulating inflammatory profiles as observed in endurance-trained individuals during EHS (68).

Dysregulation of cellular apoptosis in virtually all cell types can have deleterious effects contributing to the incidence of multiple organ dysfunction syndrome (MODS) in several clinical conditions, including sepsis (55). In addition, since cytokines such as TNF- α , IL-1, IL-6, and IL-10 have all been shown to influence apoptotic signaling (52) and are present during EHS, it may be speculated that excessive apoptosis of intestinal mucosal cells may permit inappropriate translocation of bacterial LPS into the systemic circulation, promoting a greater risk of exertional heat illness (EHI) (68).

Together, these findings suggest an interplay among the apoptotic signaling pathways, NF- κ B activation, and HSPs that may influence innate and adaptive immune responses, subsequently altering heat tolerance. It is well documented that a primary mechanism conferring improved heat tolerance in aerobically fit individuals is the ability to tolerate higher rectal temperatures (T_{re}) at exhaustion during uncompensable EHS (67, 68). Moreover, our laboratory recently reported in the same cohort of subjects a decreased heat tolerance accompanied by enhanced circulating TNF- α and endotoxin concentrations in sedentary-untrained compared with endurance-trained subjects for a given level of thermal strain (68). Therefore, the present study was designed to examine 1) whether peripheral monocytes are a contributing source of circulating inflammatory cytokines, 2) intracellular monocyte and eHSP72 content, and 3) the interplay among inflammatory activation, HSP72 induction, and cellular apoptosis in peripheral monocyte subsets during EHS at similar levels of thermal strain between endurance-trained and sedentary untrained individuals. It was hypothesized that intracellular inflammatory activation and cellular apoptosis would be reduced in endurance-trained compared with sedentary-untrained subjects at a given level of thermal strain due to enhanced cytoprotective mechanisms associated with training.

METHODS

Subjects. As detailed previously (68), 23 healthy male volunteers were recruited from surrounding universities and running clubs in the greater Toronto area, following approval by the Defence Research and Development Canada (DRDC)-Toronto and York University Human Research Ethics Committees. All subjects were medically screened, and a full explanation of procedures, discomforts, and risks were given before written informed consent was obtained. Potential subjects were excluded if they did not fit the grouping criteria described below or if they were taking any regular medications. All testing was performed in an exercise laboratory or a climatic chamber at DRDC-Toronto.

Grouping criteria. Subjects were divided into two groups of endurance-trained (TR, $n = 12$) or sedentary-untrained (UT, $n = 11$) individuals based on baseline measurements of peak O₂ consumption ($\dot{V}O_{2peak}$) expressed relative to lean body mass (LBM), percent body fat, and activity profiles as described previously (67, 68). TR subjects were defined as actively participating in a cardiovascular training

program more than three times per week and having a $\dot{V}O_{2peak} > 65$ ml·kg LBM⁻¹·min⁻¹. UT subjects were defined as being minimally active (fewer than 2 times per week) and having a $\dot{V}O_{2peak} < 50$ ml·kg LBM⁻¹·min⁻¹ (68).

Experimental design. All subjects participated in both familiarization and experimental EHS sessions, which began at ~8:00 AM. Familiarization sessions involved exposure to all dressing procedures (including venous catheter and rectal probe insertion) as well as a brief heat exposure while walking in the climatic chamber. Each familiarization session was performed at least 10 days before the experimental trial to limit any acute effects of heat acclimation. In addition, heat exposure was limited to 30 min to maintain a T_{re} below 38.0°C, since it has been shown in previous work that the HSP response is not manifested at $T_{re} < 38.0^\circ\text{C}$ (60). These restrictions on increases in T_{re} were primarily for UT subjects, since TR subjects continued their regular training regimens leading up to the experimental EHS. Subjects refrained from strenuous exercise (running, swimming, cycling, weight lifting, among others), use of alcohol, and the use of nonsteroidal anti-inflammatory drugs for 24 h, and the use of caffeine for 8 h, before each session.

Exertional heat stress model. During the familiarization and EHS sessions, subjects walked on a motorized treadmill (4.5 km/h, 2% incline, wind speed <0.1 m/s) at 40°C, with 30% relative humidity, wearing the Canadian military nuclear, biological, and chemical protective semipermeable overgarment, creating a condition of uncompensable heat stress (68). Exhaustion (Exh), which was defined by specific end-point criteria for the experimental heat stress trials, included an ethical T_{re} cutoff of 40.0°C, heart rate (HR) reaching or exceeding 95% of maximum for three consecutive minutes, Exh/thermal discomfort, dizziness or nausea precluding further participation, and/or subject or experimenter termination. Tolerance time (TT) was defined as the elapsed time from the beginning of walking on the treadmill to the attainment of one or more of the end-point criteria that resulted in termination of the EHS session. Subjects received 5 ml/kg LBM of warm water (37°C) before entering the climatic chamber and approximately every 30 min during EHS to limit heat-sink effects, reduce circulatory instability produced by progressive dehydration, and increase the level of T_{re} tolerated at Exh. HR was recorded every 5 min, and T_{re} was averaged over 1-min intervals as described previously (68).

Clothing ensemble. The Canadian military nuclear, biological, and chemical protective semipermeable overgarment was worn during familiarization and experimental sessions. In addition, combat pants and shirt, underwear, shorts, T-shirt, and running shoes were worn beneath the semipermeable overgarment. No respirator, gloves, or overboots were worn, but the hood was over the head. The total thermal resistance of this protective ensemble has been reported previously (67), but it should be noted that these values will be slightly lower in the current study due to the absence of impermeable gloves, boots, and respirator.

Blood collection. Venous whole blood was collected at six time points during the experimental EHS session, if available, by using an indwelling venous catheter and a 24-in. extension that protruded from the sleeve of the protective ensemble. After catheter insertion, subjects remained standing for 20 min to obtain postural stability before the baseline sample was collected. Subsequent samples were taken during the experimental EHS session at specific T_{re} intervals (38.0, 38.5, 39.0, 39.5, and 40°C/Exh), rather than at fixed intervals (68). Catheter patency was maintained by injecting 3 ml of a sterile saline solution between sampling intervals. Blood samples were drawn into sterile syringes and immediately transferred into corresponding vacutainers [Becton Dickinson Biosciences (BD), Franklin Lakes, NJ].

Antibodies and reagents. Mouse anti-human MAbs for cell-surface epitopes CD16⁺, CD14⁺, intracellular isotype controls, and cytokines [TNF- α , IL-1 β , IL-6, IL-1 receptor antagonist (IL-1ra), and IL-10], conjugated with fluorochromes FITC, allophycocyanin (APC), and phycoerythrin (PE), respectively, as well as PE-conjugated Annexin V

(AnV) and vital dye 7-aminoactinomycin D (7-AAD), were obtained from BD (San Jose, CA). In addition, FACS brand lysing solution, CellWASH, permeabilizing solution, binding buffer, and intracellular isotype controls were also obtained from BD (San Jose, CA). HSP72-specific FITC-conjugated mouse anti-human IgG₁ MAb (SPA-810) was obtained from StressGen (Victoria, Canada). LPS (*Escherichia coli* 026:B6), brefeldin A (BFA), and paraformaldehyde were purchased from Sigma (St. Louis, MO).

