Autologous Red Blood Cell Reinfusion: Effects on Stress and Fluid Regulatory Hormones During Exercise in the Heat

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The use of autologous erythrocyte reinfusion to improve physical performance has been evaluated during several environmental and exercise paradigms. For example, Buick et al. (1) reinfused approximately 900 ml of autologous erythrocytes and demonstrated that both maximal aerobic power (\(V_{O2 \max}\)) and endurance capacity were significantly increased 24 h after reinfusion in a normothermic and normoxic environment. Robertson and co-workers (18) confirmed these findings under normoxic conditions and extended the studies to demonstrate that exercise tolerance and \(V_{O2 \max}\) under normobaric, hypoxic conditions were both significantly improved by autologous transfusion. The same group (17) reported that similar improvements were noted for female test subjects during cycle ergometry under normoxic conditions. Further, they noted that the physiological advantages conferred by autologous reinfusion persisted for up to 2 weeks.

Gledhill (10) concluded in a recent review that increased \(O_2\) delivery to working muscles should increase physical performance if the following criteria are met: incremented blood viscosity does not significantly reduce cardiac output, blood flow distribution is unaffected, and the oxidative capacity of the working muscles is not limiting. While potential increases in total blood and plasma volume may also be implicated in the increased physical capacity of induced erythrocythemia (13). Gledhill argued that such effects may be labile and are probably normalized after the first 24 h of reinfusion, although prior investigations have not addressed these effects in reinfused subjects.

Despite the apparent interest in the use of autologous reinfusion to improve physical performance, we are unaware of any studies which have assessed the effects of reinfusion on the endocrinological responses to exercise in the heat, especially during the first 48 h and up to 9 d following reinfusion. For several years, we have studied the responses of plasma hormones to exercise/heat stress after the body fluid status of a test subject has been experimen-
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tally manipulated. Thus, we reported (7) that, while hypo-
hydration by 5% of body weight elicited significant eleva-
tions in plasma renin activity and aldosterone prior to and
during exercise in the heat, these increments were attenuated
following heat acclimation. These results were consonant with
tory, since heat acclimation is accompanied by sig-
ificant increases in intravascular fluid volume (20,21). Ana
glogously, we demonstrated (8) that heat acclimation
also moderated the stress hormone response to exercise in
the heat during hypohydration. Most recently, we have ob-
served (9) that increased severity of hypohydration is
accompanied by increased circulating levels of aldosterone,
plasma renin activity, and cortisol. Our previous results
generally indicated that experimental manipulations which
increased blood/plasma volume (i.e. heat acclimation)
tended to reduce the response of selected stress and fluid
regulatory hormones to exercise in the heat while procedures
which reduced blood/plasma volume (i.e. dehydration) in-
creased the response of these hormones to the heat stress
test.

In considering the beneficial effects of acute erythrocy-
themia induced by autologous erythrocyte infusion, it is
apparent that the physiological strain of exercise in the
heat may be reduced by either improved O₂-CO₂ systemic trans-
port or to heat dissipation. If such benefits accrued subse-
quent to erythrocythemia, then responses of stress hormones
to exercise in the heat may be attenuated, particularly in
unacclimated subjects. Alternatively, since the fluid regula-
tory hormone responses are also dependent on such varia-
tables as plasma osmolality, sodium levels, and oncotic pres-
sure, as well as blood/plasma volume, it is difficult to predict
the intensity and the direction of the responses that may
occur. Thus, the current study was designed to determine
the effects of acutely induced erythrocythemia on the
response of a representative stress hormone (plasma cortisol,
PC) and fluid regulatory hormones (aldosterone, ALD, and
angiotensin I as determined by plasma renin activity, PRA)
to a heat stress test. Unacclimated test subjects participated
so that the subjective and physiological effects of heat ac-
climation did not mask the potential effects of the reinfusion.

