86

CD4+/CD8+ T-lymphocyte ratio: effects of rehydration before exercise in dehydrated men

JOHN E. GREENLEAF, CATHERINE G. R. JACKSON, and DESALES LAWLESS

Laboratory for Human Environmental Physiology, Gravitational Physiology Branch (239-11) NASA, Ames Research Center, Moffett Field, CA 94035-1000; University of Northern Colorado, Greeley, CO 80639; and Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021-6399

ABSTRACT

GREENLEAF, J. E., C. G. R. JACKSON, and D. LAWLESS. CD4+/CD8+ T-lymphocyte ratio: effects of rehydration before exercise in dehydrated men. *Med. Sci. Sports Exerc.* Vol. 27, No. 2, pp. 194–199, 1995. Effects of fluid ingestion on CD4+/CD8+ T-lymphocyte cell ratios were measured in four dehydrated men (ages 30–46 yr) before and after 70 min of supine submaximal (71% $\dot{V}O_{2max}$) lower extremity cycle exercise. Just before exercise, Evans blue dye was injected for measurement of plasma volume. The subjects then drank one of six fluid formulations (12 $ml \cdot kg^{-1}$) in 3–4 min. All six mean post-hydration (pre-exercise) CD4+/CD8+ ratios (Becton-Dickinson Fluorescence Activated Cell Sorter and FACScan Consort-30 software program [San Jose, CA]) were below the normal range of 1.2–1.5; mean (± SE) and range were 0.77 ± 0.12 and 0.39–1.15, respectively. The post-exercise ratios increased: mean = 1.36 ± 0.15 ($P < 0.05$) and range = 0.98–1.98. Regression of mean CD4+/CD8+ ratios on mean plasma osmolality resulted in pre- and post-exercise correlation coefficients of −0.76 ($P < 0.10$) and −0.92 ($P < 0.01$), respectively. The decreased pre-exercise ratios (after drinking) were probably not caused by the Evans blue dye but appeared to be associated more with the stress (osmotic) of dehydration. The increased post-exercise ratios to normal levels accompanied the rehydration and were not due to the varied electrolyte and osmotic concentrations of the ingested fluids or to the varied vascular volume shifts during exercise. Thus, the level of subject hydration and plasma osmolality may be factors involved in the mechanism of immune system modulation induced by exercise.

DRINKS, DRINKING, IMMUNE FUNCTION, PLASMA VOLUME, PLASMA OSMOLALITY

0195-9131/95/2702-0194$3.00/0
MEDICINE AND SCIENCE IN SPORTS AND EXERCISE®

Submitted for publication June 1993.
Accepted for publication July 1994

Optimal functioning of the immune system of astronauts on long duration missions is essential for their health and performance. Some immune parameters appear to be compromised during spaceflight; post-flight immune suppression has been reported (1,4,14,19), but the mechanism and long-term consequences have not been elucidated. Lymphocyte reactivity also decreased significantly after 10 d of sedentary bed rest (3). Physical exercise training programs are being developed for astronauts to perform on extended flights as countermeasures for space flight deconditioning, but the mode, frequency, intensity, and duration of these exercise regimens are still under investigation. One recommendation is to employ alternating, high-intensity, lower extremity cycle exercise to maintain aerobic power and isokinetic exercise to maintain strength (7). Acute exercise and exercise training in particular influence immune parameters (11). Peripheral blood lymphocytes are mobilized into the circulation by moderate submaximal exercise; however, the CD4+/CD8+ lymphocyte ratio appears to be reduced significantly (immunosuppression) after a single maximal exertion and also during heavy, intensive training (11,12). Therefore, appropriate ground-based and inflight exercise-training regimens may help to ameliorate the putative spaceflight immune suppression.

Astronauts have reduced total body water with accompanying blood volume reduction (hypovolemia) during flight (6), which may be a stress on their systems until complete adaptation to microgravity occurs. More generalized stress also tends to reduce body

TABLE 1. Drink composition (per 2000 ml).