Cell preparation and staining. Whole blood was transferred from plastic syringes immediately into 3-ml sodium heparin vacutainers for HSP and apoptosis staining (60) and 3-ml sodium heparin vacutainers prespiked with BFA (10 μ l/ml) for five-panel cytokine expression (TNF- α , IL-1 β , IL-6, IL-1ra, and IL-10) (58). BFA-treated and nontreated heparinized whole blood samples were analyzed immediately using flow cytometric techniques for spontaneous intracellular

cytokine (TNF- α , IL-1 β , IL-6, IL-1ra, and IL-10) and HSP72 production as well as surface staining for apoptotic markers in CD14⁺ monocytes, classic and inflammatory subsets (see description below). Intracellular markers were analyzed at all of the sampling time points, whereas cellular apoptosis was analyzed at baseline and Exh only. In addition, at the beginning of the session and at a T_{re} of 40°C and/or Exh, 1 ml of BFA- and non-BFA-treated whole blood was aliquoted into 12 \times 75-mm Falcon tubes for *in vitro* LPS (*E. coli* 026:B6; Sigma) stimulation in an incubator (37°C, 5% CO₂, 2 h, 1 μ g/ml) for five-panel cytokine expression or was *in vitro* heat shocked in a water bath at 42°C for 2 h for HSP72 production and cellular apoptosis, respectively. Total leukocyte counts were obtained from K₂EDTA-treated whole blood using a hematology analyzer (Coulter A^{CT} diff 2; Beckman Coulter, Miami, FL) and corrected for changes in blood volume incorporating hemoglobin and hematocrit values (14). Total

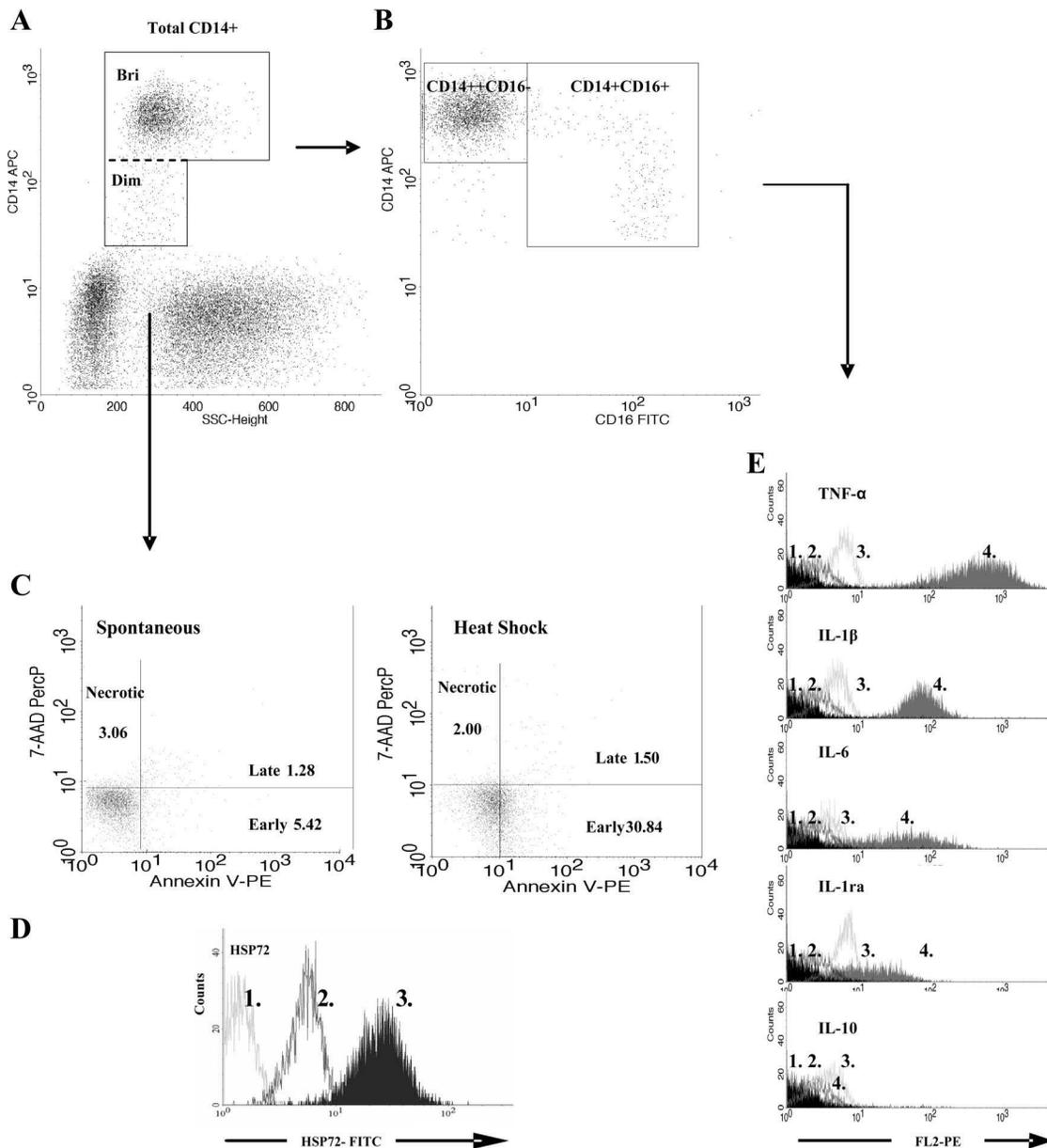


Fig. 1. Representative multiparameter flow cytometric immunofluorescence analysis of blood monocyte subsets. *A* and *B*: dot plots show sequential gating for CD14⁺CD16⁻/CD14⁺CD16⁺ and CD14^{Bri}/CD14^{Dim} subsets of total CD14⁺ monocytes in lysed whole blood. CD14 APC, allophycocyanin-conjugated CD14 MAb. *C*: dual-color dot plot [annexin V vs. 7-aminoactinomycin D (7-AAD)] representing cellular apoptosis. *D* and *E*: fluorescence histograms show intracellular cytokine (1, control; 2, isotype; 3, spontaneous; 4, *in vitro* LPS), and HSP72 (1, control; 2, spontaneous; 3, *in vitro* heat shock) staining, for CD14⁺ monocyte subsets. Representative data are from a sedentary-untrained subject.

Table 1. Total leukocytes, CD14⁺ monocytes, and CD14⁺CD16⁺ subset counts in TR and UT groups during EHS

	Baseline	38.0°C	38.5°C	39.0°C	39.5°C	Exh
Total leukocytes						
TR	6.2±0.4	8.1±0.5†	8.5±0.6†	9.2±0.6†	10.1±0.6†	10.2±0.6†‡
UT	6.1±0.4	8.4±0.4†	8.4±0.3†	8.6±0.3†		8.5±0.4†
Monocytes						
TR*	0.58±0.054	0.79±0.073†	0.79±0.083†	0.82±0.074†	0.88±0.075†	0.92±0.078†‡
UT	0.46±0.031	0.60±0.041†	0.58±0.045†	0.58±0.052†		0.58±0.052†
Inflammatory subset						
TR	0.04±0.01	0.098±0.014†‡	0.104±0.018†‡	0.11±0.02†‡	0.089±0.013†	0.089±0.014†‡
UT	0.03±0.003	0.06±0.008†	0.062±0.009†	0.06±0.01†		0.062±0.011†

Values are means ± SE of counts (×10⁹ cells/l) of total leukocytes, CD14⁺ monocytes, and CD14⁺CD16⁺ subsets between trained (TR) and untrained (UT) groups during exertional heat stress (EHS). Baseline (36.9°C) and exhaustion (Exh: UT, 39.1°C; TR, 39.7°C), *n* = 12 TR and 11 UT subjects; 39.0°C, *n* = 12 TR and 9 UT subjects; 39.5°C, *n* = 11 TR subjects. **P* < 0.05, main effect for group. †*P* < 0.05, within-group difference compared with baseline. ‡*P* < 0.05, between-group difference.

monocyte and inflammatory subset counts were obtained by multiplying the corresponding population percentages obtained from FACS analysis by the total leukocyte count.

