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TITLE

Mechanisms of Exercise-Induced Leukocytosis: Contribution of Exertional Hyperthermia to Sympathoadrenal-Mediated Lymphocyte Subset Redistribution

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Contribution of exertional hyperthermia to sympathoadrenal-mediated lymphocyte subset redistribution

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[1]*Defence and Civil Institute of Environmental Medicine, Toronto, Ontario M3M 3B9;* [2]*Faculty of Physical Education and Health,* [3]*Department of Laboratory Medicine and Pathobiology, and* [4]*Department of Public Health Sciences, University of Toronto, Toronto, Ontario, Canada M5G 1L57;* [5]*Centre de Recherches du Service des Santé des Armées, 38702 La Tronche, France; and* [6]*Hospital das Clínicas da Faculdade de Medicina, da Universidade de São Paulo, 05403-0 Ribeirõ Preto, Brazil*

Rhind, Shawn G., Greg A. Gannon, Pang N. Shek, Ingrid K. M. Brenner, Yvonne Severs, Jiri Zamecnik, Alain Buguet, Valéria M. Natale, Roy J. Shephard, and Manny W. Radomski. Contribution of exertional hyperthermia to sympathoadrenal-mediated lymphocyte subset redistribution. *J. Appl. Physiol.* 87(3): 1178–1185, 1999.—The contribution of hyperthermia to the differential leukocytosis of exercise remains obscure. This study examined changes in circulating sympathoadrenal hormone concentrations and patterns of leukocyte and lymphocyte subset ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD3^-16^+/56^+$) redistribution during exercise, with and without a significant rise of rectal temperature (T_{re}). Ten healthy men [age 26.9 ± 5.7 (SD) yr, body mass 76.0 ± 10.9 kg, body fat 13.9 ± 4.6%, peak O_2 consumption: 48.0 ± 12.4 $ml \cdot kg^{-1} \cdot min^{-1}$] exercised for 40 min (65% peak O_2 consumption) during water immersion at 39 or 18°C. T_{re} increased from 37.2 to 39.3°C ($P < 0.0001$) after 40 min of exercise in 39°C water but was held constant to an increment of 0.5°C during exercise in 18°C water. Application of this thermal clamp reduced exercise-associated increments of plasma epinephrine (Epi) and norepinephrine (NE) by >50% ($P < 0.05$) and abolished the postexercise increase in cortisol. Thermal clamping also reduced the exercise-induced leukocytosis and lymphocytosis. Multiple regression demonstrated that T_{re} had no direct association with lymphocyte subset mobilization but was significantly ($P < 0.0001$) correlated with hormone levels. Epi was an important determinant of total leukocytes, lymphocytes, and $CD3^+$, $CD4^+$, $CD8^+$, and $CD3^-CD16^+/56^+$ subset redistribution. The relationship between NE and lymphocyte subsets was weaker than that with Epi, with the exception of $CD3^-CD16^+/56^+$ counts, which were positively ($P < 0.0001$) related to NE. Cortisol was negatively associated with leukocytes, $CD14^+$ monocytes, and $CD19^+$ B- and $CD4^+$ T-cell subsets but was positively related to granulocytes. We conclude that hyperthermia mediates exercise-induced immune cell redistribution to the extent that it causes sympathoadrenal activation, with alterations in circulating Epi, NE, and cortisol.

catecholamines; cortisol; epinephrine; heat stress; hormones; immune; natural killer cells; lymphocytosis; norepinephrine; thermal physiology; water immersion

LEUKOCYTOSIS IS A CARDINAL RESPONSE to many physiological (e.g., stress, exercise) (4, 32) and pathological conditions (e.g., endotoxemia, fever) (6, 45) that influence cellular entry to, and egress from, the intravascular space. Dynamic exercise reproducibly elicits the mobilization of immunocompetent cells to the peripheral blood (23) from marginal pools residing within the microvasculature of various lymphoid and nonlymphoid organs, including the spleen, bone marrow, and lungs (24, 36). Typically, this comprises an immediate granulocytosis and monocytosis (19), followed by a neutrocytosis during the postexercise period; the magnitude of these changes depends on the intensity, duration, and type of exercise (1). Moreover, there is a differential lymphocytosis, with $CD3^-CD16^+/56^+$ natural killer (NK) cells displaying the greatest fluctuations, followed by $CD3^+$ T- and $CD19^+$ B-cell subsets (44). Because leukocyte recruitment and recirculation are essential for effective immune surveillance, the underlying mediators and immunologic relevance of these phenomena are of considerable interest (23, 37).