	Ia	II	IV	V	VI	VII
	Experimental Solutions				Commercial Solutions	
	Water	0.045% Na	NaCl/Na Citrate (0.36% Na)	9.7% Glucose +0.045% Na	Shaklee[a] Performance	Power[b] Surge
Sodium chloride (g)	—	2.24	9.00	—	—	—
Sodium citrate (g)	—	—	15.44	3.87	3.87	0.99
Dextrose (g)	—	—	—	216.00	41.12	34.81
Aspartame (g)	0.72	0.72	0.72	—	—	—
Shaklee Performance (g)	—	—	—	—	222.28	—
Power Surge (g)	—	—	—	—	—	208.70
Total	0.72	2.96	25.16	219.87	222.28	208.70
Total volume (ml)	2000	2000	2000	2000	2000	2000
Ionic concentration (meq·l^{-1}, % wt-vol^{-1})						
Na+	—	19.61/0.045	157/0.360	19.61 /0.045	19.61 /0.045	23.47 /0.055
K+	—	—	—	—	5.01 /0.020	2.51 /0.010
Cl−	—	18.95/0.060	76/0.270	18.95 /0.067	4.96 /0.018	—
Mg++	—	—	—	—	0.40 /0.002	—
Ca++	—	—	—	—	1.96 /0.016	—
P+++	—	—	—	—	0.51 /0.008	—
Total	—	38.56/0.112	233/0.630	38.56 /0.112	32.47 /0.10	26.48 /0.065
Carbohydrate (% wt-vol^{-1})						
Glucose	—	—	—	9.716	1.850	1.74
Fructose	—	—	—	—	2.426	1.44
Maltodextrin	—	—	—	—	5.440	3.47
Sucrose	—	—	—	—	—	3.07
Total	—	—	—	9.716	9.716	9.72
Osmolality	30	70	270	650	380	390

[a] Shaklee U.S., Inc., San Francisco, CA 94111.
[b] Perc Products, Moscow, PA 16444.

immune function (2). Although performance of moderate exercise (stress) may enhance some immune responses, the accompanying hypovolemic stress may depress other immune responses; hypovolemia induced by exercise is proportional to exercise intensity (10). The effect of fluid replacement (rehydration) on exercise-induced hypovolemia and immune function is not clear.

Thus, the purpose of this study was to investigate the effect of prior dehydration by fluid restriction and immediate pre-exercise rehydration on the CD4+/CD8+ lymphocyte ratio during supine submaximal exercise in men. The CD4+/CD8+ immune variables were selected as examples only. There are many other functional and surface marker immunological parameters that can change without influencing changes in CD4+/CD8+ and vice versa.

METHODS

Four men (ages 30–46 yr, height 171.0 ± SD 6.2 cm, weight 74.91 ± 8.09 kg, SA 1.87 ± 0.13 m^2) gave written informed consent and passed a comprehensive medical examination, including a treadmill stress test, before entry into the study. The protocol was approved by the Ames Human Research Experiments Review Board. Before each experiment, they dehydrated for 24 h and then exercised for 70 min in the supine position immediately after consuming one of six rehydration formulations (Table 1).

Procedure

Two wk before the rehydration experiments, peak oxygen uptake (measured with the subjects in the supine position) was extrapolated from heart rate-$\dot{V}O_2$ curves with loads of 400, 800, and 1200 kgm·min^{-1} on an Imaging Ergometer Table (model 846T, Quinton Instruments Co., Seattle, WA 98121) using our standard oxygen uptake procedures (7).

The subjects were dehydrated during 24 h before testing by eating only dry food and by restricting fluid intake voluntarily. They arrived in the laboratory at 0700 hr dressed in shorts and shoes. They urinated, were weighed, and then they lay supine on the ergometer for 45 min (resting control period). Fifteen min before exercise commenced plasma volume was measured with the Evans blue dye (T-1824) technique from one 10-min post-injection blood sample (8). The post-injection T-1824 blood sample was the pre-exercise control sample taken before drinking at −5 min.

