

# Plasma volume after heat acclimation: Variations due to season, fitness and methods of measurement

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*Purpose:* The reported magnitude of plasma volume increase ( $\Delta\%PV$ ) following heat acclimation (HA) varies widely. Variations may result from differences in measurement techniques, season and subjects' fitness. This report compares direct and indirect measurements of  $\Delta\%PV$  after 10 days of HA from studies in winter (WIN,  $n = 8$ ) and summer (SUM,  $n = 10$ ) in men, age 21–43 yr, at two fitness levels ( $VO_{2max}$ : 35 and 51 ml/min/kg). Direct measurements were made before and after HA (cycling at 30% of  $VO_{2max}$  at 50 °C, for 100 min/day) by carbon monoxide (CO) rebreathing and compared with indirect estimates from changes in hematocrit, hemoglobin and plasma protein concentration. *Results:* Overall,  $\Delta\%PV$  by CO was small (2.9%) and greater in SUM than WIN (5.0 vs. 0.3%). Red cell, blood and plasma volumes/kg lean body mass increased in SUM and decreased in WIN, the difference being significant, and  $\Delta\%PV$  by CO was similar for high and low  $VO_{2max}$ . *Conclusion:* Overall, indirect estimates of  $\Delta\%PV$  by hemoglobin and hematocrit were similar to CO, but tended to differentiate by fitness and not season. The difference in THb increase in SUM and decrease in WIN was significant. This probably accounts for the differences from the seasonal and fitness results by the direct CO method.

**Keywords:** carbon monoxide rebreathing, hematocrit, heat acclimation, hemoglobin, human subjects, plasma proteins, seasonal effects

Heat acclimation (HA) is typically achieved by exercising daily in a hot environment over a period of a week or more (28). Successful HA increases the ability to work in the heat without excessive body temperature and heat illness symptoms. Common physiological correlates of this improved ability are attenuations in heart rate (HR), body temperature rise, electrolyte loss in sweat and increased sweating during subsequent dehydrating work. An increase in plasma volume (PV) is frequently measured and presumed to be advantageous to support the increased circulatory demands of working in the heat, along with plasma protein shifts that may attenuate intravascular fluid losses (8, 14, 19, 29).

The magnitude of the increase in PV with HA varies greatly in reports, ranging from zero (3) to 30% (31). Some variability undoubtedly results from differences in the subject's fitness, number of days, exercise intensity, temperature and hydration level in HA studies (10). Seasonal temperature variations wherein the artificial HA takes place may also affect the results (29). Reported HA studies have been predominantly performed on highly fit

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individuals or workers in hot environments, with few studies comparing fitness levels during different ambient temperatures, i.e., winter and summer, which may superimpose additional variation (34).

Another source of non-uniform findings is the different methods employed to measure PV changes over the period of HA. Method comparisons are rare. Many studies have relied on indirect methods based on hemodilution of natural blood constituents such as erythrocyte (Hct), hemoglobin (Hb) and plasma protein (PP) concentrations to estimate changes from a measured or assumed pre-HA baseline value. Pre- and post-HA indicator dilution measurements are advantageous because they are not affected by changes in the blood constituents or shrinkage or swelling of erythrocytes that affect indirect methods. One direct indicator method proven to be relatively sensitive and precise is the carbon monoxide (CO) rebreathing method that measures total circulating hemoglobin (THb) (4, 11). Sawka et al. (30) noted that the CO method tends to give red cell volume (RCV) values some 15–20% higher than labelled isotope methods.

Two previous investigations used the CO method to evaluate the effects of HA on (a) the tolerance to dehydrating work in the heat (24) and (b) the tolerance to orthostatic stress before and after dehydrating exercise in the heat (21, 25). These two studies also included indirect measurement of PV changes from Hct and Hb and PP measurements before and after HA. Data from these studies were available to retrospectively determine possible contributors to the large diversity in the literature regarding the magnitude of PV and blood volume (BV) changes with HA.

In these studies the independent variable was HA and the dependent variables were a) exercise responses during dehydrating work in the heat and b) orthostatic tolerance. The resting baseline measurement of responses to HA reported here were made prior to these stressors being applied before and after HA.

The purpose of this presentation is to evaluate the effects of seasonal and fitness differences on PV changes from measurements obtained before and after successful HA by direct and indirect PV measurement techniques; the hypothesis was that no differences would be found between seasons, fitness level or methods of measurement.