The mechanisms responsible for the dynamic exchange of intravascular leukocytes reflect the propensity of immune cells to undergo rapid demargination and redistribution between compartments in response to mechanical (5, 17) and/or neuroendocrine signals occurring with exercise (32, 35). These signals are mediated, in large measure, by activation of the sympathetic nervous system (SNS), and stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, with the release of catecholamines [epinephrine (Epi) and norepinephrine (NE)] and glucocorticoids (cortisol) (4, 33). NE release from postganglionic sympathetic nerve terminals in target tissues, with circulatory spillover, stimulates marked α-adrenergic effects; these include arteriolar vasoconstriction in the skin and viscera and the redistribution of cardiac output to the working muscles and lungs (18, 40). These hemodynamic adjustments enhance vascular shear stress, thereby promoting the release of marginated cells from the walls of the vascular endothelium into the bloodstream (17).

In addition to evoking profound shifts in cardiovascular hemodynamics and blood flow to various tissues, augmented sympathoadrenal hormone release during exercise directly affects the retention and/or extrusion of selective leukocyte subpopulations within various

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immune compartments via interaction with specific cell-surface and cytosolic receptors, which are heterogeneously expressed (30, 33). Furthermore, both adrenoceptor (4, 9) and glucocorticoid-receptor stimulation are linked to alterations in cellular adhesion; thus exercise-induced hormonal stimulation may provoke leukocyte-subset-specific redistribution by modulating the affinity and/or expression of adhesion molecules on distinct immune and/or endothelial cells (19).

Heat and exercise stress interact synergistically, pushing physiological systems toward their limits (46). In the absence of thermoregulatory adjustments, increased metabolic heat production can elevate core body temperature [as measured by rectal temperature (T_{re})] by up to 1°C every 5 min (21). Exertional hyperthermia (i.e., $T_{re} \geq 39$°C) (3) is suggested to play a role in exercise-induced neuroimmunomodulation (39). However, its contribution to the sympathoadrenal-mediated lymphocyte subset redistribution is not clearly defined (7, 42). Induction of in vivo hyperthermia, by passive heat exposure (via hot-water immersion or hot-air exposure) or an exercise-induced increase in metabolism, is known to elicit significant neuroendocrine (20, 34, 41) and immune responses (8, 10, 15). Studies in which passive heating was used, in an attempt to isolate the immunologic effects of hyperthermia, demonstrate a pattern of leukocyte subset redistribution similar to that observed with exercise (12, 15, 28); however, the direction and magnitude of both hormonal and lymphocyte subset changes induced by passive heating are less consistent than those achieved by exercise (39). By contrast, extreme hyperthermia ($T_{re} \geq 42$°C) produces a marked lymphocytosis, characterized by significant increases in NK and T-cell subsets (6, 22) and is associated with altered expression of cellular adhesion molecules (22, 47). Furthermore, combined heat-exercise stress, whether induced in air or water, amplifies neuroendocrine and immune responses relative to those seen during either passive heating or exercise alone (7, 12).

Cross et al. (12) have previously demonstrated that cold-water (23°C) immersion can successfully maintain core temperature at, or near, basal levels ($T_{re} = 37.8 \pm 0.3$°C) during intensive cycle ergometer exercise. Accordingly, application of this type of thermal clamp may provide an effective means of manipulating the various hormonal and leukocyte responses associated with temperature elevation during exercise. Given the evidence that increased body temperature has a profound influence on hormone secretion during exercise (42), and the association of hormone secretion with leukocyte mobilization (7), the present study was designed to examine more extensively the contribution of exertional hyperthermia to sympathoadrenal activation and differential lymphocyte subset redistribution, by using the technique of thermal clamping. Specifically, we investigated the changes in selected circulating lymphocyte subsets ($CD3^+$ T, $CD4^+$ $T_{helper/inducer}$, $CD8^+$ $T_{cytotoxic/suppressor}$, $CD3^-CD16/56^+$ NK, and $CD19^+$ B cells), and their possible relationship to variations in circulating catecholamines and cortisol, during 40 min of cycling while the subjects were immersed in hot (39°C) or cold (18°C) water. We hypothesized that exercise-induced perturbations of lymphocyte subsets and associated sympathoadrenal hormone release would be significantly attenuated by thermal clamping.

METHODS

Subjects. Ten recreationally active [peak oxygen consumption ($\dot{V}O_{2peak}$) 48.0 ± 12.4 (SD) $ml \cdot kg^{-1} \cdot min^{-1}$] male nonsmokers (age 26.9 ± 5.7 yr, height 1.75 ± 0.07 m, body mass 76.0 ± 10.9 kg, body fat 13.9 ± 4.6%) volunteered for study. After receiving an explanation of all procedures, risks, and benefits, each volunteer gave his informed consent to participate in a research protocol approved by the Human Experimentation Committees of the Defence and Civil Institute of Environmental Medicine and the University of Toronto. An initial medical examination excluded subjects if they had a history of allergies, acute or chronic infection, or contraindications to vigorous exercise.