The 70-min supine exercise period was conducted on the Quinton ergometer at a relative load of 71 ± SE 1% (range = 68–77%); shoulder braces and heel supports were used. Sufficient practice was performed to eliminate knee pain to allow the subjects to exercise comfortably. Heart rate was monitored with a cardiotachometer (model

78203C, Hewlett-Packard, Waltham, MA 02154). Room temperature was 22.0 ± SD 0.07°C and relative humidity was 46.9 ± SD 5.9%. There was no fluid intake during exercise. A urine sample was taken and body weight was measured immediately after exercise.

Mean pre-exercise plasma osmolality (Advanced Instruments Digimatic Osmometer, model 3D II, Needham Heights, MA 02194) before drinking in experiments Ia through VII was 298, 299, 300, 294, 291, and 304 $mOsm \cdot kg^{-1}$ H_2O, respectively, indicating moderate levels of hypohydration. Normal euhydration plasma osmolality is 285 ± 2 $mOsm \cdot kg^{-1}$ (18). Additional 20 ml venous antecubital blood samples were drawn through the teflon Quik-Catheter (Travenol Laboratories, Inc., Deerfield, IL 60015) at +3, +9, +15, +30, and +70 min of exercise. The 70 min sample, taken just before exercise ceased, was the post-exercise sample. The six experiments were conducted at weekly intervals.

Drinks

Five min before exercise the men drank one of the six rehydration drinks (12 $ml \cdot kg^{-1}$; range 791-1044 ml) in about 3–4 min (Table 1). The drinks were tested in a selected-randomized order: subject 1, drinks 1–6; subject 2, drinks 2, 3, 4, 5, 6, 1, etc.

Cell Analysis

One to 2 ml of the pre-and post-exercise whole blood samples at ambient temperature were sent by overnight mail to the Rockefeller University laboratory for flow cytometric analysis of the CD4+ and CD8+ cells. On arrival, an equal volume of balanced salt solution was added to each blood sample, and this cell suspension was then layered on 3 ml of Ficoll-Paque solution (Lymphocyte Isolation Medium, Pharmacia, LKB Bio-technology, Inc., Piscataway, NJ). These tubes were centrifuged 400 × g × 20 min at room temperature; four distinct layers appeared. The upper layer was plasma; next was a thin translucent interface "buffy" layer containing lymphocytes and platelets, which was followed by the Ficoll-Paque solution. The pellet contained erythrocytes and granulocytes. The upper plasma layer was removed and saved for additional analyses. The lymphocytes were removed, washed 2× in a balanced salt solution and resuspended at a concentration of 1×10^6 $cells \cdot ml^{-1}$.

The CD4+/CD8+ cell ratios were determined with the Fluorescent Activated Cell Sorter (FACS) manufactured by Becton Dickinson Immunocytometry Systems (San Jose, CA). The FACScan Consort-30 software program, a duochrome system, gave percentages of CD4+ and CD8+ cells in a population simultaneously. Using monoclonal antibodies, the CD4+ cells were recognized by a fluoro-isothiocyanate (FITC) conjugated mouse anti-human antibody (Leu-3), and the CD8+ cells were recognized by a phycoerythrin (PE) conjugated mouse anti-human antibody (Leu-2). The fluorescent labels were activated at the same laser wave length, but they emitted light at different wave lengths.

Aliquots of the lymphocyte layer, containing approximately 100,000 cells, were incubated with 2 ul of the simultest mixture of the two antibodies for 30 min on ice. The cells were then washed twice with phosphate buffered saline/1% bovine serum albumin (PBS/BSA). They were then resuspended in 100 ul of this buffer and analyzed with the FACScan. The Becton-Dickinson simultest control was used to identify the strains negative to the cells tagged with the fluorescent antibodies.

Dot plots were produced. Percentages of CD8+ cells found in the upper left quadrant and CD4+ cells in the lower right quadrant were determined with the Consort-30 program.