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### Abbreviations

$\Delta\%$ PV: percentage change in plasma volume	PV: plasma volume (mL)
BMI: body mass index ( $\text{kg}/\text{m}^2$ )	R: recreational runners
BV: blood volume (mL)	RCV: red cell (erythrocyte) volume (mL)
BW: body weight (kg)	RH: relative humidity
CO: carbon monoxide	$S_{\text{CO}}$ : percentage of carbon monoxide saturation of hemoglobin
COHb: carboxyhemoglobin	SUM: study done in summer
HA: heat acclimation	THb: total circulating hemoglobin (g)
Hb: hemoglobin concentration of whole blood (g/dL)	TPP: total plasma protein (g)
Hct: percentage of erythrocytes in whole blood	$T_{\text{RE}}$ : rectal temperature
HR: heart rate (beats/minute)	$V_{\text{CO}}$ : carbon monoxide uptake (mL)
LBM: lean body (non-fat) mass (kg)	$\text{VO}_{2\text{max}}$ : maximal $\text{O}_2$ consumption during a ramp ergometer test ( $\text{ml}/\text{min}/\text{kg}$ body wt)
NA: unacclimated to heat	WIN: study done in winter
NR: non-runners, sedentary life style	
PP: plasma protein concentration (g/dL)	

## Materials and Methods

### Subjects

The study protocols were approved by the Human Subjects Review Committee of The Lovelace Foundation. Each subject provided written informed consent. Eight healthy men were studied in the winter (WIN-January/February) and 10 in the summer (SUM-July/August). Women were not included because the funding agency at that time required findings applicable to men. Outdoor temperature and relative humidity (RH) in WIN and SUM averaged 10 °C – 56% RH and 32 °C – 32% RH, respectively, at an altitude of 1,650 m. The subjects were known to the investigators and volunteered. Subjects in both studies were partially selected based on their recreational habits, such that in each study half were classified as runners (R), who ran 20–35 miles/week (maintained throughout the studies), and half were more sedentary, denoted non-runners (NR). Two subjects, one NR and one R, took part in both studies. The aerobic capacity ( $VO_2\text{max}$ ) of each subject was first determined by a ramp bicycle ergometer test, as described by Luft et al. (23), and lean body mass (LBM) and percent fat by hydrostatic weighing (18).

Pertinent baseline measurements are given in Table I. Their age ranged from 21–43 yr. The average  $VO_2\text{max}$  values for NR were similar to that of a healthy sedentary population (20). No means were significantly different between the eight subjects in WIN and the 10 in SUM. However, when partitioned by fitness the nine Rs had lower fat content and body mass index (BMI) and tended to have a lower weight (BW), with significantly higher  $VO_2\text{max}$  values, ranging from 44 to 55 vs. 27 to 40 ml/min/kg of BW for NRs.

Table I. Mean ( $\pm$  1.0 SD) baseline measurements of 18 subjects partitioned by season and activity

	n	Age	Ht	BW	BMI	LBM	Fat	$VO_2\text{max}$
		(yr)	(cm)	(kg)	( $\text{kg}/\text{m}^2$ )	(kg)	(%)	(ml/min/kg)
All	18	32 (7)	176.6 (7.7)	75.2 (14.4)	24.1 (4.0)	62.9 (8.7)	15.5 (8.0)	42.8 (9.1)
Study A-WIN	8	33 (7)	174.1 (7.5)	70.9 (6.7)	23.4 (2.3)	60.4 (8.2)	14.9 (7.8)	43.8 (8.8)
Study B-SUM	10	31 (7)	178.6 (7.1)	78.7 (18.1)	24.6 (5.0)	65.0 (8.9)†	16.0 (8.6)	42.0 (9.7)
Non-runners	9	30 (8)	176.6 (8.1)	81.0 (17.4)	26.0 (4.9)	63.7 (9.0)	20.4 (5.9)	35.1 (4.5)
Runners	9	34 (5)	176.6 (7.7)	69.4 (7.9)†	22.2 (0.9)*	62.1 (8.8)	10.6 (6.8)*	50.6 (4.5)*

WIN: study done in winter; SUM: study done in summer.