MATERIALS AND METHODS

Subjects: Six adult male test subjects (Ss) participated, all
members of the same military unit, and thus all exposed to
similar regimens of diet, activity, and environment through
the duration of the study. Anthropometric measures for the
experimental group were (mean ± S.D.); age: 30 ± 7 years;
weight, 79 ± 9 kg, height, 182.3 ± 4.2 cm, and percent body
fat, 15 ± 5. All test subjects received a written and oral
description of the procedures and risks of the study, and
signed a voluntary consent form signifying their agreement
to participate. All volunteers reserved the
tight consent form signifying their agreement
to participate. All volunteers reserved the
right to withdraw from the study at any time without prejudice or retribution,
but none elected to do so.

Phlebotomy and reinfusion: During the late fall and early
winter, two units of blood (900 ml) were collected from
each volunteer; the collection of each unit was separated by
at least 6 weeks. Phlebotomy, blood processing, storage and,
ultimately, reinfusion were done by personnel on the staff
of the Naval Blood Research Laboratory, Boston, MA.
Blood was collected in citrate-phosphate-dextrose, and was
stored at 4°C for 2–5 d. The erythrocytes were then separated
by centrifugation and suspended in 40% weight-to-volume
of glycerol, deep-frozen to ~80°C (22,23) and stored. At
reinfusion, the glycerolized erythrocytes were thoroughly
washed (Haemonetics Blood Processor 115) and resus-
pered in a saline-glucose-phosphate solution: approxi-
mately 600 ml of solution with autologous erythrocytes
(50% HCT) were reinfused over a 1-h time period.

Heat stress tests: Three heat stress tests (HSTs) were
conducted at an environmental temperature of 35°C and a
relative humidity of 45%. Each HST comprised a total of
180 min (three repetitions of 45 min exercise. EX1. EX2.
EX3, interspersed by 15 min rests) unless predetermined
safety criteria (heart rate >180 bpm, rectal temperature
>39.5°C) or exhaustion were achieved. The exercise com-
ponent of the HST involved walking (1.56 m·s⁻¹) on an
inclined (6%) treadmill. During the rest intervals, Ss were
reweighed and rehydrated with sufficient cool water to
maintain initial body weight. A program of scheduled re-
hydration was also employed during each exercise bout. The
subjects wore only shorts, socks, and tennis shoes during
each HST. Each HST was conducted on three separate
occasions: the first or control HST was completed at least
6 weeks after the second phlebotomy, and approximately
2 weeks prior to the autologous reinfusion during the late
spring season: the second was accomplished exactly 48 h
after completion of the reinfusion procedure; and the third
HST was 1 week following the second, which corresponded
to 9 d after the reinfusion procedure. Thus, the minimal
time interval between HSTs was 1 week.

Blood sampling: Indwelling Teflon catheters were inserted
in a superficial arm vein prior to each HST: their patency
was maintained by flushing with heparinized saline. Preex-
ercise blood samples of 10 ml were obtained after the Ss
stood quietly in a moderate environment (antechamber,
20°C, 40% rh) for at least 20 min to control for postural
effects on vascular fluid shifts (12). The remaining three
10-ml blood samples were obtained 30 min into each exer-
cise bout (EX1, EX2, EX3) of the respective HST while the
subjects continued to walk. Blood was centrifuged at 1000
G for 30 min, aliquoted, and frozen at −20°C for subse-
quent analysis. Tests were conducted between 0700 and
1100 hours to offset the effects of circadian variations on the
dependent variables (14).

Plasma analyses: Samples were analyzed for PC using
commercially prepared test kits purchased from New Eng-
land Nuclear Corp., Billerica, MA, according to standard-
ized procedures outlined in their technical bulletin. Using
these techniques intra-assay variability determined in our
labortory was just 2.5% and inter-assay variability was
7.3%. PC values are generally reported to range from
4–25 μg·100 ml⁻¹ depending importantly on the time of
day at which the blood samples are drawn (14). Angiotensin
I levels were assessed by quantifying PRA using radioim-
munoassay test kits also produced by New England Nuclear
Corp. When converting enzyme and angiotensinases are
appropriately inhibited, it has been demonstrated that the
accumulation of angiotensin I reflects plasma renin activity.
Intra-assay variability was determined to be 4%; and inter-
assay variability was 7.2% by these methods. Control levels
of PRA for healthy normotensive men range from approxi-
mately 1.0–4.0 ng Angiotensin I formed per hour per ml
plasma by this method. Aldosterone (ALD) levels were
quantified using radiomunoassay test kits purchased
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from Diagnostics Products Corp. Los Angeles, CA, by methods outlined in their technical bulletin. Intra-assay variability was 9.3% by these methods and interassay variability was recorded at 11%. Expected values for normotensive adult men range from 5-31 ng·dl⁻¹ by these methods.