The normal range for the CD4+/CD8+ ratio has not been firmly established. Golub (5) set the range at 1.8–2.2, whereas Tizard (20) suggests 1.2–1.5. We have selected the latter range because its lower limit is the lower of the two ranges, making it more difficult to conclude that our pre-exercise ratios were indeed below the lower limit of normal. As a result, the upper limit of 1.5 may be too low.

Statistical analyses

One-way analysis of variance was used to determine differences in CD4+/CD8+ ratios among pre-exercise and among post-exercise drinking experiments. Because no significant differences were found, mean ratios between pre- and post-exercise were analyzed using Student's dependent t test. Correlation coefficients were determined by linear regression. Analyses were performed with a Hewlett-Packard calculator (model 65, Cupertino, CA) and Stat-Pac programs.

RESULTS

Mean (±SE) pre-exercise and post-exercise CD4+/CD8+ ratios for the six drink formulations indicated no statistically significant changes among groups (Fig. 1). The unexpected finding was that all pre-exercise CD4+/CD8+ ratios were below the lower limit of the normal population range of 1.2, which is considered to be within the pathological range. All six post-exercise ratios tended to be greater and, with two exceptions, moved into or beyond the normal range. But there was a shift toward higher ratios (to the right) when the mean of the six pre-exercise ratios (0.77 ± 0.12) was compared with the mean of the six post-exercise ratios of 1.36 ± 0.15 ($P < 0.01$). The two drinks (IV and VII) with the lowest pre-exercise ratios tended to have the larger post-exercise percent changes of 172% and 123%, respectively.

There was no apparent relationship between drink composition and post-exercise ratios or percent change in

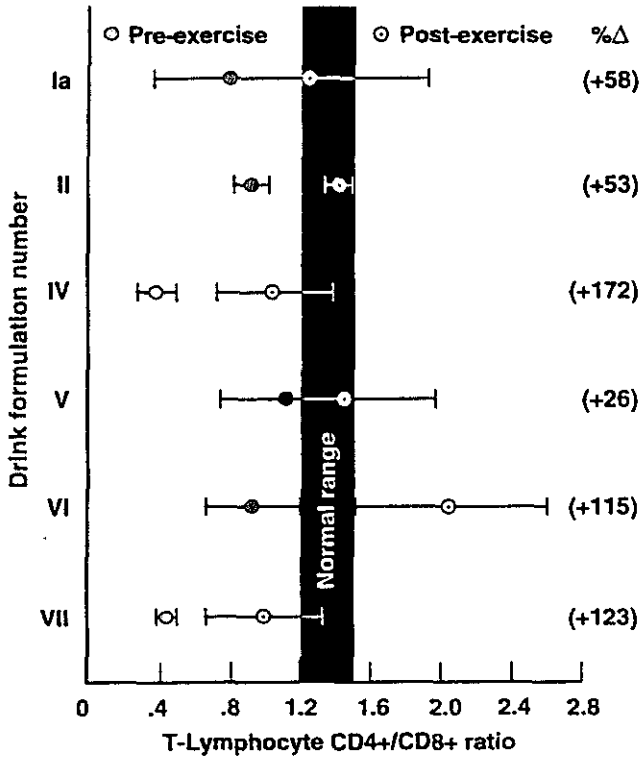

Figure 1—Mean (±SE) pre- and post-exercise CD4+/CD8+ ratios and percent changes for the six drink experiments. Roman numerals are drink designations.