Significance of difference from above group indicated by \*:  $P < 0.05$  and †:  $P < 0.10$  (trend to significance)

### Heat acclimation

In both studies HA and dehydrating ergometer exercise were performed at 50 °C dry bulb, 26 °C wet bulb (RH  $\approx$  16%) at 30% of each subject's  $VO_2\text{max}$  in an environmental chamber with air movement at 3–4 m/s. In WIN, the first day baseline resting measurements were followed by an exercise without fluid replacement to exhaustion when subjects could no longer maintain the prescribed pedalling rate. This was followed by nine consecutive days of HA, with exercise continued for 100 minutes in the heat. Water and electrolyte deficits were replaced based on BW loss with flavored 0.1% saline during short rest intervals every 20 minutes. The men rested on day 11 and then on day 12 baseline measurements were made and

exercise to exhaustion without water replacement was repeated as on day 1. In SUM, the same resting baseline measurements were followed by two orthostatic tolerance tests before and after exercise in the heat without fluid replacement. This was followed by an identical 9-day HA protocol of work in the heat as in WIN. After a rest day the baseline measurements were repeated, followed by the same two orthostatic and exercise tests. Thus, in both studies HA consisted of 10 days of work in the heat, the first day with no fluid replacement.

The effectiveness of HA was apparent in both studies. In WIN the exercise time without fluid replacement increased significantly from 77 to 112 min after HA, with the rise in HR and body temperature ( $T_{RE}$ ) being 43% and 39% lower, respectively ( $P = 0.001$ ). The average evaporative rate increased 7% and the sweat electrolyte concentration decreased 18% after HA. In SUM, where these measurements during dehydrating exercise were not made, the success of HA was confirmed by reduced HR and  $T_{RE}$  after the last (9th) HA exercise session compared to the first. The average rise in HR and  $T_{RE}$  decreased by 38% and 32%, respectively. The decrease in PV after dehydrating work in the heat before and after HA averaged 12% in both studies, with no significant difference between pre- and post-HA, or between WIN and SUM.

#### *Procedures and measurements of resting blood constituents before and after HA*

In both studies, before and after HA, the subjects were in a post-absorptive and euhydrated state. A flexible Teflon catheter was inserted into an antecubital vein and BW recorded within  $\pm 15$  g. Following 45–60 min of rest in the recumbent position, baseline blood volume (BV) was obtained by measuring THb by the CO rebreathing method (26). An infrared method (27) was used to measure blood carboxyhemoglobin (COHb) saturation ( $S_{CO}$ ) before and after rebreathing. Blood samples were also drawn for Hb, Hct and PP without stasis from the indwelling catheter. Venous Hct was determined by the microhematocrit method, with no corrections for trapped plasma or difference between venous and whole body Hct. The Hb concentration was determined by the Drabkin technique (7) using a Beckman DU spectrophotometer calibrated with Hycel methemoglobin standards. The PP concentration was determined by the Biuret method with an autoanalyzer (SMA-12, Technicon Corp) from venous samples taken during the CO rebreathing. Changes in PV, BV and RCV were also calculated from that day's baseline and post-HA baseline Hb and Hct according to equations summarized by Greenleaf et al. (13).

After CO rebreathing the THb was calculated as follows:

$$\text{THb (g)} = (V_{CO} \times 0.985 \times 100) / (1.34 \times \Delta S_{CO}) \quad (\text{Eq. 1})$$

where:  $V_{CO}$  = volume of CO in ml (STPD) introduced into the rebreathing circuit; 0.985 = average fraction of CO taken up by the blood at the end of 10 min rebreathing; 1.34 = CO capacity of 1.0 g of Hb and  $\Delta S_{CO}$  = the increase in COHb saturation after CO rebreathing. BV was then derived from THb and Hb concentration as:

$$\text{BV (mL)} = (\text{THb} / \text{Hb}) \times 100 \quad (\text{Eq. 2})$$

and PV from BV and Hct as:

$$\text{PV (mL)} = \text{BV} \times (100 - \text{Hct}) / 100 \quad (\text{Eq. 3})$$

and RCV as:

$$\text{RCV (mL)} = \text{BV} - \text{PV} \quad (\text{Eq. 4})$$

The percentage change in PV ( $\Delta\%PV$ ) after HA was calculated from PV values obtained by Eq. 3 and also calculated from PP measurements as:

$$\Delta\%PV = 100 \times [(PP_{pre} / PP_{post}) - 1] \quad (\text{Eq. 5})$$

The Hb content of red cells was estimated from the Hb/Hct ratio. Total circulating protein (TPP) was obtained from  $PP - PV/100$ .