Intracellular five-panel cytokine expression. Fresh BFA-treated whole blood (100 μl) aliquots were incubated immediately for 20 min in the dark with lineage-specific monoclonal fluorescent MABs, anti-CD14 APC, and anti-CD16 FITC for surface phenotyping. Stained white blood cells were then separated from whole blood using BD lysing solution, incubated (10 min in the dark), and centrifuged at 500 *g* for 5 min, and the supernatant was aspirated. Cell membranes were then treated with 500 μl of BD 1× FACS permeabilizing solution and incubated (10 min in the dark) and centrifuged (500 *g*, 5 min) before the supernatant was decanted and stained using intracellular cytokine-specific PE-conjugated MAb (IgG₁ normal mouse intracellular isotype control, TNF-α, IL-1β, IL-6, IL-1ra, and IL-10) and incubated (30 min in the dark). Cells were then washed (1× FACS CellWASH, 10 min) and centrifuged (500 *g*, 5 min), and the supernatant was discarded. The pellet was resuspended in 200 μl of 2% paraformaldehyde before multiparameter flow cytometric analysis was performed.

Intracellular HSP72 expression. The intracellular HSP72 staining protocol was similar to that outlined for intracellular cytokine staining, substituting a commercially available HSP72-specific FITC-conjugated mouse anti-human IgG₁ MAb (SPA-810; StressGen) or an unstained control for the BD anti-CD16 FITC, as well as non-BFA-treated whole blood samples instead of BFA-treated whole blood.

Detection of cellular apoptosis. The frequency of leukocytes undergoing apoptosis was determined by membrane phosphatidylserine exposure using AnV binding in conjunction with the vital dye 7-AAD and multiparameter flow cytometry, as previously described (60). Fresh and cultured whole blood samples were washed with 2 ml of BD binding buffer and centrifuged (500 *g*, 5 min). After centrifugation, the pellet was resuspended with 100 μl of binding buffer and incubated in the presence of PE-conjugated AnV and 7-AAD stains and anti-CD14-APC for cell surface phenotyping for 15 min in the dark, followed by a 10-min treatment with BD lysing solution. Cells

were then centrifuged (500 *g*, 5 min), and the supernatant was aspirated and resuspended in 400 μl of binding buffer. Samples were acquired within 1 h.

Flow cytometric acquisition and analyses. Stained cell suspensions were acquired on a FACSCalibur flow cytometer calibrated for four-color analyses and analyzed using CellQuest Pro software (BD, San Jose, CA). Dual-gating 90° side scatter (SSC) and APC were used to acquire ≥3,000 CD14⁺ monocytes based on cell surface staining characteristics for apoptosis, HSP72, and the cytokines (Fig. 1). Values are reported as the frequency of cells (%cytokine-positive events) and the amount of cytokine being produced per cellular population [mean fluorescence intensity (MFI)]. Quadrant markers and analysis gates for delineation of positive and negative regions were based on the corresponding isotype-matched or negative controls (see below).

Intracellular five-panel cytokine production. Analysis gates were determined using corresponding intracellular isotype-matched controls for the five-panel cytokine analyses. Additional bivariate dot plots gating on CD14⁺ monocytes were used to delineate classic (CD14⁺CD16⁻) and inflammatory monocyte (CD14⁺CD16⁺) subsets (90) (Fig. 1) and to calculate the corresponding percentage of cytokine-positive cells and MFI.

Intracellular HSP72 protein expression. Analysis gates were determined using negative control histograms. Total CD14⁺ monocyte intracellular HSP72 protein expression was determined and expressed as MFI and %HSP72-positive cells. CD14⁺ monocytes were further classified as CD14^{Bri} and CD14^{Dim} on the basis of on CD14 staining intensity and 90° SSC representative of classic and inflammatory monocyte subsets, respectively (Fig. 1). Additional back gating analyses confirmed that sole CD14^{Dim} staining produces a population representative of the CD14⁺CD16⁺ subset (data not shown).

Detection of cellular apoptosis in CD14⁺ monocytes. CD14⁺ monocytes (3,000 events) and subsets CD14^{Bri} and CD14^{Dim} were analyzed on a dual-color AnV/7-AAD fluorescence dot plot to determine the fraction of spontaneously and heat-induced necrotic (AnV⁻/

Table 2. Classic vs. inflammatory monocyte subset percentage of total CD14⁺ monocytes for TR and UT groups during EHS

	Baseline	38.0°C	38.5°C	39.0°C	39.5°C	Exh
CD14 ⁺ CD16 ⁻						
TR	92.2±1.0	87.3±1.3†	87.2±1.4†	86.9±1.7†	89.6±1.4†	90.3±1.5†
UT	92.4±1.0	89.1±1.2†	88.6±1.3†	88.7±1.6†		88.2±1.4†
CD14 ⁺ CD16 ⁺						
TR*	7.3±1.1	12.1±1.2†	12.5±1.3†	13.1±1.7†	10.1±1.4†	9.9±1.6†
UT	6.4±0.5	9.8±1.1†	10.4±1.1†	10.3±1.5†		10.4±1.5†

Values are means ± SE expressed as a percentage of total CD14⁺ monocytes for classic (CD14⁺CD16⁻) and inflammatory (CD14⁺CD16⁺) monocyte subsets. Baseline (36.9°C) and Exh (UT, 39.1°C; TR, 39.7°C), *n* = 12 TR and 11 UT subjects; 39.0°C, *n* = 12 TR and 9 UT subjects; 39.5°C, *n* = 11 TR subjects. **P* < 0.05, main effect for group. †*P* < 0.05, within-group difference compared with baseline.

7-AAD⁺), late apoptotic (AnV⁺/7-AAD⁺), early apoptotic (AnV⁺/7-AAD⁻), and total apoptotic (early + late) monocytes (Fig. 1).

Circulating HSP72. Plasma concentration of inducible eHSP72 was determined following the kit manufacturer's instructions (StressXpress; Assay Designs, Ann Arbor, MI) using a quantitative ELISA technique with a sensitivity of 0.09 pg/ml. Samples were diluted fourfold, with an additional twofold dilution as necessary.