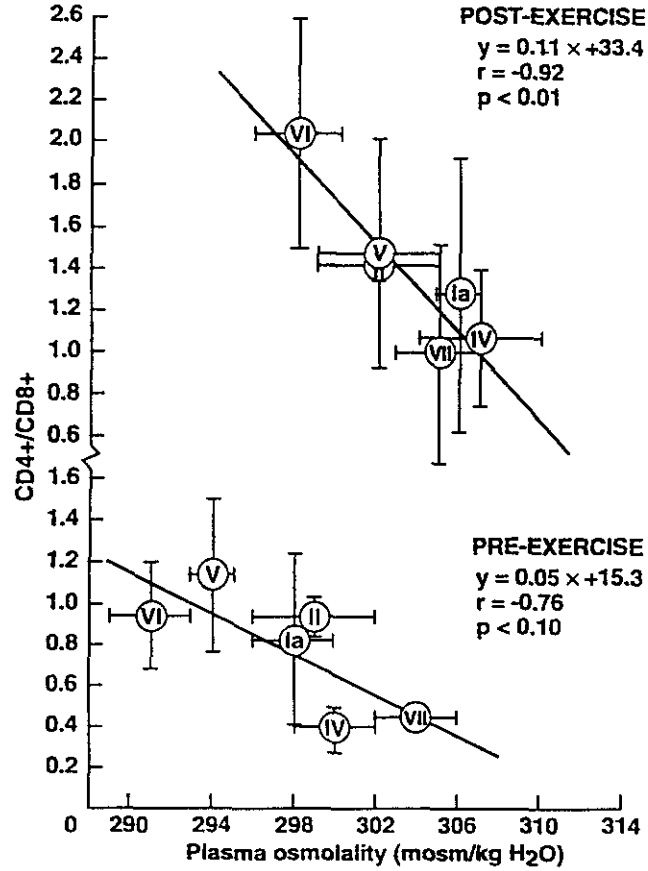

Figure 2—Regression of mean CD4+/CD8+ lymphocyte ratio on mean plasma osmolality pre-exercise, and post-exercise and post-drinking for the six experiments. Roman numerals are drink designations.

the ratios. The high sodium and citrate concentration drink (IV) had similar absolute and percent change responses as drink VII containing minimal sodium and citrate concentrations (Fig. 1). Drinks II and V, with similar sodium but different glucose concentrations, responded similarly. The two commercial drinks (VI and VII), with similar carbohydrate but different sodium concentrations, had different pre- and post-exercise ratios, but similar percent changes in their ratios. Both of the latter percent changes were different from the percent ratio change in drink V, which also contained 9.7% carbohydrate (glucose).

Regressions of mean CD4+/CD8+ ratios on mean plasma osmolality pre- and post-exercise are presented in Figure 2. The relatively high negative correlation coefficients pre-exercise ($r = -0.76$, $P < 0.10$) and post-exercise ($r = -0.92$, $P < 0.01$) indicate that higher osmotic concentrations were related to decreased ratios and suggest that 58% and 85% of the ratios variability, respectively, can be explained by change in osmolality. Linear regression analysis of percent changes in CD4+/CD8+ ratios and percent changes in plasma volume (pre- vs post-exercise) resulted in a low, nonsignificant correlation coefficient of 0.16.

A compilation of mean CD4+/CD8+ data from six studies conducted by other investigators involving moderately heavy submaximal lower extremity exercise was compared with data from the present study (Table 2). Subject hydration (fasting times) were given in only three studies (16,21,22) and time of testing (0700–1000 hr) were given in three (13,15,16). Fluid intake during exercise was not reported in any study, but presumably was zero. Fortuitously, the mean relative exercise load from the six studies was also 71%, the same as that of the present study. Percent change in CD4+ cells indicated some variability from −30 to +58%, whereas percent changes in CD8+ cells were generally positive but exhibited a greater range from −3 to +171%. All percent changes in post-exercise CD4+/CD8+ ratios in the six other studies were negative ($\bar{X}$ = 1.9 to 1.4, −25%), whereas that from the present study was positive ($\bar{X}$ = 0.8 to 1.4, +75%). With similar post-exercise ratios of 1.4 in this and the six other studies, the positive mean ratio change in the latter was due to the apparently decreased pre-exercise ratio of 0.8.

DISCUSSION

Decreased Pre-exercise Ratios

Decreased pre-exercise CD4+/CD8+ ratios below the normal range in all six drinking experiments was an unexpected finding. Two procedures that may have in-

TABLE 2. Mean T-helper, T-suppressor, and their ratio pre- and post-submaximal exercise, with data from the literature and from the present study.