### Statistics

Pre- (non-heat acclimated – NA) to post-HA differences were tested by paired-*t*-tests. Significant differences between WIN and SUM and R and NR were tested by one-way ANOVA. To test for interaction between activity level and season, a two-factor ANOVA without replication was performed on measurement differences before and after HA between R and NR and WIN and SUM. Significance of correlations between  $\Delta\%PV$  by the various methods was tested by standard linear least squares regression analyses. All *P*-values were considered significant at  $P \leq 0.05$  and trends suggested by  $P \leq 0.10$ .

## Results

### All subjects

The baseline averages and mean changes ( $\pm 1.0$  SD) in measurements after HA are given in Table II and partitioned by season and fitness level. For all 18 subjects combined there was a small, but significant, increase in PV of 92 mL (2.9%) after HA, measured by the CO method, contributing almost entirely to the significant increase in BV, and reductions in Hb ( $P < 0.05$ ), Hct (n.s.) and a trend to increasing TPP. However, the large SD of 4.8 for  $\Delta\%PV$  indicated a wide variation in  $\Delta\%PV$ , with a 95% confidence interval from  $-6.5$  to 12.3%.

### Season

Baseline Hb and Hct were significantly lower in SUM than WIN by 1.2 g/dL and 3.6%, respectively, and PV tended to be larger. The  $\Delta\%PV$  and BV increased significantly in SUM after HA, with essentially no change in WIN, a significant seasonal difference. The Hb and Hct were lower after HA in both WIN and SUM, but not significantly. The Hb/Hct ratio tended to decrease in WIN, indicating a swelling of erythrocytes with HA, but not significantly less than the small decrease in SUM. The TPP was significantly increased in SUM, mainly due to the 5% increase in  $\Delta\%PV$ .

### Fitness

Differences between R and NR were minor. The Rs had a larger PV at baseline than NRs. The NRs tended to increase PV (3.6%) after HA, with reductions in Hb and Hct from hemodilution, and TPP tended to increase. The 2.2% increase in  $\Delta\%PV$  for Rs was not significant. In Rs the only significant change with HA was a reduction in Hb/Hct ratio, attributable to their decrease in Hb and increase in RCV. The Hct did not change in Rs after HA, but decreased significantly in NR.

Because RCV, PV and BV correlate with LBM, values per LBM are shown in Fig. 1. The baseline values for RCV/LBM before HA were significantly lower during SUM than WIN. For all three measurements the values after HA decreased slightly in WIN and increased in SUM; these trend differences were all statistically significant. The same three measurements are shown in Fig. 2 for NR and R. The Rs had significantly higher baseline values for PV and BV than NR, but no changes with HA were significant.

Table II. Mean ( $\pm$  1.0 SD) baseline measurements of 18 subjects partitioned by season and activity, and changes with HA

	RCV (mL)	PV (mL)	BV (mL)	Hb (g/dL)	Hct (%)	Hb/Hct	$\Delta\%$ PV	PP (g/dL)	TPP (g)
All (n = 18)									
NA	2474 (279)	3203 (368)	5678 (580)	15.56 (0.89)	44.31 (2.67)	0.351 (0.008)	–	6.89 (0.42)	214 (26)
HA	15 (114)	92 (152)‡	106 (216)‡	-0.31 (0.48)‡	-0.54 (1.37)	-0.003 (0.007)	2.9 (4.8)‡	-0.02 (0.26)	5 (12)§
Study A-WIN (n = 8)									
NA	2542 (302)	2941 (299)	5482 (558)	16.20 (0.76)	46.32 (2.09)	0.350 (0.010)	–	6.74 (0.33)	199 (24)
HA	-29 (121)	9 (146)	-20 (197)	-0.34 (0.41)§	-0.37 (1.60)	-0.004 (0.007)§	0.3 (4.8)	0.07 (0.25)	2 (13)
Study B-SUM (n = 10)									
NA	2421 (263)	3247 (325)†	5667 (547)	15.04 (0.63)*	42.70 (1.89)*	0.352 (0.006)	–	7.00 (0.46)	227 (20)*
HA	50 (102)	158 (119)‡	207 (168)‡	-0.29 (0.56)	-0.68 (1.23)	-0.001 (0.007)	5.0 (4.1)‡	-0.09 (0.26)	8 (11)‡
HA-NA diff.	n.s.	¶	¶	n.s.	n.s.	n.s.	¶	n.s.	n.s.
NR (n = 9)									
NA	2413 (279)	2985 (277)	5397 (471)	15.70 (0.92)	44.65 (2.81)	0.352 (0.006)	–	6.94 (0.36)	27 (22)
HA	-23 (98)	107 (143)§	84 (203)	-0.39 (0.56)§	-1.09 (1.18)‡	0.000 (0.007)	3.6 (4.9)§	0.01 (0.22)	8 (12)§
R (n = 9)									
NA	2536 (282)	3237 (370)†	5773 (572)	15.41 (0.89)	43.97 (2.63)	0.351 (0.009)	–	6.83 (0.48)	221 (29)
HA	52 (123)	77 (161)	129 (228)	-0.23 (0.40)	0.01 (1.39)	-0.005 (0.007)‡	2.2 (5.0)	-0.05 (0.30)	3 (12)
HA-NA diff.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.