Statistics: Repeated measures analyses of variance were performed followed by the application of Tukey’s t test corrected for multiple group comparisons to determine the effects of exercise/heat stress on the variables of interest (15,16). During the preinfusion HST, one test subject did not complete the third exercise bout; therefore, a single calculated value was used for each variable (15, p. 228). To determine the significance of effects of red cell reinfusion, Dunnett’s t test (15, p. 422) for paired, dependent data was performed and the results for the preinfusion trial (HST1) were compared with those of the 48-h postinfusion HST (HST2) as well as the 9-d HST (HST3). Correlation coefficients were calculated by linear regression analysis. For all statistical tests, the null hypothesis was rejected at p ≤ 0.05.

RESULTS

Fig. 1 illustrates the effects of erythrocyte reinfusion and exercise/heat stress on circulating levels of cortisol in these unacclimated test subjects. The results indicate that, during all three HSTs there occurred a reduction in PC levels between the preexercise and EX1 sample which was significant for the first (preinfusion) HST (p = 0.05) and the second (48-h postinfusion) HST (p < 0.01). However, during the third (9-d postinfusion) HST, PC was at an apparently low basal level in the preexercise sample (8.1 μg·dl⁻¹) and was further reduced to 6.5 μg·dl⁻¹ during EX1; the minimum difference necessary for significance was 4.36 μg·dl⁻¹, and this change was not significant in the third HST. We have observed this decrease previously (8.9) and attributed the decrement to the normally occurring circadian reduction during this time of day. The cumulative effects of exercise in the heat apparently offset the anticipated continued circadian decline of PC until, by the third exercise interval in the preinfusion (HST1) and 9-d postinfusion (HST3) HSTs, PC levels were not significantly different from preexercise levels. However, in the 48-h postinfusion HST (HST2), PC levels were persistently and significantly (p < 0.01) depressed during all three exercise bouts from preexercise. The effects of induced erythrocythemia are best demonstrated if paired data are compared between the preinfusion (HST1) and the 48-h postinfusion (HST2) HSTs. In these comparisons, PC was significantly reduced at each of the exercise intervals in the 48-h postinfusion trial (HST2) vs. the respective sample of the preinfusion (HST1) trial (p < 0.005 EX2 and p < 0.05 EX1, EX3).

Values for aldosterone responses to reinfusion and exercise in the heat are noted in Fig. 2. The effects of exercise in the heat were apparent since, by the second exercise bout during all three HSTs, ALD levels were significantly (p < 0.01) elevated over preexercise concentrations. During EX1, ALD levels (vs. preexercise) were significantly elevated during the preinfusion (p < 0.05) and 48-h postinfusion (p < 0.01) HSTs; however, despite a consistent trend, significance was not achieved during EX1 for the 9-d postinfusion trial (preexercise level = 24.28 ng·dl⁻¹, EX1 level = 34.4 ng·dl⁻¹, minimum difference for significance = 13.8 ng·dl⁻¹). Erythrocythemia, however, apparently had no effect on the ALD responses to exercise in the heat as no significant differences were noted in comparisons between pre- and 48-h or 9d-postinfusion HSTs.

Responses of plasma levels of PRA (Fig. 3) were remarkably consistent during all HSTs, with significant (p < 0.01) increments noted even during the first exercise period (vs. preexercise). Further, these elevations persisted; for all three HSTs, the levels measured during EX2 were significantly (p < 0.01) greater than those recorded during EX1 (HST1, 7.91 vs. 9.76; HST2, 6.71 vs. 9.20; HST3, 6.59 vs. 9.02 ng·ml⁻¹·h⁻¹). Examination of the data indicates that, during the third exercise interval, the rate of increase had moderated for all three HSTs. Fig. 2 and 3 illustrate apparently analogous responses of PRA and ALD to exercise in the
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Fig. 3. Effects of erythrocythemia and exercise in the heat on circulating levels of plasma renin activity during exercise in the heat. All conditions and specifications as in Fig. 1.