Statistical analyses. An ANOVA with one repeated factor (temperature) and one between factor (fitness) was calculated on the various immunologically and physiologically dependent measures sampled during EHS as well as a separate ANOVA comparison between baseline and Exh measures. In addition, a one-factor (fitness) ANOVA was used to compare physiologically dependent measures, such as T_{re} tolerated at Exh, TT, rate of T_{re} increase, and anthropometric data. An ANOVA with two repeated factors (temperature and stimulus or shock or subset) and one between factor (fitness) was calculated on baseline and Exh measures, in vitro samples stimulated with LPS or heat shocked (HS), respectively. For all analyses, subject

numbers were as follows: baseline to 38.5°C and Exh, *n* = 12 TR and 11 UT subjects; 39.0°C, *n* = 12 TR and 9 UT subjects; and 39.5°C, *n* = 11 TR subjects. To correct for violations in the assumption of sphericity with the repeated factors, we applied the Huynh-Feldt correction to the *F*-ratio. Post hoc comparisons were performed using a Newman-Keuls procedure to isolate specific between-group mean differences at each T_{re} interval and within-group difference over temperature. All ANOVAs were performed with statistical software [StatSoft 2007 Statistica (data analysis software system), version 8.0; www.statsoft.com]. For all analyses, an α level of 0.05 was used.

RESULTS

Group characteristics. Anthropometric group characteristics for age (23 ± 4 yr), height (177 ± 6 cm), and mass (76 ± 9 kg) were not significantly different between the groups (68). However, the TR group did have a significantly higher $\dot{V}O_{2peak}$ expressed per kilogram of LBM [70 ± 2 (63–80) vs. 50 ± 1

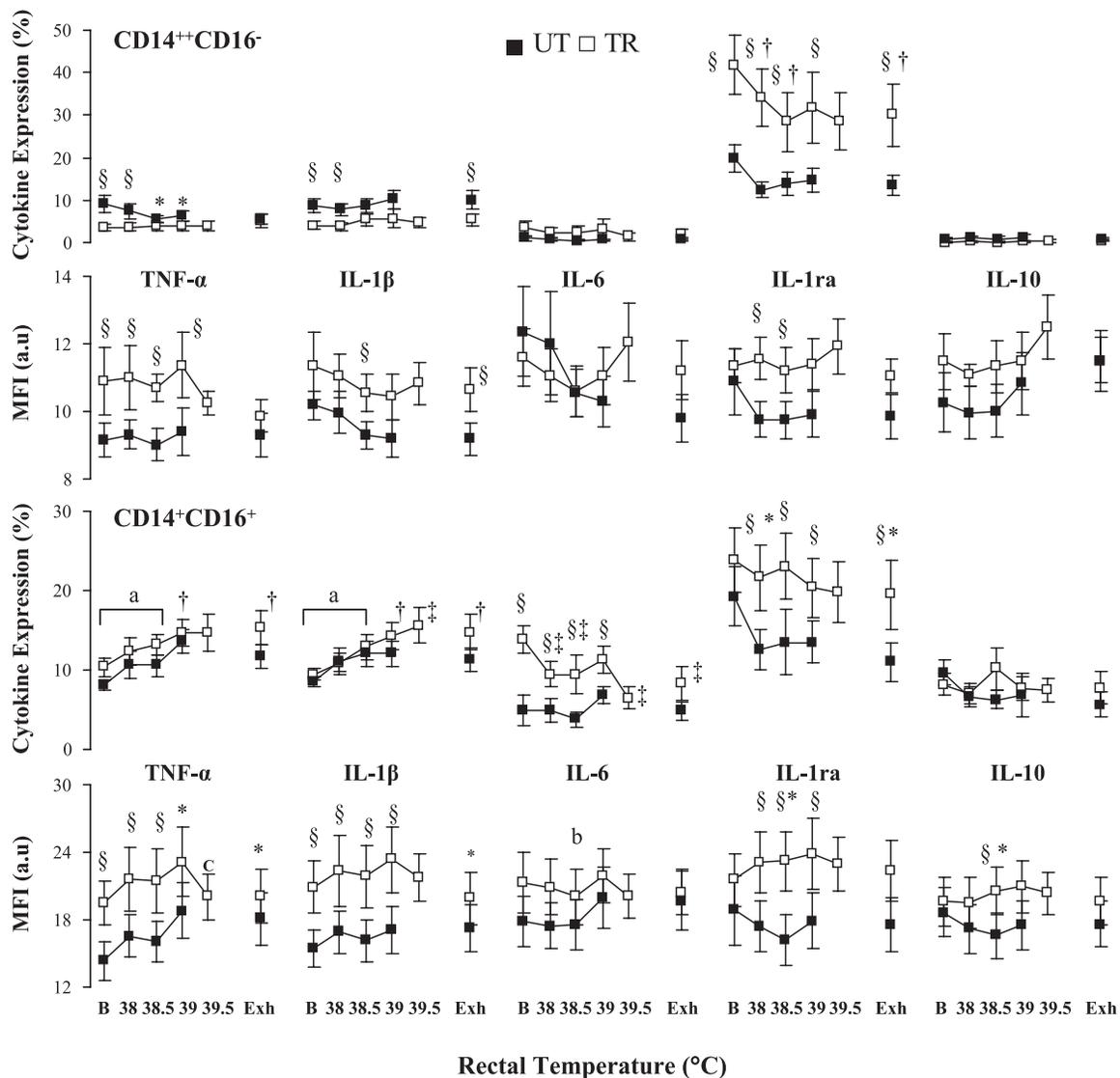


Fig. 2. Intracellular changes in the percentage (%) of cytokine-positive cells and mean fluorescence intensity (MFI; in arbitrary units, a.u.) in TNF- α , IL-1 β , IL-6, IL-1 receptor agonist (IL-1ra), and IL-10 by circulating classic (CD14⁺⁺CD16⁻) and inflammatory monocytes (CD14⁺CD16⁺) during exertional heat stress (EHS) between trained (TR) and untrained (UT) groups. Values are means \pm SE. Baseline (B; 36.9°C) to 38.5°C and exhaustion (Exh; UT, 39.1°C; TR, 39.7°C), *n* = 12 TR and 11 UT subjects; 39.0°C, *n* = 12 TR and 9 UT subjects; 39.5°C, *n* = 11 TR subjects. **P* < 0.05, UT significantly different from baseline. †*P* < 0.05, TR and UT significantly different from baseline. ‡*P* < 0.05, TR significantly different from baseline. §*P* < 0.05, between-group significance. ^a*P* < 0.05, main effect of temperature. ^b*P* < 0.05, main effect of group. ^c*P* < 0.05, TR at 39.5°C significantly different from 39.0°C.

(45–52) ml·kg LBM⁻¹·min⁻¹] and per unit of total mass [62 ± 2 (54–73) vs. 42 ± 1 (37–44) ml·kg⁻¹·min⁻¹] and lower body fatness [9.6 ± 1.0 (6–15) vs. 15.8 ± 1.7 (10–24)%] compared with the UT group (TR vs. UT, respectively). In addition, elevated blood and plasma volumes were observed in TR compared with UT groups (see Ref. 68 for values).

EHS exposure. EHS produced significant temperature-dependent increases in HR, mean skin temperature, and T_{re} in both groups, reported previously in detail (68). Briefly, HR at Exh was not significantly different between groups (159 ± 4.8 beats/min). T_{re} tolerated at Exh was higher in TR (39.7 ± 0.1°C) compared with UT subjects (39.1 ± 0.1°C), which produced significantly longer TT in TR (162.5 ± 11 min) compared with UT subjects (106 ± 10 min). However, despite the longer TT values in the TR group, the rate of T_{re} increase (1.2 ± 0.05°C/h) and time between T_{re} sampling intervals were not significantly different between groups up to 39.0°C. Reasons for trial termination consisted of six TR subjects attaining the ethical T_{re} cutoff of 40.0°C, one UT subject attaining the ethical HR cutoff, and the remaining 16 subjects reaching physical exhaustion.