Preliminary testing. This session served to acquaint participants with the laboratory equipment and procedures to be used during subsequent testing and to establish their physiological profile. Body mass was measured to the nearest 0.5 kg using an electronic scale (Setra, Acton, MA). Body density, corrected for residual lung volume, was measured by hydrostatic weighing. $\dot{V}O_{2peak}$ was determined by a progressive cycle ergometer test while the subjects were immersed in thermally neutral water (33°C). After a 4-min warm-up of pedaling (60 rpm) against water resistance alone (~75 W), loading of the ergometer was increased by 25 W/min to volitional exhaustion, which was reached within 10–12 min. Expired gas, collected breath by breath, was analyzed for respiratory minute volume and O_2 consumption, by using a metabolic measurement cart (model 2900C, SensorMedics, Yorba Linda, CA), calibrated against standard cylinder gas mixtures and a 3-liter syringe. Heart rates were recorded by using a Vantage XL heart rate monitor (Polar, Port Washington, NY).

Experimental design. Subjects reported to the laboratory 2 h before their immersion (0700) after an overnight fast. They refrained from alcohol, caffeine, medications, and exercise for 48 h before all testing. Testing was conducted at the same time of day, to avoid intertrial effects from circadian rhythms. To standardize nutritional conditions, each subject consumed 1.1 MJ (250 kcal) of a commercial liquid meal supplement (16-oz Ensure Plus, Abbott Laboratories, Saint Laurent, PQ). After instrumentation was completed, subjects rested for 30 min at a room temperature of 26–28°C before preimmersion baseline measurements.

Cycle ergometer exercise took place in a well-stirred (3.75-m^3) rectangular plastic water bath, containing an electrically braked cycle ergometer (Pedalmate, Collins, Braintree, MA), with a pressurized crank-case to prevent water infiltration. Water temperature was measured at the surface and bottom of the tank; values were held constant (±0.1°C) by means of a microcomputer-regulated thermocouple system (nanoVolt-Ohm meter, Hewlett-Packard, Toronto, ON) and a thermostatically controlled heat exchanger (Alfa-Laval, Rome, Italy). Subjects entered the water bath wearing shorts, neoprene water socks, and a weighted diver's waist belt to reduce their buoyancy while seated on the ergometer. The subjects were immersed to midchest, a technique effective in clamping the rise in core temperature observed during exercise in hot water (12). Each subject completed two randomized 40-min submaximal (65% $\dot{V}O_{2peak}$) exercise bouts in hot (39°C) and cold water (18°C); sessions were separated by an interval of

1 wk. T_{re} values were recorded continuously throughout each session, by using a thermistor probe (Baxter Pharmaseal, Valencia, CA) inserted 0.14 m into the rectum. The intensity of effort was adjusted as necessary, on the basis of repeated measurements of heart rate and oxygen consumption during a given trial. One arm was supported comfortably above the water surface (shoulder level) to allow blood sampling. After exercise, the subjects were dried, and recovery measurements continued in air (28°C) for a further 120 min. Subjects consumed an additional 500 ml of water during this period.

Blood sampling and hematologic analyses. Peripheral venous blood samples were drawn from a 21-gauge intravenous catheter (Insyte, BD Vascular Access, Sandy, UT) inserted into a superficial forearm vein. Six specimens of 20 ml each were collected preimmersion (30 min); at 0, 20, and 40 min of exercise while immersed; and at 30 and 120 min postexercise. Patency was maintained by using a heparinized saline solution. Total leukocyte numbers, differential counts, Hb, and hematocrit (Hct) determinations were performed on 3 ml of K_3EDTA-treated blood, by using a Coulter JT hematology analyzer (Coulter Electronics, Hialeah, FL). Venous blood samples for catecholamine determinations were drawn into 4.5 ml K_3EDTA vacutainers containing glutathione (Amersham, Oakville, ON). After gentle mixing, they were placed into an ice-water bath before being centrifuged (Beckman Instruments, Mississauga, ON) for 15 min (4°C, 2,250 *g*). The separated plasma was then transferred to prechilled polypropylene Eppendorf tubes (GIBCO Life Technologies, Burlington, ON), frozen, and stored at 70°C for later analysis. Reported leukocyte and lymphocyte subset counts were adjusted for percent changes in blood volume (Δ%BV), and hormonal concentrations were adjusted for changes in plasma volume (Δ%PV), as calculated from Hb and Hct (14).

Immunophenotyping. Determination of lymphocyte subpopulations was performed by using dual-parameter immunofluorescence labeling of K_3EDTA-treated whole blood and optimal concentrations of FITC and phycoerythrin-conjugated monoclonal antibodies (44). Stained cell suspensions were enumerated on a FACScan flow cytometer equipped with a 15-mW air-cooled 488-nm argon-ion laser, by using standard operating methods (Becton-Dickinson Immunocytometry Systems, San Jose, CA). Daily instrument calibration used a mixture of monosized FITC- and phycoerythrin-conjugated and unconjugated latex particles (4.8-mm CaliBRITE beads), in conjunction with AutoCOMP software. Digitized data were acquired and analyzed on a Macintosh microcomputer system by using Cell Quest and Attractors softwares (Becton-Dickinson Immunocytometry Systems). Counts for individual subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD3^-CD16^+/56^+$, $CD19^+$, $CD14^+$) were obtained by multiplying the corresponding percentages of cells derived from the FACScan by the total leukocyte counts on the Coulter counter.