				T-helper ($CD4^+$)			T-suppressor ($CD8^+$)			$CD4^+/CD8^+$		
				Exercise			Exercise			Exercise		
Reference	Subject	Mode	Load	Pre	Post	% Δ	Pre	Post	% Δ	Pre	Post	% Δ
Kendall et al. (13)	30 M 20–31 yr	Cycle	75%	1.9	2.8	+47	0.7	1.9	+171	2.7	1.5	−44
Landmann et al. (15)	11 M 4 F 17–25 yr	Cycle	75%	3.1	4.9	+58*	1.8	3.7	+106*	1.7	1.3	−24
Nieman et al. (16)	12 F 24–45 yr	TM (walk)	60%	0.97	0.96	−1	0.55	0.76	+38	1.8	1.3	−28*
Ricken et al. (17)	27 M 26 ± 4 yr	Cycle	63%			−10*			+10*	1.1	0.9*	−18
Tvede et al. (21)	20 M 23–28 yr	Cycle	80%			−30*			−3*	2.3	1.6	−30
Tvede et al. (22)	16 M 23–28 yr	Cycle	75%	0.98	1.37	+40*	0.55	0.83	+51*	1.8	1.7	−6
Mean			71%	1.7	2.5	+17	0.9	1.8	+62	1.9	1.4	−25
Present study	4 M 30–46 yr	Cycle	71%							0.8	1.4*	+75*

M = males; F = females; cycle = ergometer; TM = treadmill; Load = % maximal $\dot{V}O_2$.
*$P < 0.05$, authors' designation.

fluenced this reduction were the injection of the Evans blue dye (T-1824) just before drinking and the 24-h dehydration.

About 3.8 g of the 0.5% (5 $mg \cdot ml^{-1}$) aqueous T-1824 dye solution was injected just before drinking each of the fluid formulations, so residual dye was present after the first injection in all subsequent experiments. Previously, mean (± SE) residual dye absorbances (615 mμ), determined from approximately 5 g of T-1824 injected into seven men at 3 weekly intervals, was 0.013 ± 0.001, 0.033 ± 0.004, and 0.043 ± 0.003 units, respectively (23). When expressed as percentages of the subsequent injected doses, the absorbances were 12.8 ± 1.7%, 25.3 ± 3.0%, and 37.9 ± 2.8%, respectively. Linear regression analysis, using zero and the 3 weekly absorbance values (r = 1.0), indicated that residual dye absorbances for an additional two weekly injections (5 and 6) would have been 0.006 and 0.007 units, respectively, with corresponding percentages of 50.6% and 63.2%. Thus, with an injection dose of about 5 $g \cdot wk^{-1}$, the residual plasma dye content increases by about 10 percentage $units \cdot wk^{-1}$. But, selective randomization of the tests would have essentially equalized the high and low dye content in the plasma. Mean post-exercise ratios (1.4) were similar in the present and six prior studies. If Evans blue dye had a selective depressive effect on the pre-exercise ratio, it was not apparent from the post-exercise ratios. Thus, there is no obvious reason to attribute the decreased pre-exercise ratio to the presence of the dye.

Reduction of total body water and the accompanying hypovolemia resulting from dehydration should not have caused the decreased ratios. Hemoconcentration would not affect either the CD4+ or CD8+ cell counting procedure selectively because the buffy layer was washed, and the cells were resuspended repeatedly. The cells ratio would not be affected by any consistent change in the number of all cells, e.g., from hemodilution or hemoconcentration. However, total body dehydration imposes a generalized stress on the body that has been reported to depress immune function (2) via an unspecified glucocorticoid mechanism. However, the high negative correlations between CD4+/CD8+ ratios and plasma osmolality suggest that increased osmolality (hyperosmotic stress) may have contributed to the decreased ratios perhaps, but not necessarily, via the generalized stress response.