Total leukocytes and subset changes. EHS produced a temperature-dependent increase in total leukocytes, monocytes, and inflammatory (CD14⁺CD16⁺) monocyte subset (×10⁹ cells/l) (Table 1). Main effects of temperature were also observed in classic (CD14⁺CD16⁻) and inflammatory (CD14⁺CD16⁺) subset percentages (Table 2). There were no differences observed between CD14⁺CD16⁻ and CD14^{Bri} or CD14⁺CD16⁺ and CD14^{Dim} subset proportion in relation to total CD14⁺ monocytes. However, a significantly greater increase was observed in CD14⁺CD16⁺ cell counts in TR compared with UT subjects (Table 1).

Intracellular five-panel cytokine production. Intracellular changes in the percentage of cytokine-positive cells and protein content (MFI) for CD14⁺CD16⁻ and CD14⁺CD16⁺ monocyte subsets are depicted in Fig. 2. There were no changes observed in proinflammatory TNF-α or IL-1β by CD14⁺CD16⁻ cells in TR subjects, although UT subjects displayed a higher percentage of expression and TR subjects a greater intracellular protein content, respectively (Fig. 2). EHS produced a temperature-dependent increase in CD14⁺CD16⁺ TNF-α and IL-1β expression and protein content. A main effect of temperature was seen from baseline to 38.5°C for intracellular TNF-α and IL-1β cytokine-positive cells. Of note were the significant within-group difference observed in cytokine-positive cells at 39.0°C in both TR and UT subjects, yet MFI increases in TNF-α (39.0°C) and IL-1β (Exh) by CD14⁺CD16⁺ cells were observed only in UT subjects. In contrast, MFI was greater in TR compared with UT subjects for TNF-α and IL-1β, but the only within-group difference observed in TR subjects during EHS was a decrease in TNF-α in CD14⁺CD16⁺ cells at 39 to 39.5°C. Although not significant, a similar reduction in TNF-α MFI was also observed in TR CD14⁺CD16⁻ cells (trend *P* = 0.11). IL-6 and IL-1ra expression were elevated in CD14⁺CD16⁺ cells of TR compared with UT subjects, with a temperature-dependent reduction during EHS. The only change observed in intracellular IL-10 was a decreased MFI in UT CD14⁺CD16⁺ inflammatory monocytes during EHS. In vitro LPS stimulation produced significant increases in both the percentage of cytokine-positive cells and MFI in TNF-α, IL-1β, IL-6, and IL-1ra compared with baseline, pre-EHS, and post-EHS,

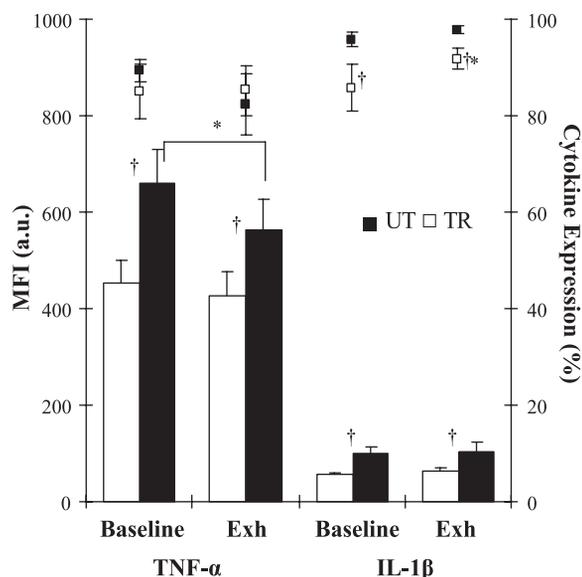


Fig. 3. LPS tolerance in TR and UT groups represented as changes in intracellular expression (squares) and MFI (columns) of TNF-α and IL-1β by circulating total CD14⁺ monocytes following in vitro LPS stimulation (1 μg/ml, 2 h) before (baseline) and after (Exh) acute EHS exposure. Values are means ± SE. Baseline (36.9°C) and Exh (UT, 39.1°C; TR, 39.7°C), *n* = 12 TR and 11 UT subjects. **P* < 0.05, within-group significance. †*P* < 0.05, between-group significance.

respectively (Fig. 3 and Table 3). Reduced proinflammatory TNF-α and IL-1β protein contents were observed in TR compared with UT subjects following LPS stimulation, indicative of a greater LPS tolerance within the TR group. However, a significant reduction in the percentage of cytokine-positive cells was only observed for IL-1β (Fig. 3). After EHS, a reduction in TNF-α MFI was observed in UT subjects compared with baseline, in addition to an increase in the percentage of IL-1β-positive cells. Conversely, significantly elevated levels of IL-1ra expression and protein content following LPS stimulation were observed in TR compared with UT subjects post-EHS despite reduced IL-1ra expression during acute exposure (Fig. 2). For LPS-induced IL-6, a tendency (*P* = 0.06) toward higher IL-6 expression in TR subjects from pre- to post-EHS was observed (Table 3). There were no changes observed in intracellular IL-10 following LPS stimulation, a result that may have been affected by the length of stimulation as well as cell type examined (27, 72).

Intracellular HSP72 protein expression. CD14⁺ monocytic HSP72 intracellular MFI at baseline was not significantly different between groups, whereas the percentage of cells expressing HSP72 was greater in UT subjects at baseline. EHS produced a temperature-dependent increase in both the percentage of expression and MFI in CD14⁺ monocytes in TR subjects only (Fig. 4). A similar pattern of response was observed in CD14^{Bri} and CD14^{Dim} subsets compared with CD14⁺ total monocytes (Fig. 5). Interestingly, the physiological percentages of cells expressing HSP72 were reduced in CD14^{Bri} in TR subjects, similar to that in CD14⁺ monocytes; however, this reduction was not observed in the CD14^{Dim} subset. In contrast, intracellular HSP72 protein content was significantly elevated in TR CD14^{Dim} compared with TR CD14^{Bri} and UT CD14^{Dim} at baseline, 39.5°C, and Exh. There were no differences in physiological expression of HSP72 in

Table 3. Percentage of positive cells and MFI of total CD14⁺ monocytes following in vitro LPS stimulation

	IL-6	IL-1ra	IL-10
<i>Cells positive for intracellular cytokine, %</i>			
Baseline			
TR	62.8±5.2*	57.3±7.1*	1.2±0.6
UT	61.1±4.0*	50.1±4.8*	0.7±0.4
Exhaustion			
TR	69.0±3.5*	65.7±8.2*†	0.5±0.4
UT	63.6±4.0*	49.2±4.3*	0.6±0.4
<i>MFI of cells positive for Intracellular cytokine, a.u.</i>			
Baseline			
TR	67.1±10.4*	25.3±2.2*	13.3±1.1
UT	70.8±9.3*	22.2±1.9*	14.7±1.4
Exhaustion			
TR	72.0±8.5*	28.6±3.1*†	12.8±0.8
UT	61.8±8.1*	22.0±1.3*	12.5±2.2

Values are means ± SE of percentages of cells positive for intracellular cytokines IL-6, IL-1 receptor agonist (IL-1ra), and IL-10 and mean fluorescence intensities (MFI, in arbitrary units, a.u.) of total CD14⁺ monocytes following LPS stimulation. Baseline (36.9°C) and Exh (UT, 39.1°C; TR, 39.7°C), *n* = 12 TR and 11 UT subjects. **P* < 0.05, significant change with stimulation. †*P* < 0.05, between-group difference.