Hormonal analyses. Unbound plasma catecholamine concentrations were quantitated by gas chromatograph-mass spectrometry as previously described (50). Total plasma concentrations of cortisol were measured in duplicate by commercial solid-phase ^{125}I radioimmunoassay kits (ICN Biomedicals, Irvine, CA). All specimens from a given subject were analyzed in the same assay run to minimize interassay variations. The intra- and interassay coefficients of variations were ≤10% for both hormones.

Statistical analyses. Significance of changes in leukocyte subsets and stress hormones were analyzed by two-way repeated-measures ANOVA. When the *F* ratio showed significant interaction effects, post hoc pairwise multiple-contrast comparisons were computed to identify sources of differences between time points. Associations between individual cell counts, hormone levels, and T_{re} were explored by using stepwise multiple regression. An alpha level of 0.05 was accepted as indicating significance. Calculations were performed by using StatView and SuperANOVA microcomputer software packages (SAS Institute, Cary, NC).

RESULTS

Core temperature response and thermal clamping. The effectiveness of thermal clamping is illustrated by the T_{re} vs. time profile shown in Fig. 1. After 40 min of exercise with hot (39°C)-water immersion, the average T_{re} increased significantly ($P < 0.0001$) from 37.2 to 39.3°C. By contrast, during exercise in cold (18°C)-water, there was only a minor increment (0.5°C) of T_{re}.

Δ%BV and Δ%PV. Results of the ANOVA revealed that changes in the concentration of Hb and Hct, Δ%BV, and Δ%PV were all significant ($P < 0.0001$) across time during both hot- and cold-water immersions (Table 1). A significant ($P = 0.03$) interaction effect between conditions was only found for changes in the Hct. Forty minutes of exercise under both hot- and cold-water conditions elicited similar reductions in Δ%BV (5.6 ± 0.75 vs. 5.3 ± 0.80) and Δ%PV (9.7 ± 1.4 vs. 9.2 ± 1.3). There were no significant changes in any of the above-mentioned parameters during the recovery period.

Plasma hormone concentrations and core temperature. Resting, preimmersion plasma free-catecholamine concentrations were within the normal control levels (Epi = 0–480; NE = 615–3,240 pmol/l) for young adult men (50) (Table 2). Both Epi and NE peaked at the end of 40 min of exercise. During hot-water immersion, Epi and NE levels increased by >500% ($P < 0.001$) and 300% ($P < 0.05$) of their preimmersion values, respectively. Clamping significantly reduced these increases to <200 and <100% for Epi and NE, respectively. Total resting cortisol concentrations fell within the normal range of 138–635 nmol/l (12). Cortisol levels rose relatively slowly, peaking 63% ($P < 0.0001$) above baseline 30 min after exercise (Table 2). In contrast to hot-water immersion, exercise in cold water was followed by a significant ($P < 0.05$) reduction in plasma cortisol concentration.

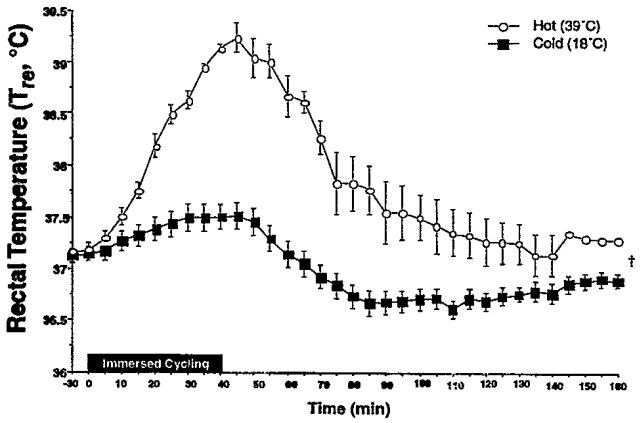

Fig. 1. Rectal temperature (T_{re}) vs. time during and after exercise with hot- and cold-water immersion. Values are mean ± SE for 10 subjects. † Statistically significant difference in mean value over time for the entire session, $P < 0.0001$.