Increased Post-exercise Ratios

Post-exercise restoration to the normal CD4+/CD8+ ratio of 1.4 may have been a normalizing rehydration response to drinking the various fluid formulations; the mean ratio from the six prior exercise studies was also 1.4, and some subjects in those experiments were probably not dehydrated before their exercise. In a similar *resting* dehydration experiment, consumption of these six fluids resulted in plasma volume increases of 1.1% to 7.6% after 70 min (9). Changes in plasma volumes (Hb-Hct transformation) after 70 min of exercise with drinks Ia to VII were: −14.1%, −7.5%, −7.6%, −5.2%, −9.2%, and −13.5%, respectively. Plasma volume during sitting ergometer exercise without drinking decreases linearly with increasing load, reaching −18 to −20% at maximal exertion; the plasma volume shift at 71% of peak oxygen uptake without drinking is about −13% (10). Thus, there would have been a net fluid gain in the vascular space with drinks having a percent change greater (less negative) than −13%; i.e., all except drinks Ia (water, −14.1%) and VII (Power Surge, −13.5%). The nonrestored levels of vascular volumes with drinks Ia and VII did not influence the magnitude of the post-exercise increases in the CD4+/CD8+ ratios of +58%

and +123%, respectively, when compared with those of the other drinks (Fig. 1). Thus, these findings and the low correlation coefficient between percent changes in the ratios and plasma volumes suggest that vascular fluid shifts of different magnitudes do not seem to be the major mechanism for the mean increased post-exercise ratio to 1.4.

Despite plasma volume and body water restoration toward normal after drinking, the negative relationship between CD4+/CD8+ ratios and plasma osmolality persisted post-exercise (Fig. 2, *upper curve*); drinking and exercise did not influence the slope of the curve, but they did affect the magnitude by shifting it to a higher level for both ratio and osmolality. Thus, the level of plasma osmolality, but probably not volume, may be a factor influencing immune system modulation by exercise.

The authors thank A. Dearborn, J. Evans, D. Fegan-Meyer, R. Gottfried, J. Harris, L. Juhos, L. Keil, S. Kravik, R. Looft-Wilson, R. Pelligra, and V. Rimer for valuable assistance, Drs. C. Wade, F. Sonnenfeld, and G. Taylor for constructive comments, and A. Hardesty and T. Eames for manuscript preparation.

This work was supported by NASA Task 199-18-12-07.

Address for correspondence: John E. Greenleaf, Ph.D., Laboratory for Human Environmental Physiology (239-11), NASA, Ames Research Center, Moffett Field, CA 94035-1000.