Fig. 4. Linear regression and scatter plot of values for PRA and ALD during all three heat stress tests and before and during all exercise bouts.

Heat and erythrocythemia: statistical analysis confirmed the correlation between these two covariates: n = 72, r = 0.84, t = 12.76, p < 0.001 (Fig. 4).

Discussion

We have previously reported (8,9) that when euhydrated subjects exercise in the heat under conditions similar to those selected in the current experiments, physiological stress response, as manifested in circulating cortisol levels, is minimal. In euhydrated subjects tested between 0700-1100 hours, the decrement between the preexercise and the EX1 and EX2 samples had been a consistent observation in our earlier studies (8,9). In the current investigation, wherein the metabolic rate had been increased from approximately 30% (8,9) to about 50% \( VO_{2\text{max}} \), PC levels increased in the preinfusion and 9-d postinfusion trials by the third exercise interval. These observations imply that, by the third exercise interval, Ss were experiencing physical discomfort as a result of the combination of the physical work rate and environment. Follenius et al. (6) had earlier reported that heat stress induced an adrenocortical response only in those individuals who experienced physical discomfort during the exposure.

Alternatively, erythrocyte reinfusion had repressive effects on this cumulative stress response; even during the first exercise interval, plasma levels of cortisol during the second HST (48-h postreinfusion) were significantly reduced by approximately 24% compared with the preinfusion trial. Further, these reductions persisted throughout the second and third exercise bouts. This may be interpreted in terms of an attenuation of the physiological strain of this exercise/heat regimen following reinfusion. It had been previously demonstrated that the increased arterial oxygen content induced by erythrocythemia can translate to a reduced requirement for skeletal muscle blood flow (24, 25), in turn permitting greater cutaneous perfusion for heat dissipation.

We have reported (19) that erythrocythemia not only provided a thermoregulatory benefit during exercise in the heat but also elicited a 11% increase in maximal \( VO_{2\text{max}} \) consumption. Thus, it can be hypothesized that the reduced cortisol concentrations observed in the 48-h reinfusion trial could be a reflection of the reduced physiological strain engendered by this regimen of exercise in the heat which, during HST2, represents a reduced percentage of each subject's maximal aerobic capacity.

Levels of ALD and PRA were unaffected by the induced erythrocythemia during exercise/heat stress. We had originally hypothesized that an increased blood volume, anticipated particularly during the 48-h postinfusion HST (20), might alleviate the increments in ALD and PRA reported during and immediately subsequent to exercise in the heat (2, 4, 11). However, our hemodynamic measurements demonstrated (19) that, immediately prior to the second HST (48-h postinfusion), the acute erythrocythemia had elicited a marked (~7%) decrease in plasma volume compared to the preinfusion level, an observation which had not been reported previously. However, the increased erythrocyte volume was apparently offset by a compensatory decrement in plasma volume so that total blood volume was unchanged. Thus, the responses of the fluid regulatory hormones to exercise in the heat following erythrocythemia (Fig. 2, 3) are not inconsistent with our previous observations (7-9). The present results, with particularly sharp increases in PRA and ALD at each exercise interval in all three HSTs, confirm the intensity of these responses in unacclimated men. Our previous data (7, 9), as well as that of other investigators (3-5), have indicated that the acquisition of heat acclimation moderates the intensity of the hormonal response to exercise in the heat. Clearly, induced erythrocythemia had no effects on the heat/exercise responses of these fluid regulatory hormones in our euhydrated, but unacclimated, subjects.

We have concluded from this study that autologous reinfusion of 2 units of erythrocytes attenuated the stress response to exercise in the heat as manifested in PC levels. Further, induced erythrocythemia had no effects on the incremental response pattern of the fluid regulatory hormones ALD and PRA to the heat/exercise regimen because the anticipated increase in blood volume effected by the red blood cell infusion was compensated by a slight decrease in plasma volume. Thus, the response pattern of the infused, euhydrated, and nonacclimated subjects is consistent with our earlier observations of response profiles in nonreinfused, euhydrated, and unacclimated test volunteers.
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