UT subjects during EHS. In vitro heat shock produced significant increases in the percentage and MFI of all subsets across groups at baseline and post-EHS (Fig. 5); however, only an increase in the TR CD14^{Dim} percentage of HSP72-positive cells increased baseline to post-EHS. HSP72 MFI was consistently higher in TR compared with UT subjects pre- and post-EHS across all subsets. A lower percentage of HSP72-positive cells was observed in CD14^{Dim} compared with CD14^{Bri} in both groups, whereas a decreased CD14^{Dim} MFI was observed in UT subjects only.

Cellular apoptosis. Spontaneous apoptosis in monocytes and monocyte subsets are depicted in Table 4 and Fig. 6, respectively. EHS produced a significant increase in the percentage of spontaneous apoptotic cells in UT subjects, whereas in vitro heat shock significantly increased the percentage of apoptotic cells in both groups. A reduction in cellular apoptosis in TR compared with UT subjects following heat shock corresponded with the greater intracellular HSP72 expression in TR subjects at baseline (Table 4). CD14⁺ late apoptotic and necrotic percentages were below 5% and did not change during EHS or following in vitro heat shock (data not shown), although a greater percentage of necrotic cells was observed in UT compared with TR subjects. A comparison of the percentage of apoptotic CD14^{Bri} and CD14^{Dim} depicted a response similar to that for the total CD14⁺ monocyte population, although the significant increase in apoptotic cells at Exh was not observed in UT subjects (trend *P* = 0.13). In addition, UT CD14^{Dim} apoptotic responses were significantly elevated compared with UT CD14^{Bri} as well as TR CD14^{Dim}.

Circulating plasma HSP72. A temperature-dependent increase in plasma HSP72 concentration was observed during EHS (Fig. 4). Although baseline circulating levels were not significantly different, TR plasma HSP72 elevations were significantly greater than UT levels during EHS and occurred at a lower level of thermal strain (38.5 vs. 39.0°C).

DISCUSSION

The present study demonstrates that intracellular TNF-α and IL-1β expression is increased in inflammatory (CD14⁺CD16⁺) but not classic (CD14⁺⁺CD16⁻) monocytes during EHS. Enhanced intracellular HSP72 expression in circulating monocytes was accompanied by reduced intracellular inflammatory activation in endurance-trained individuals at a given level of thermal strain and is concordant with our recently published findings examining circulating inflammatory mediators in the same cohort of subjects (68). In addition, observed impairment of HSP induction in sedentary untrained subjects coincided with increased EHS-induced cellular apoptosis and lower T_{re} tolerated, providing additional insight into the mechanism(s) associated with enhanced intestinal barrier function observed with endurance training.

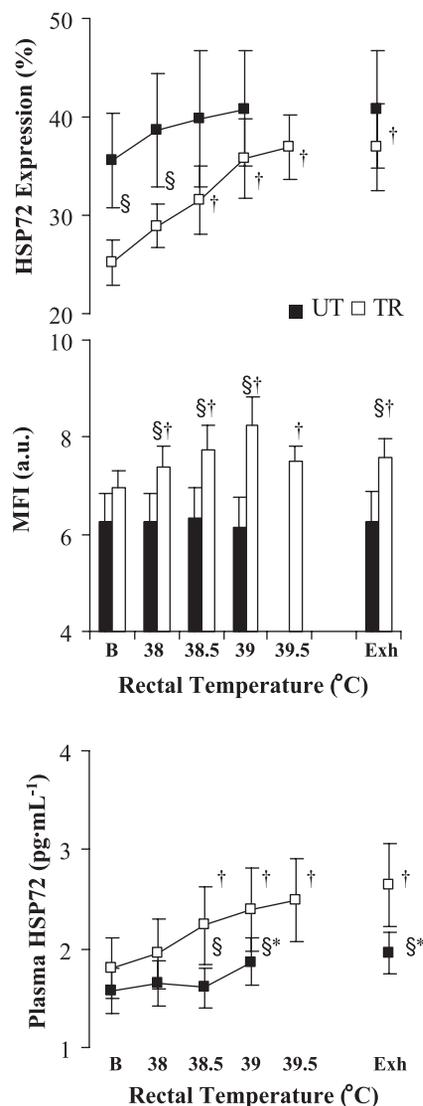


Fig. 4. Changes in total CD14⁺ monocytes intracellular HSP72 expression, MFI, and concentration of HSP72 in plasma during EHS between TR and UT groups. Values are means ± SE. Baseline (36.9°C) to 38.5°C and Exh (UT, 39.1°C; TR, 39.7°C), *n* = 12 TR and 11 UT subjects; 39.0°C, *n* = 12 TR and 9 UT subjects; 39.5°C, *n* = 11 TR subjects. **P* < 0.05, UT significantly different from baseline. †*P* < 0.05, TR significantly different from baseline. ‡*P* < 0.05, between-group significance.