WIN: study done in winter; SUM: study done in summer; NR: non-runners; R: runners; RCV: red cell volume; PV: plasma volume; BV: blood volume (all obtained by CO method); PP: plasma protein concentration; TPP: total plasma protein. n. s.: no significant difference

Significance of difference in NA (baseline) value from above group indicated by \*:  $P < 0.05$  and †:  $P < 0.10$  (trend to significance).

Significance of change after HA indicated by ‡:  $P < 0.05$  and §:  $P < 0.10$  (trend significance).

Significance of difference from above group in change after HA indicated by ¶:  $P < 0.05$  and \*\*:  $P < 0.10$  (trend to significance)

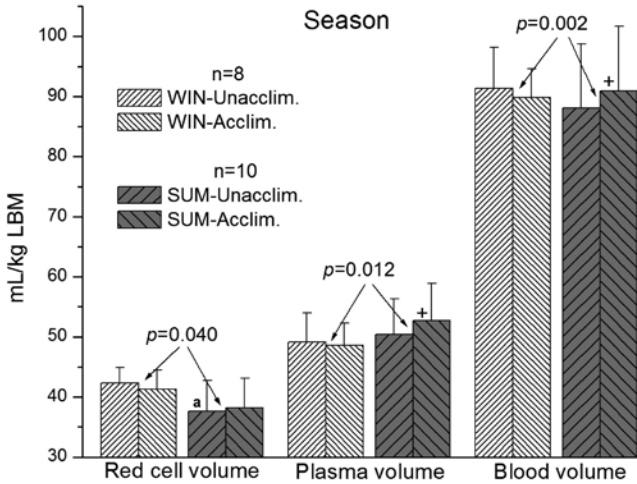


Fig. 1. Mean ( $\pm 1.0$  SD) values of red cell, plasma and blood volume per kg of lean body mass (LBM) before and after heat acclimation for winter and summer studies. a: baseline value significantly ( $P < 0.05$ ) lower in summer than winter; +: value significantly ( $P < 0.05$ ) higher after heat acclimation than before. The  $P$ -levels are shown for significance of difference of changes with heat acclimation between seasons

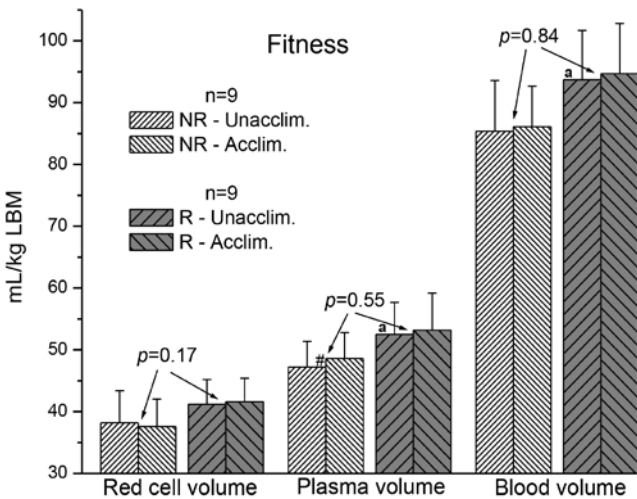


Fig. 2. Mean ( $\pm 1.0$  SD) red cell, plasma and blood volume per kg of lean body mass (LBM) before and after heat acclimation for runners (R) and non-runners (NR) from both studies. a: baseline values significantly ( $P < 0.05$ ) higher in R than NR; #: value tends to be higher ( $P < 0.10$ ) in NR after heat acclimation than before. The  $P$ -levels are shown (n.s.) for significance of differences of changes between R and NR with heat acclimation