REFERENCES

1. Cogoli, A. Hematological and immunological changes during space flight. *Acta Astronautica* 8:995–1002, 1981.
2. Degiorgi, L. and D. Altomare. Modification of the immune system following mental and physical stress. *Bull. Inst. Pasteur* 90:99–107, 1992.
3. Gmünder, F. K., F. Baisch, B. Bechler, et al. Effect of head-down tilt bedrest (10 days) on lymphocyte reactivity. *Acta Physiol. Scand.* 144 604(Suppl.):131–141, 1992.
4. Gmünder, F. K., G. Lorenzi, B. Bechler, et al. Effect of long-term physical exercise on lymphocyte reactivity: similarity to spaceflight reactions. *Aviat. Space Environ. Med.* 59:146–151, 1988.
5. Golub, E. S. and D. R. Green. *Immunology, A Synthesis*, 2nd Ed. Sunderland, NY: Sinauer Associates Inc., 1991, pp. 1–744.
6. Greenleaf, J. E. Importance of fluid homeostasis for optimal adaptation to exercise and environmental stress: acceleration. In: *Perspectives in Exercise Science and Sports Medicine, Vol. 3: Fluid Homeostasis During Exercise*, C. V. Gisolfi and D. R. Lamb (Eds.). Carmel, IN: Benchmark Press, Inc., 1990, pp. 309–346.
7. Greenleaf, J. E., R. Bulbulian, E. M. Bernauer, W. L. Haskell, and T. Moore. Exercise-training protocols for astronauts in microgravity. *J. Appl. Physiol.* 67:2191–2204, 1989.
8. Greenleaf, J. E., V. A. Convertino, and G. R. Mangseth. Plasma volume during stress in man: osmolality and red cell volume. *J. Appl. Physiol.* 47:1031–1039, 1979.
9. Greenleaf, J. E., G. Geelen, C. G. R. Jackson, et al. Vascular uptake of rehydration fluids in hypohydrated men at rest and exercise. Moffett Field, CA: NASA Tech. Memo. 103942, 1992, pp. 1–46.
10. Greenleaf, J. E. and M. H. Harrison. Water and electrolytes. In: *Nutrition and Aerobic Exercise*, D. K. Layman (Ed.). Washington, DC: American Chemical Society, 1986, pp. 107–124.
11. Hickson, R. C. and J. B. Boone. Physical exercise and immunity. In: *Stress and Immunity*, N. Plotnikoff, A. Murgo, R. Faith, and J. Wybran (Eds.). Boca Raton, FL: CRC Press, 1991, pp. 211–234.
12. Keast, D., K. Cameron, and A. R. Morton. Exercise and the immune response. *Sports Med.* 5:248–267, 1988.
13. Kendall, A., L. Hoffman-Goetz, M. Houston, B. MacNeil, and Y. Arumugam. Exercise and blood lymphocyte subset responses: intensity, duration, and subject fitness effects. *J. Appl. Physiol.* 69:251–260, 1990.
14. Konstantinova, I. V., Y. N. Antroipova, V. I. Legenkov, and V. O. Zazhirey. Study of reactivity of blood lymphiod cells in crew members of the Soyuz 6, 7, and 8 space ships before and after flight. *Kosm. Biol. Med.* 7:35–40, 1973.
15. Landmann, R. M. A., F. B. Muller, C. Perini, M. Wesp, P. Erne, and F. R. Buhler. Changes of immunoregulatory cells induced by psychological and physical stress: relationship to plasma catecholamines. *Clin. Exp. Immunol.* 58:127–135, 1984.
16. Nieman, D. C., S. L. Nehlsen-Cannarella, K. M. Donohue, et al. The effects of acute moderate exercise on leukocyte and lymphocyte subpopulations. *Med. Sci. Sports Exerc.* 23:578–585, 1991.
17. Ricken, K. H., T. Rieder, G. Hauck, and W. Kindermann. Changes in lymphocyte subpopulations after prolonged exercise. *Int. J. Sports Med.* 11:132–135, 1990.
18. Sagawa, S., K. Miki, F. Tajima, et al. Effect of dehydration on thirst and drinking during immersion in men. *J. Appl. Physiol.* 72:128–134, 1992.
19. Taylor, G. R., L. S. Neale, and J. R. Dardano. Immunological analyses of U. S. Space Shuttle crew members. *Aviat. Space Environ. Med.* 57:213–217, 1986.
20. Tizard, I. *Immunology: An Introduction*, 3rd Ed. New York: Saunders College Publishing, 1992, pp. 1–512.
21. Tvede, N., C. Heilmann, J. Halkjaer-Kristensen, and B. K. Pedersen. Mechanisms of B-lymphocyte suppression induced by acute physical exercise. *J. Clin. Lab. Immunol.* 30:169–173, 1989.
22. Tvede, N., B. K. Pedersen, F. R. Hansen, et al. Effect of physical exercise on blood mononuclear cell subpopulations and *in vitro* proliferative responses. *Scand. J. Immunol.* 29:383–389, 1989.
23. Young, H. L., L. Juhos, B. L. Castle, J. Yusken, and J. E. Greenleaf. Body water compartments during bed rest: evaluation of analytical methods. Moffett Field, CA: NASA Tech. Rept. R-406, 1973, pp. 1–19.

VOL. 27, NO. 2, FEB. 1995

MEDICINE & SCIENCE IN SPORTS & EXERCISE®

OFFICIAL JOURNAL OF THE AMERICAN COLLEGE OF SPORTS MEDICINE

ISSN: 0195-9131

Williams & Wilkins
A WAVERLY COMPANY