Table 1. *Hemoglobin concentration, hematocrit, and blood and plasma volumes during cycle ergometer exercise with hot (39°C)- and cold (18°C)-water immersion*

		Sample Time					
		Rest		Exercise		Recovery	
Parameter	Condition	−30 min	0 min	20 min	40 min	70 min	160 min
Hemoglobin, g/dl	Hot	14.3 ± 0.11	14.6 ± 0.19	15.3 ± 0.23†	15.4 ± 0.21†	14.8 ± 0.31	14.7 ± 0.24
	Cold	14.4 ± 0.12	14.6 ± 0.22	14.8 ± 0.25	15.1 ± 0.27†	14.8 ± 0.32	14.7 ± 0.28
Hematocrit, %	Hot	44.2 ± 0.42	44.5 ± 0.44	46.9 ± 0.63†	47.0 ± 0.73†	44.9 ± 0.63	44.8 ± 0.74
	Cold	44.3 ± 0.61	44.7 ± 0.64	45.3 ± 0.71‡	46.1 ± 0.78*	45.5 ± 0.83	44.9 ± 0.85
Blood volume, Δ%	Hot	0.0 ± 0.0	−0.3 ± 0.54	−4.8 ± 0.93†	−5.6 ± 0.75†	−1.4 ± 0.96	−0.6 ± 1.2
	Cold	0.0 ± 0.0	−0.2 ± 0.45	−3.4 ± 0.76*	−5.3 ± 0.80†	−1.2 ± 1.3	−1.3 ± 1.3
Plasma volume, Δ%	Hot	0.0 ± 0.0	−1.6 ± 1.1	−8.6 ± 1.6†	−9.7 ± 1.4†	−1.7 ± 1.6	−0.8 ± 2.2
	Cold	0.0 ± 0.0	−2.1 ± 1.3	−6.3 ± 1.2*	−9.2 ± 1.3†	−3.1 ± 2.3	−3.4 ± 2.2

Values are means ± SE for 10 men. Δ, Change. Significant change from rest: *$P < 0.01$; †$P < 0.001$. ‡Significant change between hot and cold exercise trials, $P < 0.05$.

Total leukocytes and subsets changes. Initial resting values for total peripheral blood leukocytes and leukocyte subsets did not differ significantly between trials (Fig. 2, *A–D*). Combined exercise-heat stress induced significant biphasic mobilization of circulating leukocytes (55%) and granulocytes (88%), with values peaking 2 h postexercise ($P < 0.001$). As in the earlier trial of Cross et al. (12), the leukocyte and granulocyte responses to exercise were largely abolished by thermal clamping, with the exception of a substantial rise ($P < 0.001$) in granulocyte concentration 2 h after exercise in the cold. The peak increase in lymphocyte count during exercise was also smaller ($P < 0.05$) and less well sustained with clamping (35 vs. 45%). Circulating monocyte counts did not differ between conditions.

Lymphocyte subsets changes. During the hot-water immersion, there were significant increases in circulating $CD3^+$ ($P < 0.001$), $CD4^+$ ($P < 0.05$), and $CD8^+$ ($P < 0.001$) counts during exercise and a significant drop in $CD3^+$ ($P < 0.05$) and $CD8^+$ ($P < 0.05$) counts after exercise (Fig. 3, *A–E*). However, thermal clamping largely abolished both the initial increase and the subsequent decline in these counts. In the case of the circulating $CD3^-CD16^+/56^+$ NK cells, exercise-heat stress produced a 125% increase after 20 min, which persisted until the end of exercise. Clamping significantly reduced ($P < 0.05$) the NK cell response, to ~50% of the unclamped value, but there remained a significant ($P < 0.05$) response to exercise. The response of the circulating $CD19^+$ cells showed no difference between clamped and unclamped conditions, with both trials demonstrating a significant ($P < 0.05$) increase only 2 h postexercise.

Relationships between core temperature, hormones, and leukocyte subsets. The relationships between circulating leukocyte and lymphocyte subsets and plasma hormone concentrations derived from stepwise multiple regression analyses are summarized in Table 3. The proportions of the total variance (R^2) attributed to catecholamines and cortisol ranged from 8 to 36% for the different subsets. Stepwise multiple-regression analyses demonstrated a strong overall relationship ($R^2 = 0.530$; $P < 0.0001$) between T_{re} and hormone levels, but T_{re} had no direct influence on variations in any circulating leukocyte or lymphocyte subsets. Individual hormone and T_{re} regression coefficients were found to be significant for Epi ($P < 0.0001$) and NE ($P < 0.0001$) concentrations but not for cortisol ($P = 0.609$). Epi concentration was an important determinant of total leukocytes and lymphocytes, along with $CD3^+$, $CD4^+$, $CD8^+$, and $CD3^-CD16^+/56^+$ subset counts. The relationship between NE and lymphocyte subsets was weaker than for Epi, with the exception of $CD3^-CD16^+/56^+$ counts, which showed a positive relationship to NE levels. Apart from granulocytes, the relationships of circulating cortisol levels were found to be negatively associated with total leukocytes, monocytes, $CD19^+$ B cells, and $CD4^+$ subsets.