Two-factor ANOVA analyses of all changes induced by HA shown in Table II and Figs. 1 and 2 did not indicate significant interaction between season and fitness level (all  $P$ -values for interaction  $> 0.20$ ).



### Methods of measurement

The  $\Delta\%PV$  values are presented in Table III for the four methods. For all 18 subjects  $\Delta\%PV$  was quite similar, as determined by the CO, Hb+Hct and Hct calculations (13). The  $\Delta\%PV$  based on PP shows trivial changes with HA in all groups. In WIN and SUM the two indirect Hb+Hct and Hct estimates gave similar results for each season, in contrast to the CO values being significantly higher in SUM. Both indirect methods indicate a larger increase after HA for NR than R (Hb+Hct – n.s. and Hct –  $P < 0.10$ ), which is not apparent by the CO method. The correlations of CO measurements with those of Hb+Hct and Hct are significant, but low, while the latter methods correlated closely with a slope near 1.0, indicating no overall change in erythrocyte volume (Hb/Hct ratio) during HA. Overall there was little change in THb with HA; however in WIN the loss of 23 g was significantly different ( $P = 0.038$ ) than the 14 g gain during SUM. Opposite, but non-significant, changes in THb were noted for Rs and NRs. The overall changes in THb were closely associated with the CO minus Hb+Hct method differences in  $\Delta\%PV$  ( $n = 18, r = 0.99$ ); they were also closely associated with the CO minus Hct method differences ( $r = 0.90$ ), while the correlation of THb changes with the Hb+Hct minus Hct methods was low ( $r = 0.15$ ).

Table III. Mean ( $\pm 1.0$  SD) changes in  $\Delta\%PV$  and THb with HA and correlations ( $n = 18$ ) between methods

Methods								Correlations	
Group	n	CO	Hb+Hct	Hct	PP	THb (g)	CO vs. Hb+Hct	r	P
All	18	2.9 (4.8)‡	3.2 (5.6)‡	2.4 (5.7)§	0.3 (3.7)	–2 (38)	CO vs. Hct	0.63	0.005
WIN	8	0.3 (4.8) ¶	3.0 (5.6) n.s.	1.7 (6.7) n.s.	–0.9 (3.6) n.s.	–23 (36)§ ¶	CO vs. PP	0.17	0.51
SUM	10	5.0 (4.1)‡	3.3 (5.9)	2.9 (5.1)	1.3 (3.6)	14 (33)	Hb+Hct vs. Hct	0.94	<0.001
NR	9	3.6 (4.9)§ n.s.	4.7 (6.0)‡ n.s.	4.6 (5.1)‡ **	–0.1 (3.2) n.s.	–9 (34) n.s.	Hb+Hct vs. PP	0.39	0.11
R	9	2.2 (5.0)	1.6 (5.0)	0.1 (5.6)	0.7 (4.2)	4 (43)	Hct vs. PP	0.28	0.26

$\Delta\%PV$ : percentage change in plasma volume

WIN: study done in winter; SUM: study done in summer

NR: non-runners; R: runners

CO: carbon monoxide rebreathing

Hb+Hct: calculated from Hb and Hct, assuming no change in total circulating Hb or red cell volume

Hct: calculated from Hct, assuming no change in total circulating Hb

PP: calculated from PP concentration, assuming no change in total circulating PP

n.s.: no significant difference

THb: total circulating Hb

Significance of change after HA indicated by ‡:  $P < 0.05$  and §:  $P < 0.10$  (trend to significance)

Significant difference between groups in change after HA indicated by ¶:  $P < 0.05$  and \*\*:  $P < 0.10$  (trend to significance)

### Additional observations

As mentioned previously, the decrease in PV after dehydrating work in the heat before and after HA averaged 12% in both studies, with no significant difference between pre- and post-HA, or between WIN and SUM or R and NR. The correlation between  $\Delta\%PV$  measured by the CO method and the difference in PV lost during dehydrating work in the heat before and after HA for all subjects ( $n = 18$ ) was low ( $r = 0.09$ ).