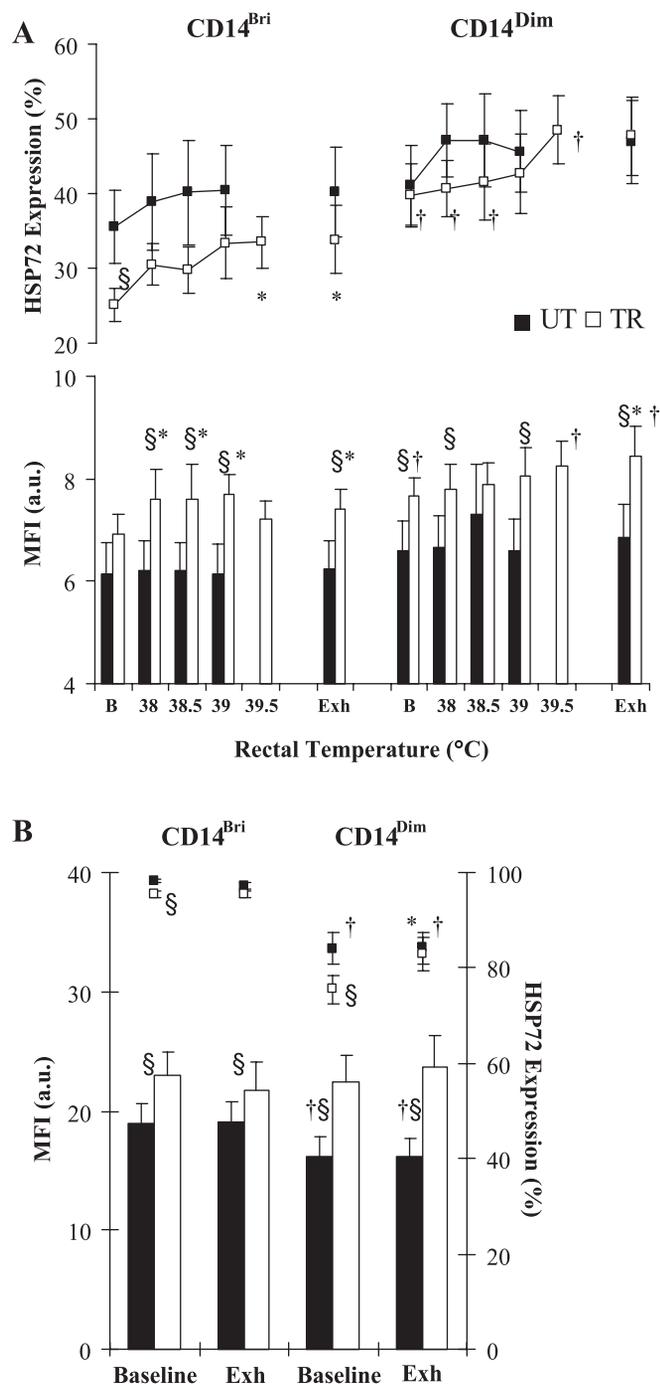


Fig. 5. Changes in HSP72 intracellular expression (squares) and MFI (columns) by circulating CD14^{Bri} and CD14^{Dim} monocytes during EHS (A) and following in vitro heat shock (42°C, 2 h; B) between TR and UT groups. Values are means \pm SE. Baseline (36.9°C) to 38.5°C and Exh (UT, 39.1°C; TR, 39.7°C), $n = 12$ TR and 11 UT subjects; 39.0°C, $n = 12$ TR and 9 UT subjects; 39.5°C, $n = 11$ TR subjects. * $P < 0.05$, TR significantly different from baseline. † $P < 0.05$, between-subset significance. § $P < 0.05$, between-group significance.

Classic (CD14⁺⁺CD16⁻) vs. inflammatory (CD14⁺CD16⁺) monocytes. CD14⁺CD16⁺ inflammatory monocytes are intrinsically involved in host defense, responding directly to invading pathogens through their transcription and release of multiple cytokines, promoting initiation of the acute phase response. In contrast, CD14⁺⁺ classic monocytes

primarily act as scavengers, aiding in the neutralization of unbound LPS (65, 86) and removal of apoptotic cells as part of the mononuclear phagocyte system (46). At rest, a majority of the CD14⁺CD16⁺ monocytes are not present in the peripheral circulation but reside in the marginal pool and are released into the peripheral circulation via catecholamine-mediated sympathetic mechanisms (78). The time course of peripheral blood inflammatory monocyte expansion during disease (>50%) (25) has been found to follow systemic cytokine appearance (7, 91); however, with physical stress, an immediate mobilization of CD14⁺CD16⁺ cells from the marginal pool is observed (31, 78). EHS elicited a similar increase in CD14⁺CD16⁺ subsets, with a greater increase observed in TR subjects. This response may be a specific adaptation to training, analogous to the enhanced β_2 -adrenoreceptor-mediated natural killer (NK) cell mobilization reported with exercise training (68), mediated through improved β -adrenergic receptor sensitivity and binding (43).

Peripheral monocytes and intracellular cytokine production. Early studies examining intracellular cytokine production have suggested that peripheral monocytes may not be a primary source of inflammatory cytokines released during strenuous exercise, despite well-documented increases in circulating TNF- α (74, 77). This conclusion is also supported by more recent findings by Zaldivar et al. (89) and Vassilakopoulous et al. (84) suggesting that monocytes do not contribute significantly to augmented cytokine production following cycling exercise (30–45 min, 75–80% $\dot{V}O_{2peak}$). Importantly, however, previous studies failed to distinguish between specific monocyte subsets, employing only single-color staining with CD14⁺ (58, 84), CD33⁺ (74–77), or CD4^{Dim} (89) for monocyte identification, all of which are specific for the classic monocyte subset (5, 12).

Early examination of the classic vs. inflammatory monocyte subsets using polymerase chain reaction (PCR) revealed that CD14⁺CD16⁺ blood monocytes produce high levels of proinflammatory cytokine mRNA transcripts compared with classic monocytes (27). Recent analysis using intracellular staining confirmed that CD14⁺CD16⁺ monocytes are major producers of TNF- α and possess a higher sensitivity for LPS-induced TNF- α production in vivo compared with CD14⁺⁺CD16⁻ monocytes (6, 62, 64). Therefore, it seems plausible that CD14⁺CD16⁺ monocytes are responding to mild endotoxemia accompanying strenuous exercise (9, 50) or EHS (68). A common finding when examining intracellular cytokine profiles with exercise, with or without hyperthermia, has been either no change or a slight reduction in the level of spontaneous TNF- α in cytokine-positive peripheral blood monocytes in trained (74, 77, 89) and untrained subjects (84), similar to the present study's responses in CD14⁺⁺CD16⁻ monocytes. By contrast, Rhind et al. (58) found corresponding increases in intracellular monocyte expression and circulating TNF- α during exhaustive exercise and subsequent cold exposure, changes that were suggested to be reflective of the inflammatory status of the cells before the exposure, or perhaps specific immune modulating influences accompanying exercise (58).

Limited data has been reported examining inflammatory monocytes with exercise; however, cycling at 75% $\dot{V}O_{2peak}$ to exhaustion has been reported to produce significant increases in CD14⁺CD16⁺ intracellular protein expression of TNF- α , IL-1 β , and IL-6 in healthy donors (21). The latter exercise

Table 4. Percentage of apoptotic CD14⁺ monocytes and intracellular HSP72 expression in TR and UT groups before and after EHS and following in vitro heat shock

	Spontaneous			In Vitro Heat Shock (2 h, 42°C)		
	HSP72		Apoptosis, %	HSP72		Apoptosis, %
	Positive cells, %	MFI, a.u.		Positive cells, %	MFI, a.u.	
Baseline						
TR	25.2±2.2	7.0±0.3	4.9±1.1	93.1±0.8*	23.1±2.0*‡	20.8±1.8*‡
UT	35.6±4.8‡	6.2±0.6	6.1±0.8	95.2±1.9*	18.2±1.5*	31.5±4.2*
Exhaustion						
TR	37.0±4.4*	7.6±0.4*‡	7.7±1.5‡	94.0±1.1†	22.0±2.5†‡	18.3±2.6†
UT	40.7±5.9	6.3±0.6	15.1±2.1*	94.1±1.8†	17.3±1.7†	19.5±2.2†

Values are means ± SE of percentages of CD14⁺ monocytes positive for heat shock protein 72 (HSP72), MFI for HSP72, and percentages of apoptotic CD14⁺ monocytes in TR and UT groups before (baseline) and after exhaustion EHS and following in vitro heat shock. Baseline (36.9°C) and Exh (UT, 39.1°C; TR, 39.7°C), $n = 12$ TR and 11 UT subjects. * $P < 0.05$, within-group difference compared with baseline. † $P < 0.05$, within-group difference compared with Exh. ‡ $P < 0.05$, between-group difference.

intensity is comparable to previous work examining total monocytes, which suggests that CD14⁺CD16⁺ intracellular inflammatory activation may have occurred in these previous studies. In the present study, EHS was accompanied by an increase in CD14⁺CD16⁺ cells positive for proinflammatory TNF- α and IL-1 β , but an increase in protein content did not occur until temperatures approached 39.0°C (see Fig. 2), possibly due to the appearance of endotoxin in the circulation of these subjects (68). As previously suggested (76), it does not appear that circulatory IL-6 or IL-1ra increases originate from peripheral monocyte subsets; in fact, a reduction in protein content was observed during EHS, possibly reflecting the inflammatory status of these cells. In contrast, minimal changes in intracellular IL-10 were observed in the present study, which may be due to signaling kinetics and/or current gating profiles, since it has recently been shown that the inflammatory subset can be subdivided into CD14^{Bri}CD16⁺ and CD14^{Dim}CD16⁺

(31, 72), where CD14^{Bri}CD16⁺ and CD14^{Dim}CD16⁺ are the major producers of IL-10 and TNF- α , respectively (72).