Table 2. *Concentration of circulating hormones during cycle ergometer exercise with hot (39°C)- and cold (18°C)-water immersion*

		Sample Time			
				Recovery	
Hormone	Condition	Rest, 0 min	Exercise, 40 min	70 min	160 min
Epinephrine, pmol/l	Hot	284 ± 33	1,776 ± 203†	1,072 ± 236*	468 ± 71
	Cold	242 ± 29	716 ± 127*‡	311 ± 47	259 ± 41
Norepinephrine, pmol/l	Hot	2,528 ± 243	11,535 ± 1,718†	5,054 ± 2,458*	2,663 ± 262
	Cold	2,291 ± 196	4,491 ± 540†‡	3,684 ± 513*	2,822 ± 272
Cortisol, nmol/l	Hot	470 ± 44	608 ± 60	768 ± 68*	373 ± 35
	Cold	535 ± 67	551 ± 63	384 ± 47‡	295 ± 37

Values are means ± SE for 10 men. Significant changes from rest; *$P < 0.05$; †$P < 0.001$. ‡Significant change between hot and cold exercise trials, $P < 0.05$.

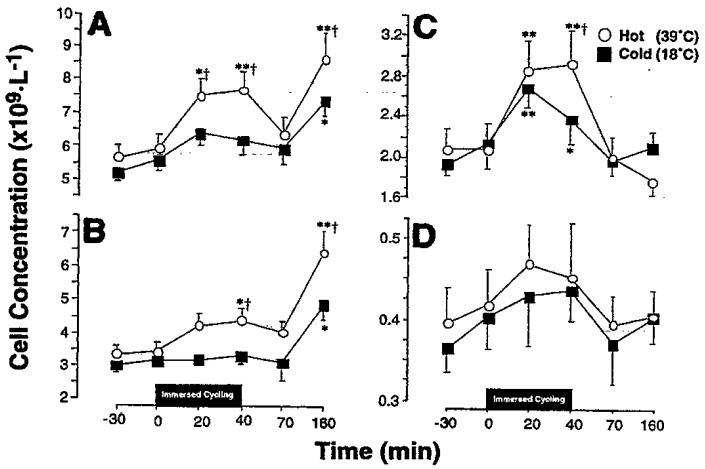

Fig. 2. Changes in total circulating leukocyte subsets during and after exercise with hot- and cold-water immersion. *A*: total leukocytes. *B*: granulocytes. *C*: lymphocytes. *D*: monocytes. Values are means ± SE for 10 subjects. Statistically significant differences from resting: $*P < 0.05$; $**P < 0.001$. †Significant intertrial differences, $P < 0.05$.

DISCUSSION

In this study, we used midchest water immersion as a method to investigate the impact of exertional hyperthermia on sympathoadrenal activation and consequent redistribution of circulating immunocompetent cells within the peripheral blood. The procedure we adopted allows manipulation of core body temperature while a constant exercise intensity is maintained (49). Our hypothesis was that exertional hyperthermia, acting as a direct physiological stimulus of sympathoadrenal activation was, at least partially, responsible for the differential lymphocytosis of exercise. Accordingly, thermal clamping by cold-water immersion should result in diminished exercise-induced catecholamine and cortisol release, with a concomitant reduction in lymphocyte subset redistribution.

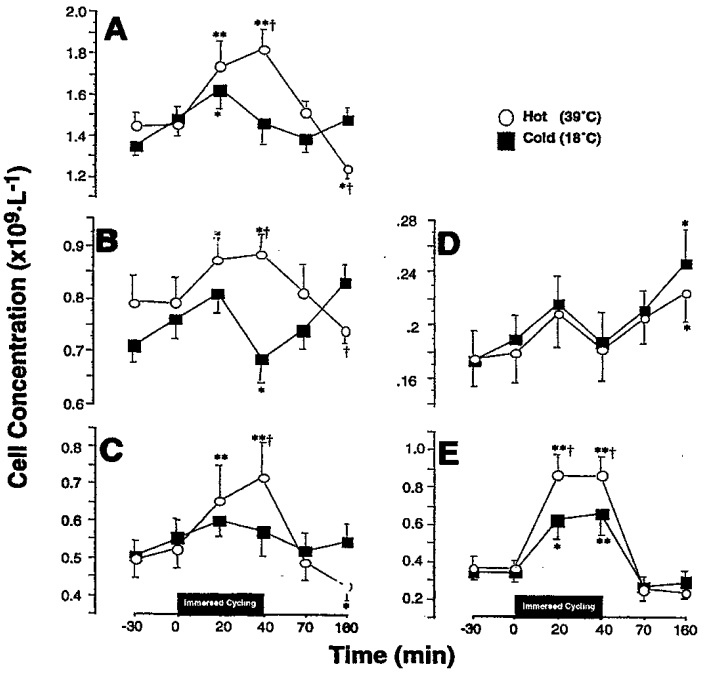

Fig. 3. Changes total circulating lymphocyte subsets during and after exercise with hot- and cold-water immersion. *A*: $CD3^+$ T cells. *B*: $CD4^+$ $T_{cytotoxic/suppressor}$. *C*: $CD8^+$ $T_{helper/inducer}$. *D*: $CD19^+$ B cells. *E*: $CD3^-/CD16/56^+$. Values are means ± SE for 10 subjects. Statistically significant differences from resting: $*P < 0.05$; $**P < 0.001$. †Significant intertrial differences, $P < 0.05$.