## Discussion

Direct measurements by CO rebreathing demonstrated a small increase in  $\Delta\%PV$  (Table II) over 10 days of HA in all 18 subjects, with a significant increase in BV and insignificant increase in RCV. These changes occurred almost entirely in SUM. The changes in these measurements were essentially independent of the fitness level. The small changes in  $\Delta\%PV$ , even in SUM, bring into question the significance of a PV increase during HA. It did not prevent or change the decrease in PV during the dehydrating work in the heat after HA. The physiological benefits of the PV increases during HA are also questionable based on the low correlation observed between  $\Delta\%PV$  during HA and the differences in PV lost during dehydrating work in the heat before and after HA for all subjects.

The TPP values tended to be larger after HA, mainly in SUM and in NR. This increase has been considered to be an important result of HA, by affecting plasma oncotic pressure to maintain intravascular volume and thereby delay circulatory failure with dehydration (14). The lack of  $\Delta\%PV$  noted by the PP estimation and its lack of relationship to the other three methods (Table III) suggests that protein flux in or out of the vascular space during HA invalidates estimates of PV changes by PP under these conditions.

Lower baseline Hb and Hct levels before HA in SUM (Table II) may be related to the seasonal difference in  $\Delta\%PV$ . A number of reports have noted similar seasonal differences in samples from the general population (15, 16, 17, 31, 33). Part of the reduction of Hb and Hct in the warmer summer is likely attributable to natural HA occurring because of higher ambient temperatures increasing PV for sweating or evaporative heat loss demand. Recreational training before and after HA by Rs in this study, superimposed on the artificial HA, may have attenuated their PV increase by contributing to their higher baseline BV and PV before HA. The contribution of training in warmer ambient temperatures superimposed on artificial HA has been pointed out by Shapiro et al. (34), who found that PV expanded more in the summer, as shown here (Table II). Other reports have also pointed out the influence of training on Hb and Hct (2).

Higher fitness levels are an important asset in performing in the heat and improving the effectiveness of HA (1, 5, 6). Conversely, HA seems effective in improving endurance performance in temperate environments (9, 22). However this report found no significant difference in PV changes after HA related to fitness; nor was the exercise time in dehydrating heat increased more for Rs than NRs.

Differences in experimental protocols and measurement techniques also contribute to variability of reported PV changes with HA. Baseline PV is often measured by indicator dilution methods like Evans blue or isotopes to label plasma albumin, or CO rebreathing or isotopes to label erythrocytes and measure RCV. Short term changes due to exercise, dehydration, etc. are then commonly estimated by changes in Hb+Hct or only Hct, to obtain  $\Delta\%PV$ . These two should give similar results if THb is unchanged and there is no change in erythrocyte volume resulting from changes in plasma osmolality (13). However, the values in Table III indicate that the difference between the THb decreases with HA in WIN and the increase in SUM was significant. The close association of changes in THb with the CO minus Hb+Hct method differences in  $\Delta\%PV$  and the close association with the CO minus Hct method differences indicate that the assumption of no change in THb from baseline after 10 days of HA was not valid, making these indirect estimations suspect for measuring  $\Delta\%PV$  after HA. The low correlation of THb changes with the Hb+Hct vs. Hct method differences was low, probably indicating random measurement error of Hb and Hct. THb may be expected to decrease with HA exercise in NRs because of increased erythrocyte turnover resulting

from daily exercise to which they were not accustomed (32). The reduction in THb in WIN and increase in summer is more difficult to explain; differences in diet and nutrition may be involved (35), or perhaps the HA exercises in WIN were more stressful than in SUM when superimposed on a background of relative inactivity for both R and NRs (12).

### Conclusions

This report indicates that the mean PV increase after 10 days of HA is small but can vary widely when measured directly with the CO method, and of questionable benefit for subsequent dehydrating work. The PV increase by the CO method is larger in summer than winter and independent of aerobic capacity. Indirect methods based on Hb and Hct give generally similar results, but may not differentiate between seasons and tend to introduce questionable differences based on aerobic capacity because of changes in THb during HA.

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