Intracellular signaling pathway and cytokine kinetics. A variety of mediators stimulate proinflammatory intracellular signal transduction via kinase-dependent phosphorylation of the NF- κ B/I κ B pathway, resulting in NF- κ B p65 nuclear translocation (19), including but not limited to cytokines, reactive oxygen species, LPS, and complement (1). NF- κ B p65 translocation occurs within minutes of extracellular stimulation, and subsequent intracellular protein accumulation follows within 30–60 min (26). Typically, intracellular proteins appear before or simultaneously with extracellular protein (38). Thus the appearance of circulating TNF- α in our subjects (68) before intracellular accumulation (Fig. 2) supports the notion that intestinal epithelial/mucosa and the liver may be the primary source of inflammatory cytokines during EHS induced-endotoxemia (10, 56), with the peripheral CD14⁺CD16⁺ cells responding later, after the appearance of proinflammatory TNF- α or endotoxin in the systemic circulation (68).

Anti-inflammatory/antiapoptotic effects through induction of the stress response. It is well documented that HSP induction within the cell can downregulate the inflammatory cascade by reducing TNF- α production to inflammatory stimuli (11, 18, 36, 73, 79). The mechanism responsible for the upregulation of HSP during inflammatory stimuli results from serine kinase and mitogen-activated protein kinase (MAPK) activation of the NF- κ B pathway, coupled with the production of reactive oxygen species and subsequent oxidative stress in the mitochondria. This cascade activates HSF-1 in the cytoplasm, which leads to an increase in HSP72 mRNA transcription in the nucleus (1). HSP72 is released back into the cytosol, where it forms a complex with NF- κ B and I κ B, inhibiting nuclear translocation of NF- κ B (11) and stabilizing I κ B (88).

Although limited data on intracellular HSP72 expression exist for EHS, significant increases in the percentage of positive cells and protein content have been reported in peripheral monocytes in trained subjects following strenuous exercise (22, 23) and EHS (23, 60). Training adaptations associated with intracellular HSP72 expression have been observed previously in CD14⁺ monocytes (60); however, this is the first study to examine the differences between CD14⁺ monocyte subsets and their associated intracellular inflammatory activation at a given level of thermal strain. It appears that the

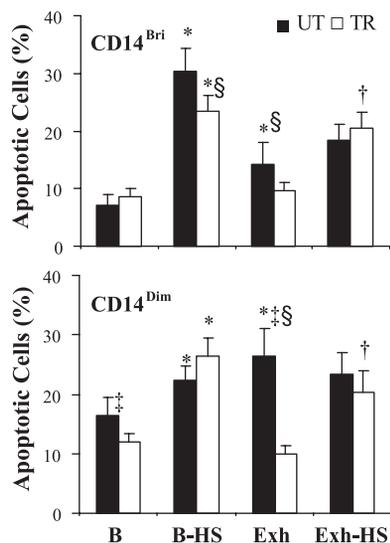


Fig. 6. Changes in percentage of apoptotic cells in circulating CD14^{Bri} and CD14^{Dim} monocytes before and after acute EHS and following in vitro heat shock (HS: 42°C, 2 h) between TR and UT groups. Values are means ± SE. Baseline (36.9°C) to 38.5°C and Exh (UT, 39.1°C; TR, 39.7°C), $n = 12$ TR and 11 UT subjects. * $P < 0.05$, significantly different from baseline. † $P < 0.05$, significantly different from Exh. ‡ $P < 0.05$, between-subset significance. § $P < 0.05$ between-group significance.

training-induced reduction in the percentage of HSP72-positive cells (22) and leukocyte protein content (69) is not uniform across all CD14⁺ monocyte subsets (see Fig. 5). Furthermore, enhanced spontaneous basal HSP72 protein content and HSP72 induction in TR CD14^{Dim} cells suggests an adaptive response in the inflammatory subset, perhaps as a result of repeated low-level LPS exposures during training (68), a mechanism that also may be contributing to the greater LPS tolerance observed in TR subjects (see Fig. 3).

The blunted response to EHS in untrained subjects has been reported previously (60) and has been linked to heat intolerance (49) as well as disease (16); however, the mechanism responsible for the reduced HSP response is less clear. Findings by Melling et al. (43) suggest that enhanced extracellular signal-regulated kinase (ERK)1/2, a suppressive secondary signal for HSF-1 transcription and HSP72 expression, may contribute to the blunted HSP72 in UT subjects, possibly mediated through increased NF- κ B signaling (68). The blunted intracellular response has been found to improve following a single bout of EHS (data not shown) as well as to increase with training (43, 60, 87). Although training-induced increases in HSF-1 DNA binding have been observed, an important finding reported was that training altered ERK1/2 signaling pathways such that concurrent activation of ERK1/2 and HSP72 expression was achieved following acute exercise (43).

Circulating levels of eHSP72 have been suggested to play an important systemic role, acting as an extracellular danger signal to activate immune responses (24, 33). Released from hepatosplanchnic tissues (20) in an intensity- and duration-dependent manner (24, 45), eHSP72 can induce LPS tolerance in THP-1 cells (2) and has been related to the inflammatory status during heat illness (51, 61). We observed a greater eHSP response in TR compared with UT subjects, similar to the intracellular differences and findings reported by others (60). However, it remains to be seen whether a link exists between eHSP72 release from hepatosplanchnic tissue and the endotoxemia associated with EHS (68).

Increased rates of organ-specific cell death are considered to be involved in the pathophysiology of MODS in a variety of systemic disorders, including heatstroke (35, 52), contributing to the breakdown of intestinal barrier function observed during endotoxemia (13, 15, 39, 66, 80, 85). Increased physiological levels of inflammatory stimuli can promote an increase in cellular apoptosis (52, 63), which is supported by the finding that TNF receptor shares intracellular signaling domains with FAS/CD95, a crucial receptor involved with apoptotic signaling (48). Inhibition of HSF-1 signaling (63) also has been linked to TNF- α , which supports the theory of transcriptional dysfunction related to heat intolerance as presented by Moran et al. (49). Induction of HSP has been shown to regulate apoptosis directly through c-Jun NH₂-terminal kinase (JNK) and p38 pathways, which is critical for cellular apoptosis inhibition while promoting ERK survival pathways (60). Furthermore, enhanced levels of circulating IL-10 as observed in our TR subjects (68) also may help to potentiate HSP expression (34) while reducing TNF- α -induced apoptosis at Exh despite significantly greater levels of thermal strain.

Perspectives

This is the first study to substantiate the link between endotoxin translocation and intracellular activation of peripheral inflammatory monocytes during EHS. Identification of the contribution of peripheral inflammatory monocytes in response to endotoxemia supports the initiation of "heat sepsis" (40) within physiologically relevant temperature thresholds. Furthermore, there appears to be intricate anti-inflammatory/anti-apoptotic cytoprotective adaptations associated with endurance training, which are accompanied by an enhanced stress response. Based on the current surrogate observations of apoptosis profiles in peripheral monocytes, differences in organ-specific apoptosis profiles may be contributing to the differences in rectal temperatures tolerated, intestinal barrier dysfunction, and the proinflammatory profile associated with the progression of EHI observed between trained and untrained individuals during EHS.

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