Table 3. *Relationship between circulating leukocyte and lymphocyte subset counts and plasma hormone concentrations by stepwise multiple regression*

Independent Variables	R^2	Probability Values		
		Epi	NE	Cortisol
Total leukocytes	0.255	0.008	0.02	0.0005*
Granulocytes	0.136	NS	NS	0.004
$CD14^+$ monocytes	0.317	NS	NS	0.02*
Lymphocytes	0.209	0.003	NS	NS
$CD3^+$ T cells	0.161	0.001	NS	NS
$CD4^+$ $T_{helper/inducer}$ cells	0.197	0.01	NS	0.01*
$CD8^+$ $T_{cytotoxic/suppressor}$ cells	0.075	0.03	NS	NS
$CD19^+$ B cells	0.178	NS	0.04	0.0005*
$CD3^-CD16^+/56^+$ NK cells	0.363	0.001	0.0001	NS

Probabilities are expressed as *P* values for individual regression coefficients. R^2, coefficient of determination; NK, natural killer; NS, not significant ($P > 0.05$). *Negative association between variables.

A key finding of this report was that exertional hyperthermia mediates the differential lymphocytosis of exercise indirectly. Our data suggest that temperature elevation does not exert a significant independent effect on immune cell redistribution, but rather that the responses are induced by exercise and thermally mediated stimulation of the SNS and HPA axis with the release of catecholamines and cortisol. This is supported by the close association between increases in core temperature and the five- to sixfold increments of circulating Epi and NE observed when exercise was performed in hot water.

Core temperature was observed to increase at a rate of ~0.05°C/min during exercise in hot water, reaching 39°C by the completion of 40 min of exercise and 39.3°C 5 min postexercise. By contrast, during exercise with cold-water immersion, thermal clamping effectively held core temperature constant. This finding supports previous assertions that induction of hormone secretion is associated with the magnitude of exercise-induced increases in core temperature (18). Our results also corroborate several investigations documenting that in vivo hyperthermia can provoke considerable neuroendocrine immunomodulation (7, 8, 39). In humans, circulating concentrations of catecholamines (34, 41, 48) and cortisol (11, 20, 34) are greatly augmented by combined exercise-heat stress.

The relationship between changes in circulating leukocyte subsets and hormone concentrations largely parallels the differential pattern of glucocorticoid and adrenoceptor expression by specific immune cells (30, 33). Increases in total leukocytes and $CD3^-CD16^+/56^+$ NK cells were related to both Epi and NE concentrations, whereas fluctuations of total lymphocytes, $CD3^+$,

$CD4^+$, and $CD8^+$ T-cells subsets were related only to Epi, and fluctuations of $CD19^+$ B cells were related to NE. These changes are consistent with studies identifying Epi-induced β_2-adrenoceptor stimulation as a primary mediator of lymphocyte subset redistribution (4). Conversely, granulocyte and $CD14^+$ monocyte counts were unrelated to catecholamines but were strongly associated with circulating cortisol concentrations. Physiological increases in glucocorticoids probably contributed to the exercise-induced monocytopenia by provoking their sequestration within the bone marrow, while at the same time inducing neutrophilia by eliciting the deployment of recently differentiated neutrophils from the bone marrow and their reduced extravasation to the tissues (24). In addition, elevated cortisol levels may have also contributed to the postexercise lymphocytopenia by selective retention of recirculating lymphocytes within the spleen and lymph nodes (33, 36). Thus it seems likely that exercise-induced increases in blood flow velocity and intravascular shear stress, combined with the direct effects of sympathoadrenal hormones on cell-cell interactions, work in a synergistic fashion to elicit differential leukocyte mobilization patterns during exercise and thermal stress.

This report is the first to demonstrate that nonpharmacological hormonal blockade by thermal clamping can substantially reduce exercise-elicited increases in circulating Epi and NE concentrations as well as in NK cell counts. $CD14^+$ monocytes and $CD19^+$ B cell counts were unchanged, but increases in the other circulating leukocyte and lymphocyte subset counts were greatly diminished when core temperature was clamped. As in previous studies, the delayed increase in circulating cortisol was comparatively small (39) and was abolished by thermal clamping (12). The abolition of cortisol release during thermal clamping appears to play an important role in limiting the exercise-induced rise in leukocyte and granulocyte counts. In addition, the lower cortisol levels during the clamped condition are likely to have contributed to the reduced lymphocytopenia and the significant reductions of $CD3^+$, $CD4^+$ and $CD8^+$ T-cell counts postexercise.

Acute reductions (6%) in BV, due to fluid shifts out of the intravascular space (hemoconcentration), were not a major factor contributing to the overall and differential leukocytosis that occurred with exercise, because no significant differences were found between measured and corrected cell counts. Similarly, the modest reduction of PV (10%) did not significantly influence circulating hormonal changes with exercise under either hot or cold conditions. These findings concur with previous reports demonstrating that fluid shifts accompanying acute swimming exercise make only a minor contribution to the changes in immune cell or bloodborne hormone concentrations (29) and with evidence that the hydrostatic pressure associated with water immersion may reduce that magnitude of volume shifts (49). Furthermore, our choice of midchest water immersion is unlikely to have produced a significant degree of central hypervolemia, as is common with water immersion to the neck, which can inhibit sympathoadrenal activation (16).

The fact that thermal clamping failed to completely abolish exercise-induced increases in circulating catecholamines and NK cells, despite the maintenance of core temperature at near basal levels, reinforces the idea that the factors controlling lymphocyte mobilization are complex and that isolated sympathoadrenal hormonal fluctuations do not account entirely for the observed responses (39). Such a conclusion is supported by the results of Kappel et al. (27, 28), who demonstrated that, although hot-water immersion and the selective infusion of Epi and NE mimicked the pattern of exercise-induced leukocyte and lymphocyte subset redistribution, neither produced the degree of leukocytosis seen in relation to intense exercise. Furthermore, recent studies (26) employing various forms of pharmacological hormonal blockade during in vivo whole body heating have failed to abrogate the hyperthermia-induced leukocytosis, suggesting that multiple mechanisms and mediators probably contribute to the rapid exercise-induced redistribution of leukocyte subsets. Therefore, on the basis of the present results, we cannot exclude the impact of other immunomodulatory hormones, including adrenocorticotrophic hormone, β-endorphin, and growth hormone, which are known to be augmented by hyperthermia and to affect leukocyte mobilization (26, 39).

Reductions in noncutaneous regional blood flow are an important component of the hemodynamic response to exertional hyperthermia. During intense exercise, splanchnic circulation is reduced to ~40% of its resting value (40) and may drop to as low as 20% during combined exercise-heat stress (46). This sharp reduction in visceral blood supply leads to significant intestinal ischemia, rendering the gastrointestinal walls permeable to bacterial lipopolysaccharide (LPS) endotoxin (38). Because of the high LPS gradient (31), this type of insult results in translocation of LPS into the systemic circulation (21).

Our results are compatible with an LPS-induced pattern of leukocyte redistribution, including a strong biphasic neutrophilia (45), which is augmented by the synergistic action of Epi and tumor necrosis factor-α (2). LPS also regulates the expression of cellular adhesion molecules on the vascular endothelium (25) and interacts additively with cortisol to produce a marked lymphopenia, with T cells being most severely affected (13, 45). Moreover, several recent studies support the concept that LPS-induced immune dysregulation is critically involved in the pathophysiology of heatstroke (6, 21, 22). Because leakage of LPS is suggested to begin at temperatures as low as 39°C (21), it is conceivable that mild endotoxemia may have contributed to the observed changes in leukocyte kinetics during immersed exercise in the heat (31).

Exertional hyperthermia may also play an active role in directing cellular migration by altering the function of adhesion molecules directly at the level of immune cells and/or the vascular endothelium (22). For example, hyperthermia markedly enhances intercellular

adhesion molecule-1 and L-selectin-mediated adhesion of lymphocytes to the endothelium and their emigration into tissues (47). As such, hyperthermia may have an integral role in the generation of an efficient immune response via amplification of lymphocyte transmigration into lymphoid tissues, i.e., the lymph nodes and Peyers patches, as well as into injured or inflamed tissues (23). Furthermore, changes in cellular adhesion in response to thermal stimuli may be regulated by other soluble factors, including cytokines such as tumor necrosis factor-α and interleukin-6, which are triggered in response to exercise (43) and hyperthermia (7).

Conclusions. Thermal clamping enabled us to isolate the effects of exercise on the redistribution of leukocyte and lymphocyte subpopulations. Induction of hyperthermia by combined exercise-heat stress increased the degree of sympathoadrenal activation and mobilization of immune cells relative to exercise alone. Multiple-regression analyses suggest that a rise of core temperature exerts much of its effect on leukocyte subset counts by modulating the output of stress hormones. Conversely, clamping of the thermal response to exercise by cold-water immersion greatly reduced catecholamine and cortisol responses, and smaller changes in the hormonal milieu largely explain the lesser redistribution of leukocyte and lymphocyte subsets observed under such conditions. Collectively, our findings demonstrate that elevation of core temperature during exercise is a critical mediator of SNS activation but that it is unlikely to be the sole stimulus of leukocyte redistribution. Therefore, although hyperthermia-induced hormonal release contributes to the mechanisms that regulate leukocyte mobilization with exercise, multiple factors are likely to be involved, including hemodynamic changes, alterations in gut permeability with the translocation of LPS, along with changes in cytokine induction and cellular adhesion molecule expression. Future studies should examine the possible contribution of other such mediators to the mechanism of exercise-induced leukocytosis.

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